PUCRS

ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR DOUTORADO EM BIOLOGIA CELULAR E MOLECULAR

STÉFANIE INGRID DOS REIS SCHNEIDER

AVALIAÇÃO DA MODULAÇÃO COGNITIVA, LOCOMOTORA, NEUROMETABÓLICA E DA LATERALIDADE ENCEFÁLICA EM RATOS WISTAR COM DIABETES MELLITUS INDUZIDOS POR ESTREPTOZOTOCINA

Porto Alegre

2020

PÓS-GRADUAÇÃO - *STRICTO SENSU*

Pontifícia Universidade Católica
do Rio Grande do Sul

ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR
DOUTORADO EM BIOLOGIA CELULAR E MOLECULAR

STÉFANIE INGRID DOS REIS SCHNEIDER

AVALIAÇÃO DA MODULAÇÃO COGNITIVA, LOCOMOTORA, NEUROMETABÓLICA E DA LATERALIDADE ENCEFÁLICA EM RATOS WISTAR COM DIABETES MELLITUS INDUZIDOS POR ESTREPTOZOTOCINA

PORTO ALEGRE

2020

STÉFANIE INGRID DOS REIS SCHNEIDER

AVALIAÇÃO DA MODULAÇÃO COGNITIVA, LOCOMOTORA, NEUROMETABÓLICA E DA LATERALIDADE ENCEFÁLICA EM RATOS WISTAR COM DIABETES MELLITUS INDUZIDOS POR ESTREPTOZOTOCINA

Tese apresentada como requisito para a obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientador: Prof. Dr. Léder Leal Xavier

PORTO ALEGRE

2020

Ficha Catalográfica

S359a Schneider, Stéfanie Ingrid dos Reis Schneider

Avaliação da modulação cognitiva, locomotora, neurometabólica e da lateralidade encefálica em ratos wistar com diabetes mellitus induzidos por estreptozotocina / Stéfanie Ingrid dos Reis Schneider Schneider . – 2020.

110.

Tese (Doutorado) – Programa de Pós-Graduação em Biologia Celular e Molecular, PUCRS.

Orientador: Prof. Dr. Léder Leal Xavier.

1. Diabetes Mellitus. 2. MicroPET. 3. 18F-fluorodeoxyglucose. 4. Lateralidade. I. Xavier, Léder Leal. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da PUCRS com os dados fornecidos pelo(a) autor(a).
Bibliotecária responsável: Clarissa Jesinska Selbach CRB-10/2051

STÉFANIE INGRID DOS REIS SCHNEIDER

AVALIAÇÃO DA MODULAÇÃO COGNITIVA, LOCOMOTORA, NEUROMETABÓLICA E DA LATERALIDADE ENCEFÁLICA EM RATOS WISTAR COM DIABETES MELLITUS INDUZIDOS POR ESTREPTOZOTOCINA

Tese apresentada como requisito para a obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Aprovada em: 31 de março de 2020.

BANCA EXAMINADORA:

_____

Jarbas Oliveira - PUCRS

_____

Rodrigo Braccini Madeira da Silva- PUCRS

_____

Adroaldo Lunardelli

PORTO ALEGRE

2020

A minha pequena Iza, pelo simples fato de existir
e colorir os meus dias...

AGRADECIMENTOS

À minha família, meus pais Roque e Marisa pelo amor e investimento que fizeram por mim ao longo de toda a minha vida desde a graduação, mestrado e doutorado.

Aos meus irmãos por serem meu incentivo a buscar o melhor sempre.

À Iza, unicamente pelo fato de existir e por me esperar chegar todos os dias em casa depois de um dia cheio de trabalho, sorrir e me fazer feliz.

Ao meu amor, meu melhor amigo, parceiro e companheiro Rodrigo, pela dedicação, carinho e apoio. Obrigada pela convivência diária, por me ouvir, por me incentivar e por estar em todos os momentos ao meu lado.

À Andrea Wieck, que sorte a minha tu teres aparecido na minha vida, me ensinar a respirar, e além de tudo ser uma ótima pesquisadora, que se dedica de corpo e alma, disposta a estabelecer discussões científicas e enriquecer o nosso trabalho de uma forma única. Tu foste a peça que faltava para mim, "a chavezinha". Eu lhe admirarei pelo resto da minha vida como mulher e como profissional que tu és. Me espelharei em ti como pesquisadora e espero que tua essência permaneça sempre a mesma.

Ao meu orientador Professor Léder Leal Xavier meu agradecimento por ter me aceito como sua estudante de doutorado e por ter colaborado no desenvolvimento deste projeto.

Aos meus queridos e excelentes professores Régis Mestriner, Jarbas Oliveira, Elke Bromberg, Clarice Alho, Rosane Silva, Carla Bonan, Fernanda Morrone, Moisés Bauer e Márcio Donadio do Programa de Pós-Graduação em Biologia Celular e Molecular da PUCRS pelo intenso trabalho e contribuição na minha formação. Sentirei falta das aulas,

das discussões sobre os artigos e de todas as aulas que vocês me proporcionaram. Minha admiração por esses mestres da ciência e do ensino será eterna.

À Raquel, técnica do Laboratório de Biologia Celular e Tecidual, obrigada pela sua amizade, seu amor, seus conselhos, seu apoio, sem você eu não conseguiria.

À Zingara, por desde o início ter me instruído, me orientado e ter me auxiliado para contemplar todas as exigências do PPG. Agradeço ao seu apoio e sua amizade.

Ao Cássio Loss, por ter me recebido na UFRGS de braços abertos com toda a sua dedicação e ainda por ter doado seu tempo para me instruir e me orientar. Agradeço por ter sido um amigo, um parceiro e um ótimo profissional.

À Paula Feltes por me orientar e ter um papel fundamental na minha orientação. Uma grande amiga e uma excelente profissional. Te admiro muito.

Às minhas colegas de laboratório Bruna, Rafa, Lisiê, Vic e Isadora obrigada por tornarem meus dias mais divertidos dentro do período de convivência no laboratório, vou lembrar destes momentos para sempre.

Aos colegas Samuel e Gianina do Centro de Pesquisa pré-clínica do Instituto do Cérebro (INSCER) do Rio Grande do Sul - PUCRS por me auxiliar a desenvolver este trabalho.

A todos que de alguma forma contribuíram para a realização desse trabalho, principalmente aos pacientes diabéticos que de alguma forma se beneficiam com este estudo.

Aos meus alunos, aos meus colegas de trabalho, de profissão e pesquisadores, todos que de alguma forma me deram a mão e me ajudaram a ser mais forte.

À CAPES, CNPq, FAPERGS e PUCRS pelo financiamento ao longo desses anos.

RESUMO

Os estágios avançados da diabetes mellitus (DM) estão associados à disfunção locomotora e cognitiva. Os déficits motores observados estão relacionados à neuropatia diabética envolvendo nervos periféricos, o que aumenta o risco de morbidade em pacientes diabéticos. Além disso, as complicações do sistema nervoso central (SNC) durante a progressão do diabetes são relativamente mais sutis quando comparadas às alterações periféricas. Até o presente momento, poucos estudos investigaram os efeitos neurológicos nos estágios iniciais do diabetes, principalmente em modelos experimentais. Considerando isto, nosso estudo teve como principal objetivo avaliar a atividade locomotora, a memória espacial e de curto prazo e o metabolismo da glicose no encéfalo antes e após a indução do DM em ratos wistar adultos. O DM foi induzido por injeção intraperitoneal de 60mg/kg de estreptozotocina (STZ), o que leva à ruptura das células beta pancreáticas e consequente indução do DM experimental 48 horas após a injeção. A atividade locomotora foi avaliada através do teste de campo aberto (OF); a memória pelo novo teste de reconhecimento de objetos (NOR) e o metabolismo da glicose no encéfalo pela micro tomografia por emissão de pósitrons (PET) com o uso da fluorodeoxiglucose ([^{18}F]FDG). O peso corporal, a ingestão de alimentos e água, a produção de urina e fezes e os níveis de glicose no sangue também foram avaliados ao longo do experimento. Os resultados da análise comportamental mostraram que há um comprometimento cognitivo leve no grupo DM em comparação aos animais controle, sugerindo alterações na memória espacial de curto prazo. Em relação à atividade locomotora, não foram observadas alterações significativas entre os grupos. A análise global do metabolismo da glicose no encéfalo medida pela captação de ^{18}F-FDG indicou pequenas alterações em áreas importantes para memória de curto prazo e de trabalho, controle emocional e locomoção. Os animais com DM não alteraram a captação de ^{18}F-FDG no cerebelo, apesar de os animais controles apresentarem aumento

do metabolismo. Enquanto isso, os resultados mostraram um aumento da captação nos DM observada na amígdala quando comparada aos grupos controle. Captação de hipocampo não foi significativamente alterada quando analisada globalmente. No entanto, ao segmentá-lo no hipocampo dorsal e ventral, foi possível observar uma captação aumentada de ^{18}F-FDG no hipocampo dorsal em ratos diabéticos. Esses resultados sugerem possíveis mecanismos compensatórios nos estágios iniciais do DM em relação à cognição e ao metabolismo da glicose cerebral induzido. A conclusão do nosso estudo é de que as regiões do encéfalo obtiveram diferentes padrões de distribuição do metabolismo de glicose quando analisadas globalmente e em secções. Com esses resultados fomos avaliar a diferença dos padrões de distribuição do metabolismo de glicose, de acordo com a sua lateralidade, usando ^{18}F-FDG como marcador de metabolismo de glicose nas regiões encefálicas. Os resultados demonstraram que a distribuição de ^{18}F-FDG no encéfalo antes e após a indução do DM em ratos Wistar adultos não é homogênea. As áreas encefálicas que demonstraram diferença no metabolismo de glicose em relação a lateralidade entre diabéticos e controles foram as do córtex auditivo, córtex orbitofrontal, núcleo accumbens core e hipocampo posterior. Com esses resultados demonstramos que a avaliação do metabolismo de glicose analisado de forma global deve ser substituída por análise por meio de lateralidade de cada região encefálicas para a utilização do microPET em modelo experimental de diabetes induzido por STZ. Mais estudos são necessários para avaliar possíveis alterações avaliados em longo prazo após a indução de DM nesse modelo animal.

Palavras-chave: microPET, ^{18}F-FDG, Ratos Wistar, Diabetes mellitus

ABSTRACT

Advanced stages of Diabetes Mellitus (DM) are associated to locomotor and cognitive dysfunction. Motor deficits observed are related to diabetic neuropathy involving peripheral nerves, which increases morbidity risk in diabetic patients. In addition, central nervous system (CNS) complications during diabetes progression are relatively more subtle compared to peripheral changes. To the present moment, few studies investigated the neurological effects during the early stages of diabetes onset, mainly in experimental models. Considering the above, our study evaluated the locomotor activity, short-term and spatial memory and brain glucose metabolism before and after DM induction in adult Wistar rats. The DM was induced by intraperironeal injection of 60mg/kg of streptozotocin (STZ), which leads to pancreatic beta cells disruption and consequent induction of experimental DM 48 hours after injection. Locomotor activity was evaluated through the open field (OF) test; the memory by the novel object recognition (NOR) test and brain glucose metabolism by [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography (PET). Body weight, food and water intake, urine and fecal output as well as blood glucose levels were also evaluated throughout the experiment. Behavioral analysis demonstrated in DM group compared to control animals, alterations in short-term spacial memory observed through NOR. Regarding locomotor activity, no significant alterations were observed between groups. Global analysis of brain glucose metabolism measured by ^{18}F-FDG uptake indicated slight alterations in important areas for short-term and working memory, emotional control and locomotion. Controls animals had increased ^{18}F-FDG uptake in the cerebellum, whereas DM animals had no alterations. DM animals had a increase was observed in the amygdala when compared to the control groups. Hippocampus uptake was not significantly altered when analyzed

whole. However, when segmenting it into dorsal and ventral hippocampus, it was possible to observe increased tracer uptake in the dorsal hippocampus. These results suggest possible intrinsic compensatory mechanisms in early stages of DM regarding cognition and brain glucose metabolism induced. Thus, in our second part of the study, the main objective was to assess the difference in the distribution patterns of glucose metabolism, according to its laterality, using ^{18}F-FDG as a marker in brain regions. The results demonstrated that the distribution of ^{18}F-FDG in the brain before and after the induction of DM in adult wistar rats is not homogeneous. The brain areas that showed a difference in glucose metabolism in relation to laterality between diabetics and controls were those of the auditory cortex, orbitofrontal cortex, nucleus accumbens core and hippocampus Posterior. We demonstrate with these results that the assessment of glucose metabolism analyzed globally should be replaced by analysis using the laterality of each brain region for the use of microPET in an experimental model of diabetes induced by STZ. Further studies are necessary to evaluate possible long-term alterations after DM induction in this animal model.

Keywords: microPET, ^{18}F-FDG, Wistar Rats, Diabetes mellitus

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

^{18}F-FDG	^{18}F-2-fluoro-2-deoxy-glucose
DM	Diabetes Mellitus
DMT1	Diabetes Mellitus Tipo 1
DMT2	Diabetes Mellitus Tipo 2
DP	Doença de Parkinson
ERO	Espécies reativas de oxigênio
GFAP	Proteína Glial Fibrilar Ácida
GLUT 1	Transportador de Glicose tipo 2
GLUT2	Transportador de Glicose tipo 2
GLUT 3	Transportador de Glicose tipo 2
GLUT4	Transportador de Glicose tipo 4
HPA	Hipotálamo-Pituitária-Adrenal
IGF-1	Fator de Crescimento semelhante à Insulina tipo 1
microPET	Micro tomografia por emissão de pósitrons
OMS	Organização Mundial da Saúde
PET	Tomografia de emissão de pósitron
SNC	Sistema Nervoso Central
SNc	Substância Nigra pars Compacta
STZ	Estreptozotocina
UI	Unidades Internacionais

SUMÁRIO

Capítulo I

1. INTRODUÇÃO

2. JUSTIFICATIVA

3. OBJETIVOS

1. INTRODUÇÃO

1.1 Diabetes Mellitus

A diabetes mellitus (DM) é uma disfunção metabólica complexa que impacta os sistemas de saúde, a economia e o crescimento global. É considerada uma das doenças mais incapacitantes em todo o mundo, com alta prevalência, grande mortalidade e altos custos médicos (Aguilar, Bhuket, Torres, Liu, & Wong, 2015; Fava, 2013; GBD, 2015; NCD-RisC, 2016; Roglic & World Health Organization, 2016). Assim, a DM é caracterizada como um grupo de doenças metabólicas em que o paciente apresenta hiperglicemia, que afetará consequentemente o metabolismo encefálico, contribuindo para alterações neuropatológicas (Atkinson, Eisenbarth, & Michels, 2014; Sonneville et al., 2012). Aproximadamente 90% dos casos de DM se enquadram em duas categorias principais: DM tipo 1 (DMT1) e DM tipo 2 (DMT2) *(American Diabetes Association, 2016*).

No Brasil, os dados epidemiológicos de diabetes apontam que até o ano de 2019 o número de pessoas com a doença era de 16,8 milhões, segundo a *International Diabetes Federation* (IDF, 2019). É o quarto país com o maior número de pessoas com diabetes tipo 2 (de Almeida-Pititto et al., 2015). Na região da América do Sul e Central, 9,43% da população adulta têm diabetes, com 41,9% dos pacientes não- diagnosticados. Em 2019, na América Latina, quase 243.200 adultos entre 20-79 anos morreram como resultado do diabetes, dos quais metade das mortes ocorreu no Brasil. No Brasil, 46% dos pacientes são não-diagnosticados, além disso há 95.846 pacientes de d0 até os 19 anos com DMT1 (IDF, 2019).

Os custos médicos para DMT1, que compreendem o rastreio, diagnóstico e tratamento, nos Estados Unidos, são em torno de U$$ 150 bilhões de dólares ao ano,

custando US$ 2.692 por indivíduo, demonstrando assim o impacto econômico significativo da doença (Egede, Bishu, Walker, & Dismuke, 2016). No Brasil, em um estudo multicêntrico, o custo médico anual direto para cada paciente é de U$$ 1.319,15, relacionados à terapia, principalmente para a aquisição de insulina e suprimentos para monitorização da glicemia (Cobas et al., 2013).

Conforme as Diretrizes da Sociedade Brasileira de Diabetes, a classificação atual da diabetes está relacionada ao mecanismo fisiopatológico subjacente que inclui a DM do tipo 1 (DMT1), DM do tipo 2 (DMT2), DM gestacional e DM de outros tipos como neonatal, secundária a infecções ou a medicamentos (SBD, 2020) . Entretanto, uma nova classificação proposta por pesquisadores escandinavos divide a diabetes em 5 categorias como: DM auto-imune grave, DM insulino-deficiente grave, DM insulino-resistente grave, DM leve relacionado à obesidade, DM leve relacionado à idade. Esta nova classificação tem como objetivo observar os fatores prognósticos que permitam personalizar o tratamento desde o início da doença (Ahlqvist et al., 2018).

A DMT1 compreende 5 a 10% dos casos de DM, sendo causada principalmente como resultado de uma destruição auto-imune das células beta pancreáticas, secretoras de insulina. A destruição celular leva a deficiência total de insulina tornando os pacientes dependentes de uma terapia insulínica durante toda a vida (Atkinson et al., 2014). A etiologia da DMT1 envolve variáveis genéticas e fatores relacionados a exposição ambiental que desencadeiam uma resposta imune inata direcionada a auto-destruição das células (Cabrera, Henschel, & Hessner, 2016). O aparecimento de DMT1 fulminante tem sido relatado durante infecção aguda com papeira, parainfluenza, vírus da herpes humano 6, citomegalovírus e enterovirus (De Beeck & Eizirik, 2016). Por sua vez, a suscetibilidade genética para o desenvolvimento da DMT1 envolve os indivíduos que possuem a presença dos alelos DR3 e DR4 correspondentes ao antígeno leucocitário

humano (HLA) de classe DRB1 que apresentam maior risco de desenvolver a doença (Noble et al., 2010; Redondo, Steck, & Pugliese, 2018). Além disso, a hipótese da higiene vem sendo postulada pelos pesquisadores que apontam uma correlação positiva entre a exposição a doenças infecciosas e a escassez de estimulação da imunidade inata no início da vida. Isto explicaria a ativação do sistema imune adaptativo, como o fator para o desencadeamento de uma resposta auto-imune nas células beta, uma vez que ocorre uma relação inversa entre a incidência de DMT1 e as taxas de mortalidade por doenças infecciosas (Bach & Chatenoud, 2012; Fava, 2013). Os mecanismos da DMT1 podem relacionar-se com as reações de hipersensibilidade do tipo tardio, falhas dos mecanismos centrais e periféricos que permitem a expansão de células T auto-reativas, presença de citocinas inflamatórias como a interleucina-1 (envolvida na imunidade inata), que altera a biossíntese de insulina e acarreta morte de células beta induzida por hiperglicemia, com redução ainda mais expressiva deste hormônio (Atkinson et al., 2014; Cabrera et al., 2016).

Os indivíduos mais acometidos pela DMT1 são os com idade inferior a 30 anos. A destruição auto-imune das células beta pancreática em pacientes infantis ocorre de forma rápida, enquanto que em adultos ocorre de forma lenta. Os sintomas clínicos clássicos DMT1 decorrentes da hiperglicemia aparecem em dias ou semanas, como a presença de poliúria com glicosúria (*American Diabetes Association*, 2012; Funk, 2007; Pirot, Cardozo, & Eizirik, 2008). O diagnóstico clínico da DM inclui os sintomas clássicos juntamente com o diagnóstico laboratorial baseado nos critérios de dosagem de hemoglobina glicada ≥6,5%, glicemia em jejum com os níveis ≥ 126mg/dL e teste oral de tolerância a glicose ≥200 mg/dL (*American Diabetes Association*, 2012).

A DMT2 é caracterizada pela hipo-responsividade dos receptores de insulina, gerando resistência desse hormônio nos tecidos-alvo. Cerca de 90 a 95% dos casos de

DM são do tipo 2. Como há apenas uma deficiência relativa na produção de insulina pelas células beta do pâncreas os pacientes não fazem terapia insulínica para a sua sobrevivência. Como na DMT2 há secreção residual de insulina, a hiperglicemia se desenvolve gradualmente, fazendo com que muitos pacientes com DMT2 sejam assintomáticos e, por esse motivo, acabam sendo diagnosticados tardiamente. Outros pontos relevantes são que a DMT2 apresenta uma importante predisposição genética, sendo fortemente correlacionado com a idade e também com a obesidade (responsável por cerca de 80% dos casos de DMT2), principalmente em pacientes com obesidade abdominal central (*American Diabetes Association*, 2012; Funk, 2007).

A única terapia eficaz para DMT1 com a capacidade de controlar a hiperglicemia é a insulina, porém esse tratamento também tem levado a resistência à insulina e ao prejuízo de tecidos-alvo como o encéfalo (Blázquez, Velázquez, Hurtado-Carneiro, & Ruiz-Albusac, 2014). Utilizando ratos que tornaram-se diabéticos em 2 semanas, tratados com insulina em diferentes doses, foi possível avaliar a melhor dose para terapia insulínica. Neste estudo as doses de insulina administradas aos ratos diabéticos variaram de 1 até 9 unidades internacionais (UI), por dia, ao longo de 7 dias. A dose intermediária de 3 UI/dia de insulina foi a que melhor induziu a máxima sensibilidade à insulina, embora o controle glicêmico não tenha sido atingido adequadamente quando comparado as dosagens de 6UI/dia e 9UI/dia (Okamoto et al., 2011). Também foi observado que no tecido adiposo, a expressão da translocação de GLUT-4 no músculo esquelético de ratos diabéticos foi mais efetiva com a dose intermediária de insulina intermédia de 3UI/dia, do que com doses mais elevadas, de 6UI/dia e 9UI/dia, que produziam uma diminuição da translocação do transportador GLUT-4 (Okamoto et al., 2011).

Outros estudos verificaram a expressão e translocação de transportadores de glicose no encéfalo na presença do diabetes mellitus, demonstrando que o GLUT-4 está

localizado em pequenas quantidades em áreas específicas do encéfalo, como bulbo olfatório, giro dentado do hipocampo e hipotálamo, o GLUT-1 é abundante em distintas regiões do encéfalo, o GLUT-2 é encontrado em neurônios específicos no hipotálamo, como; o núcleo paraventricular, núcleo arqueado e região lateral, enquanto o GLUT-3 é o principal transportador de glicose nos neurônios do cerebelo, estriado, córtex e hipocampo (Kang, Routh, Kuzhikandathil, Gaspers, & Levin, 2004; Levin, Routh, Kang, Sanders, & Dunn-Meynell, 2004; Simpson et al., 1999).

1.1.1 Modelo Experimental de Diabetes Mellitus por estreptozotocina

Os modelos experimentais para indução de diabetes dependerão principalmente do objetivo do estudo. No caso da DMT1, a deficiência na produção de insulina é alcançada por uma variedade de mecanismos diferentes, desde a ablação química das células beta até a criação de roedores que espontaneamente desenvolvem diabetes auto-imune. Dentre os modelos o quimicamente induzido utiliza a estreptozotocina (STZ) ou aloxano para indução da DMT1 (Bansal, Ahmad, & Kidwai, 2017; Dufrane et al., 2006). Em relação aos modelos auto-imunes espontâneos para esta patologia são encontrados o camundongo diabético não obeso (NOD) e o rato Biobreeding (BB). O primeiro apresentando insulite por volta de 3 a 4 semanas de idade e o último respectivamente que desenvolve diabetes entre 8 e 16 semanas de idade, com fenótipo diabético bastante grave e necessitando de insulinoterapia para sobreviver. O grau de translação entre o modelo experimental de diabetes para os pacientes está relacionado com a similaridade da progressão da diabetes em humanos, como visto na indução química em primatas que contribuem para o entendimento dos mecanismos envolvidos nesta patologia (Dufrane et al., 2006).

O antibiótico STZ, derivado da bactéria gram positiva *Streptomycetes achromogene* é uma toxina paras as células β pancreáticas utilizada como indutor de diabetes mellitus em roedores, podendo ser administrada por via intravenosa, intraperitoneal ou lingual (Ates et al., 2007; Pamela Brambilla Bagatini et al., 2014; Damasceno et al., 2014; De Senna et al., 2011; Do Nascimento et al., 2011; Haider, Ahmed, Tabassum, et al., 2013; Radenković, Stojanović, & Prostran, 2016; Venturini et al., 2010a). A indução da DM por meio de STZ não ocasiona a perda total de produção de insulina, que tornaria o animal dependente de terapia insulínica (Geert Jan Biessels, 2013). A ação tóxica de STZ requer a sua absorção celular, apresentando atividade alquilante, com a transferência de grupos metil para as moléculas de DNA, resultando em quebras nas cadeias de DNA. Além disso, há um aumento na geração de espécies reativas de oxigênio (ERO) e liberação de óxido nítrico (NO) após a metabolização da STZ, contribuindo para a morte das células beta-pancreáticas. A STZ é seletivamente acumulada em células beta do pâncreas devido a baixa afinidade do transportador de glicose tipo 2 (GLUT2) na membrana plasmática. Assim, as células produtoras de insulina que não expressam este transportador de glicose são resistentes à (Elsner, Guldbakke, Tiedge, Munday, & Lenzen, 2000; Ledoux & Wilson, 1984; Schnedl, Ferber, Johnson, & Newgard, 1994). A importância do GLUT2 no presente processo é também demonstrado pela observação de que STZ afeta outros órgãos que expressam este transportador, particularmente nos rins e fígado. Como a barreira hematoencefálica não expressa GLUT2, não ocorre efeito direto da STZ no SNC após a administração sistêmica (Kumagai, 1999).

Os animais geralmente apresentam um pico hiperglicêmico 72 h após a indução do DM com STZ. Após esse período, eles permanecem hiperglicêmicos, com níveis de glicose sanguínea entre 350 a 450 mg/dL (o nível considerado normal é de

aproximadamente 90- 200mg/dL). Assim como os pacientes diabéticos, os animais desenvolvem danos micro e macrovasculares, havendo comprometimento de funções visuais, renais, cardíacas, do sistema nervoso periférico e também do sistema nervoso central (SNC) (Geert Jan Biessels, van der Heide, Kamal, Bleys, & Gispen, 2002; Gispen & Biessels, 2000).

1.1.2 Alterações neurológicas envolvidas com a DM

São observadas alterações neurológicas em modelos animais e em pacientes que apresentam a DM. No início da década de 20, os pesquisadores Miles e Root observaram que os pacientes diabéticos apresentavam um desempenho insatisfatório em tarefas de atenção, aprendizado e memória quando comparados a indivíduos saudáveis (MILES e ROOT, 1992). Com base nisso, a avaliação dos mecanismos que desencadeiam o dano neurológico progressivo na diabetes mellitus e nas terapias de reversão têm sido o foco de muitos estudos (Bagatini et al., 2017, 2014; De Senna et al., 2015; Do Nascimento et al., 2011; Tesfaye et al., 2010).

As evidências clínicas em humanos apontam que quanto mais precoce é o aparecimento dos sintomas e sinais da diabetes mellitus maior e a gravidade da patologia, sendo que se a doença for desenvolvida durante a infância haverá um maior comprometimento dos processos neurológicos. Tanto a DMT1 quanto a DMT2 têm efeitos adversos sobre as funções do SNC, incluindo a cognição (Sima, 2010). Observa-se que os pacientes infantis com DMT1 possuem níveis menores de desempenhos escolares, sendo que as alterações neurocomportamentais nas crianças com DMT1 incluem déficit de atenção, da inteligência, da memória e velocidade de processamento (Northam et al., 2009; Northman E., Anderson P, Rani J, Hughes M, 2001; Schoenle, Schoenle, Molinari, & Largo, 2002).

Por outro lado, pacientes com DMT2 exibem prejuízos cognitivos independentemente da idade e possuem maior chance de desenvolvimento de demência, uma condição fortemente associada ao envelhecimento (G. Biessels, Strachan, Visseren, Kappelle, & Whitmer, 2014; G J Biessels, 2013; Koekkoek, Kappelle, Berg, Rutten, & Biessels, 2015). A presença de comorbidades relacionadas ao DMT2, como dislipidemia, hipertensão e doença cerebrovascular exerce uma importante influência para o desenvolvimento de deficiências na memória desses pacientes (McCrimmon, Ryan, & Frier, 2012; Moheet, Mangia, & Seaquist, 2015).

Crianças que apresentam DMT1 antes dos seis anos de idade possuem uma maior incidência de esclerose mesial temporal, não associado a episódios anteriores de hipoglicemia. O início precoce de hipoglicemia grave nestes pacientes causa uma redução no volume de massa cinzenta, que pode ser observada por ressonância magnética (Ho et al., 2008). O comprometimento encefálico é evidenciado também na progressão da doença. Na avaliação cognitiva e de neuroimagem com ressonância magnética, os pacientes com DMT1 há 12 anos demonstram reduções dos volumes de massa branca nos lobos para-hipocampal, temporal e frontal, bem como diminuição dos volumes de massa cinzenta do tálamo, hipocampo e córtex insular (Northam et al., 2009).

Os pacientes que apresentaram DMT1 com um período de duração de 15-25 anos possuem diminuição da densidade da massa cinzenta nas regiões do tálamo, giro temporais superior e médio e giro frontal (Musen et al., 2006). Déficits cognitivos são mais prevalentes em pacientes com aparecimento de diabetes em uma idade jovem. Corroborando tal ideia, pacientes diabéticos jovens desde quatro anos de idade e que sucumbiram à cetoacidose mostraram perda neuronal acentuada no hipocampo e no córtex frontal e atrofia de substância branca de regiões frontal e temporal. Pacientes

diabéticos também apresentam diminuição dos receptores de insulina e fator de crescimento semelhante à insulina tipo 1 (IGF-I) (Van Duinkerken et al., 2009).

A funcionalidade da barreira hemato-encefálica, sua permeabilidade e a relação desta com doenças neurodegenerativas e diabetes mellitus são temas de diferentes estudos (Hawkins, Lundeen, Norwood, Brooks, & Egleton, 2007; Huber, VanGilder, & Houser, 2006). A presença de hiperglicemia ocasiona o aumento da permeabilidade da barreira hemato-encefálica em roedores e humanos e redução nas expressões de proteínas como a claudina-5 e a aquaporina, sendo o exercício físico capaz de reverter parcialmente a queda dos níveis de claudina 5 mas não de aquaporina (De Senna et al., 2015). O diabetes mellitus também é capaz de alterar o funcionamento de vias extrapiramidais, com a redução de tirosina hidroxilase, uma enzima limitante da síntese de catecolaminas, na região da substância nigra pars compacta (SNc) de ratos diabéticos (Bagatini et al., 2014).

A hiperglicemia se destaca como uma das principais e mais conhecidas causas de complicações no DM. Embora o encéfalo utilize a glicose como sua principal fonte energética, em situações de hiperglicemia prolongada o transporte de glicose pode ser desequilibrado, fazendo com que a glicose seja desviada para vias metabólicas que podem culminar em neurotoxicidade (Mergenthaler, Lindauer, Dienel, & Meisel, 2013; Tomlinson & Gardiner, 2008). O aumento no fluxo da via dos polióis pode comprometer o ciclo da glutationa e a atividade da enzima glutationa peroxidase (GPx), levando a um aumento no conteúdo de EROs. Concomitantemente, ocorre um aumento na produção de superóxido devido à sobrecarga na cadeia transportadora de elétrons mitocondrial, e todos esses eventos levam ao estresse oxidativo, um desequilíbrio entre a produção de agentes oxidantes e as defesas antioxidantes, capaz de causar danos em macromoléculas, como lipídeos, proteínas e ácidos nucléicos. Por sua vez, o aumento no fluxo da via da hexosamina gera uridina difosfato N-acetilglicosamina, que pode se combinar com

resíduos de serina e treonina de proteínas intracelulares e, consequentemente, comprometer suas funções biológicas. Por fim, o aumento no fluxo da via dos produtos finais de glicação avançada (AGEs) pode promover alterações bioquímicas em qualquer macromolécula. Além disso, a ligação de AGEs aos seus receptores (RAGEs) promove respostas inflamatórias via *up regulation* de RAGEs e via ativação do fator nuclear kappa B (NF-κB); a ativação deste fator de transcrição leva à ativação de cascatas de sinalização pró-inflamatórias e pró-apoptóticas, aumentando a produção de ERO e contribuindo para o dano de células endoteliais vasculares, neurônios e células gliais (Callaghan, Cheng, Stables, Smith, & Feldman, 2012; Tomlinson & Gardiner, 2008).

Além da hiperglicemia, existem outros fatores relacionados às disfunções neurológicas no DM, incluindo diminuição da secreção e/ou ação da insulina, alteração na atividade do eixo hipotálamo-pituitária-adrenal (HPA), obesidade e inflamação (Gaspar, Baptista, Macedo, & Ambrósio, 2016). A insulina tem um papel importante para a homeostase do SNC, pois se liga a receptores de insulina e também a receptores do fator de crescimento semelhante à insulina tipo 1 (IGF-1) - ambos distribuídos em diferentes regiões encefálicas - promovendo a ativação de vias de sinalização relacionadas à expressão de genes anti-apoptóticos, à síntese de proteínas ligadas à plasticidade sináptica
- envolvidas no estabelecimento da potenciação de longa duração (LTP) e da depressão de longa duração (LTD) - e estabilização dos microtúbulos. No DM, a diminuição da secreção e/ou ação da insulina pode inibir as referidas vias de sinalização, prejudicando a plasticidade neural e aumentando a vulnerabilidade neuronal (Banks, Owen, & Erickson, 2012; Blázquez, Velázquez, & Hurtado-carneiro, 2014; Gaspar et al., 2016).

Em relação às alterações na função e atividade do eixo HPA, o consequente aumento dos níveis basais de glicocorticoides é capaz de promover prejuízos sobretudo na plasticidade hipocampal, incluindo inibição da arborização dendrítica, da plasticidade

sináptica e da neurogênese (Reagan, Grillo, & Piroli, 2008; Wrighten, Piroli, Grillo, & Reagan, 2009b). Ademais, a obesidade se destaca como fator contribuinte para o desenvolvimento de demência e outros prejuízos cognitivos, pois está relacionada a um aumento na incidência de patologias vasculares no SNC e atrofia cortical. Nesse sentido, o aumento dos níveis de hormônios produzidos no tecido adiposo, como a leptina, também pode contribuir para o déficit cognitivo no DM, impedindo vias de sinalização e prejudicando a plasticidade sináptica no hipocampo (Bruehl, Sweat, Tirsi, Shah, & Convit, 2011; Mueller et al., 2012). Por fim, a presença de microglia com fenótipo reativo e o aumento na expressão de citocinas pró-inflamatórias no encéfalo diabético podem promover um *up regulation* de RAGEs e ativação do NF-κB, capazes de gerar inflamação e estresse oxidativo, culminando em eventos neurodegenerativos e disfunções cognitivas (Gaspar et al., 2016; Pugazhenthi, Qin, & Reddy, 2016).

1.2 Regiões encefálicas envolvidas com a diabetes mellitus

O DM experimental induzido por STZ está associado ao desenvolvimento de alterações neuroestruturais em roedores, demonstradas em estudos que relatam morte neuronal no córtex frontal (Jakobsen, Sidenius, Gundersen, & Østerby, 1987) e no hipocampo (Jing, Chen, Kuo, Pao, & Chen, 2013). Além disso, é observado também que animais apresentam desmielinização e degeneração axonal no estriado e no córtex (Huang, Gao, Yang, Lin, & Lei, 2012). A presença de alterações estruturais dos animais diabéticos na barreira hematoencefálica (Mooradian, 1997), diminuição no tamanho das células e nos níveis de proteínas, ácidos graxos e colesterol no córtex de ratos jovens (4 a 8 semanas de vida), indicam um menor conteúdo cortical de mielina (Malone, Hanna, & Saporta, 2006).

Em humanos, o estado do diabetes está associado a uma redução leve, de todo o volume do córtex, principalmente no lobo frontal, com efeitos estruturais mais proeminentes em pacientes com consciência prejudicada ocasionada por hipoglicemia, devido à exposição a longo prazo a episódios recorrentes de hipoglicemia (Bednarik et al., 2017). O encéfalo é sensível em relação a expressão de receptor de insulina e quanto à distribuição seletiva em regiões do bulbo olfatório, hipotálamo, hipocampo, amígdala, cerebral córtex e cerebelo. Assim, episódios de hipoglicemia e hiperglicemia afetam o sistema nervoso central e periférico e levam a disfunções graves (Özdemir, Akbaş, Kotil, & Yilmaz, 2016).

1.2.1 Amigdala e DM

A amigdala é considerada o centro emocional do encéfalo e possui um papel importante na consolidação da memória em contextos emocionais, também chamados de memórias emocionais (Janak & Tye, 2015; Roozendaal, McEwen, & Chattarji, 2009). A conectividade da amígdala se expande para várias regiões do encéfalo, como o córtex frontal e o hipocampo que regulam os efeitos da excitação emocional e da exposição ao estresse nessas áreas do encéfalo (Roozendaal, McEwen, & Chattarji, 2009).

Sabe-se que o estresse prejudica a recuperação da memória e a memória de trabalho, principalmente via modulação da amígdala da exposição ao estresse hipocampal a hormônios do estresse como noradrenalina e glicocorticóides (Roozendaal, Griffith, Buranday, De Quervain, & McGaugh, 2003; Roozendaal, McReynolds, & McGaugh, 2004). Um estudo demonstrou que os animais diabéticos tipo 2 apresentam déficit de memória e reconhecimento social com alteração da neurotransmissão dopaminérgica na região da amigdala (Parashar, Mehta, & Malairaman, 2018).

1.2.2 Córtex Frontal e DM

Em humanos, o córtex frontal, juntamente com as outras áreas corticais associativas, executa funções relacionadas à cognição como aprendizagem e memória, atenção, motivação e na memória de trabalho. Essa região tem um papel fundamental no planejamento de respostas comportamentais apropriadas a estímulos externos e internos, e funciona integrando outras áreas para realizar tarefas mentais individuais (Petty & Mitchell, 2004).

Em roedores, os sinais relacionados à decisão são predominantes no córtex frontal. Especificamente, no córtex frontal medial (incluindo o córtex agranular medial em ratos e o córtex motor secundário em camundongos), muitos neurônios mostram atividade de disparo diferencial para uma escolha em relação a outra (Atilgan & Kwan, 2018; Siniscalchi, Phoumthipphavong, Ali, Lozano, & Kwan, 2016; Sul, Jo, Lee, & Jung, 2011).

É no córtex frontal, uma das regiões mais afetadas pelo tratamento com STZ que após 8 semanas de hiperglicemia, as células piramidais no córtex parietal, córtex pré-frontal medial e hipocampo de ratos diabéticos induzidos por STZ apresentam atrofia dendrítica e densidade espinhal reduzida (Malone et al., 2006; Martínez-Tellez, Gómez-Villalobos, & Flores, 2005).

1.2.3 Córtex Motor e DM

A principal função do córtex motor é de sinalização para orientação do movimento do corpo. Esta região do encéfalo está localizada na parte do lobo frontal e é anterior ao sulco central. É constituído por estruturas denominadas de córtex motor primário, córtex pré-motor e área motora suplementar. O córtex motor primário atua como mensageiro

enviando a maioria dos impulsos elétricos que saem do córtex motor. O córtex pré-motor está localizado anteriormente ao córtex motor primário na área 6. de Brodmann. Sua função é preparar o movimento, principalmente a musculatura proximal. A área motora suplementar está na superfície medial da fissura longitudinal, na porção anterior à representação da perna no córtex motor primário. Embora não totalmente compreendidas, as funções propostas incluem estabilização e coordenação postural do corpo (Teka et al., 2017).

A relação entre o córtex motor e a DM é devido a indução de reduções no tamanho da área motora cortical, lesões axonais nos neurônios corticoespinais que projetam-se na coluna lombar, além desses disturbios corticoespinal contribuírem para distúrbios motores em pacientes diabéticos (Muramatsu, Ikutomo, Tamaki, Shimo, & Niwa, 2018). Segundo Emerick et al., (2005) foi demonstrado que animais diabéticos em estágio inicial possuem reduções de tamanho na área cortical motora do membro anterior. Segundo Huang et al., (2012) avaliando histologicamente as regiões do córtex motor e o estriado, os animais diabéticos induzidos por STZ apresentam desmielinização e degradação axonal está associado a lesões estriatais/ corticais observáveis na ressonância magnética.

1.2.4 Cerebelo e DM

O cerebelo é uma região subcortical que se conecta a outras regiões do encéfalo, como o córtex motor, envolvido na regulação da coordenação motora, cognição e atenção, recebendo informações de várias regiões do córtex cerebral (Allali et al., 2018; Koziol et al., 2014). Estudos com seres humanos mostram que o hipermetabolismo cerebelar usando o marcador ^{18} F-fluorodesoxyglucose (^{18}F-FDG) PET está associado a uma atividade compensatória para manter funções cognitivas em doenças neurodegenerativas, como doença de Parkinson, doença de Alzheimer, demência com corpos de Lewy entre outras (Blum et al., 2018). Alterações cerebrais estruturais e ultraestruturais foram

demonstradas em animais diabéticos e a depressão foi relacionada com as regiões do córtex cerebral, cerebelo e hipotálamo. Nessas regiões as alterações incluiam aumento de mitocôndrias inchadas e vacuolizadas presentes na diabetes (Hernández-Fonseca et al., 2009).

1.2.5 Hipocampo e DM

O hipocampo está associado, entre outras funções, à cognição, principalmente à memória, e essa área pode ser segmentada em porções anterior e posterior (dorso-ventral em roedores) que são estruturalmente e funcionalmente distintas (Collin, Milivojevic, & Doeller, 2015; Dalton, Zeidman, Barry, Williams, & Maguire, 2017; Fanselow & Dong, 2010). O hipocampo dorsal tem um papel preferencial em certas formas de aprendizado e memória, principalmente no aprendizado espacial, mas o hipocampo ventral pode ter um papel preferencial nos processos cerebrais associados a comportamentos relacionados à ansiedade. O papel deste último no processamento emocional também é distinto da amígdala, que está associada especificamente ao medo (Dalton et al., 2017).

De acordo com alguns estudos, a diabetes induzida por STZ apresenta diminuição de neurogênese no hipocampo e alterações nas tarefas comportamentais que incluem o aprendizado e a memória espacial em testes no labirinto de água de Morris e reconhecimento de novos objetos (Piazza et al., 2011; Revsin et al., 2009). A diminuição da proliferação celular do hipocampo em camundongos diabéticos resulta em déficits cognitivos leves na tarefa de reconhecimento de posicionamento de objetos (Revsin et al., 2009). Visto que os animais diabéticos submetidos ao teste de labirinto de água de Morris só apresentam algum déficit quando há uma disfunção hipocampal mais grave.

1.2.6 Micro Tomografia por emissão de pósitrons (microPET-CT)

A micro tomografia computadorizada por emissão de pósitrons em (microPET-CT) é uma ferramenta importante para o estudo de doenças cerebrais, devido à sua técnica ser considerada não-invasiva. Usando este método é possível medir os níveis de consumo de glicose e quantificá-los em momentos diferentes com o animal *in vivo* (Kopschina et al., 2019).

O marcador do metabolismo encefálico da glicose mais utilizado é o ^{18}F-FDG que demonstra maior eficiência e precisão para o mapeamento cerebral em tempo real.
De acordo com os estudos, o FDG é uma molécula análoga de glicose que é utilizada como marcador metabólico e permite identificar a distribuição da sua captação em diferentes áreas do encéfalo. Normalmente múltiplas vias *metabólicas* de glicose no encéfalo podem ser observadas por exames de imagem (Rooijackers, Wiegers, Tack, Van Der Graaf, & De Galan, 2016).

Nesse sentido, tanto em modelos experimentais quanto em humanos após aaplicação intravenosa, o FDG é transportado para as células como um análogo da glicose (figura 1). O primeiro passo após a entrada da glicose ou FDG na célula é a fosforilação em glicose-6-fosfato (Glc-6-P) pela enzima hexoquinase. A Glc-6-P pode entrar em várias vias metabólicas no encéfalo. Esse monossacarídeo pode ser metabolizado para produzir energia via glicólise ou a Ciclo de Krebs. Os intermediários dos ciclos glicolítico e Krebs também são usados para a síntese de aminoácidos e neurotransmissores. Além disso, Glc-6-P é um precursor do glicogênio. Por fim, o metabolismo do Glc-6-P através do via da pentose fosfato (PPP) fornece pentose para nucleotídeo síntese e NADPH, necessários para reações redutivas, como lipídios síntese e proteção contra o estresse oxidativo.

O exame de microPET quando utiliza o marcador ^{18}F-FDG que fica preso no início do metabolismo (por exemplo fluorodeoxiglucose-6-fosfato / FDG-6-P), para estimar as taxas captação do metabolismo de glicose. Com isso quando o FDG entra pela via glicolítica, será feita a fosforilação da molécula realizada pela enzima hexoquinase, que limita a taxa, o FDG-6-fosfato não é mais metabolizado. Devido à sua carga negativa, esse marcador metabólico permanece preso nas células e seu acúmulo é proporcional à taxa glicolítica celular. Deste modo, quando ocorre o acúmulo de FDG no tecido consequentemente isto reflete o transporte celular de glicose e a atividade da hexoquinase. Normalmente, a captação fisiologicamente aumentada de FDG é observada no trato urinário, no trato intestinal, nos músculos, no encéfalo e no coração. Especialmente, a atividade intestinal pode mostrar padrões imprevisíveis, o que pode ser confuso (Rooijackers, Wiegers, Tack, Van Der Graaf, & De Galan, 2016).

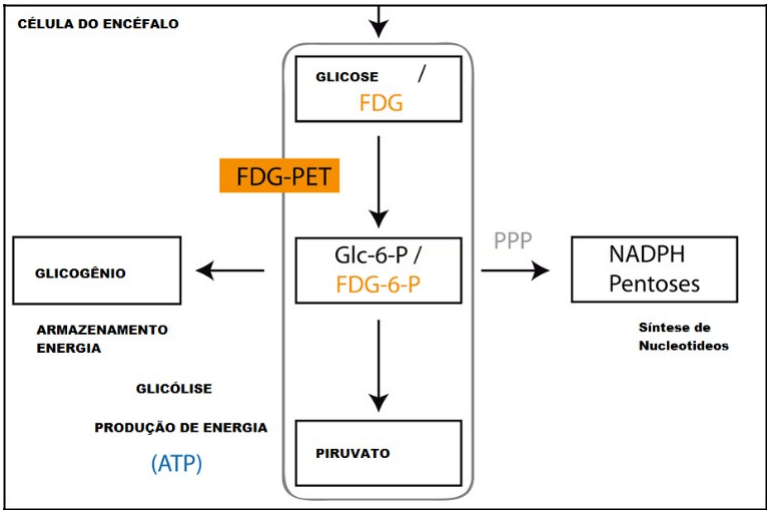


Figura 1. Desenho esquemático de uma célula do encéfalo humano mostrando a entrada do FDG ou glicose pela via glicolítica (Modificado de Rooijackers et al., 2016 para língua portuguesa, sob licença CC BY, disponível em http://creativecommons.org/licenses/by/4.0/)

2. JUSTIFICATIVA

As perspectivas das pesquisas científicas no que tangem a patogênese, diagnóstico e o tratamento da diabetes mellitus tipo I auxiliam os pacientes no desafio de manejar a doença, seja por meio de bombas de insulina, monitoramento da glicose ou a administração da insulina. Contudo, apesar do investimento maciço da comunidade científica ainda não existem meios de retardar a evolução das disfunções cognitivas, motoras e neurometabólicas em pacientes diabetes mellitus tipo I que seguem o tratamento de forma efetiva.

Dados da OMS apontam que o número de pacientes com diabetes tem aumentado significativamente juntamente com as complicações secundárias relacionadas principalmente aos órgãos alvos que captam a glicose. Compreender e avaliar os mecanismos envolvidos no metabolismo encefálico e quando as alterações iniciam são passos cruciais para o mapeamento e entendimento da patogênese das complicações neurodegenerativas induzidas pelo diabetes mellitus.

Acreditamos que as análises de alterações comportamentais e neurometabólicas induzidas pelo diabetes mellitus são partes importante para o conhecimento e também para entendermos como esta doença comporta-se em seus estágios precoces. Assim, é válido ressaltar que poderemos criar estratégias para conter o avanço das disfunções cognitivas e motoras nesta patologia.

3. OBJETIVO

3. 1 Objetivos gerais

a) Avaliar os aspectos comportamentais cognitivos e locomotores; o metabolismo encefálico em ratos Wistar machos adultos, submetidos indução de diabetes por STZ.

b) Avaliar o ^{18}F-FDG como marcador de lateralidade em ratos Wistar machos adultos, submetidos indução de diabetes por STZ.

3.2 Objetivos específicos

a) Avaliação das alterações comportamentais/locomotoras com o uso do teste do campo aberto e o teste de reconhecimento do novo objeto.

b) Avaliação do metabolismo encefálico com uso da técnica de microPET-CT, com ^{18}F-FDG como marcador metabólico nas regiões do hipocampo, amígdala, córtex frontal, córtex motor e cerebelo.

c) Avaliação da lateralidade do metabolismo encefálico com uso da técnica de microPET-CT, com ^{18}F-FDG como marcador metabólico em ratos wistar machos.

d) Verificar as alterações metabólicas de glicose utilizando ^{18}F-FDG através da técnica de microPET nas regiões do córtex auditivo, córtex orbitofrontal e núcleo accumbens core e hipocampo posterior entre ratos diabéticos e controles.

Capítulo II

"Early diabetes melittus effects on cognition, behavior and glucose brain metabolism in wistar rats" Artigo a ser submetido ao periódico Psychoneuroendocrinology

EARLY DIABETES MELITTUS EFFECTS ON COGNITION, BEHAVIOR AND GLUCOSE BRAIN METABOLISM IN WISTAR RATS

Stéfanie Ingrid dos Reis Schneider[a], Bruna Bueno Milanesi[a], Paula Fernanda Ribas Neves[a], Lisie Valeria Paz[a], Gabriele Zenatto Lazzari[a], Laura Tartari Neves[a], Samuel Greggio[b], Gianina Teribele Venturin[b], Paula Kopschina Feltes[b], Cassio Morais Loss [c], Pamela Brambilla Bagatini[a], Jaderson Costa Da Costa[b], Matilde Achaval Elena[d], Andréa Wieck[a*]; Léder Leal Xavier[a].

aLaboratório de Biologia Celular e Tecidual, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brazil.

bInstituto do Cérebro do Rio Grande do Sul – INSCER-PUCRS, Porto Alegre - RS, Brazil.

c Departamento de Bioquímica, Instituto de Biociências, Universidade Federal do Rio Grande Rio Grande do Sul, Porto Alegre - RS, Brazil.

d Laboratório de Histofisiologia Comparada, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS , Brazil.

*CORRESPONDING AUTHOR

Andrea Wieck, PhD.
Laboratório de Biologia Celular e Tecidual
Escola de Ciências da Saúde e da Vida
PUCRS
Avenida Ipiranga, 6681
Prédio 12, Sala 104
Porto Alegre, RS, Brasil
CEP:90619-900
E-mail: andrea.wieck@edu.pucrs.br
Telephone: +55 (51) 3320-3545

ABSTRACT

Advanced stages of Diabetes Mellitus are associated to locomotor and cognitive dysfunction. Motor deficits are related to diabetic neuropathy involving peripheral nerves, which increases morbidity risk in diabetic patients. In addition, central nervous system (CNS) complications during diabetes progression are relatively more subtle compared to peripheral changes. To the present moment, few studies investigated the neurological effects during the early stages of diabetes onset, mainly in experimental models. Considering the above, the main objective of the present study was to evaluate the locomotor activity, short-term and spatial memory and brain glucose metabolism before and dafter DM induction in adult Wistar rats. The DM was induced by intraperitoneal injection of 60mg / kg of streptozotocin, which leads to pancreatic beta cells disruption and consequent induction of experimental DM 48 hours after injection. Locomotor activity was evaluated through the open field (OF) test; memory by the novel object recognition (NOR) test and brain glucose metabolism by [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography (PET). Body weight, food and water intake, urine and fecal output as well as blood glucose levels were also evaluated throughout the experiment. Behavioral analysis demonstrated in DM group compared to control animals, suggesting alterations in both working and short term memory. Regarding locomotor activity, no significant alterations were observed between groups. Global analysis of brain glucose metabolism measured by [^{18}F]FDG uptake indicated slight alterations in important areas for short-term and working memory, emotional control and locomotion. DM animals had increased [^{18}F]FDG uptake in the cerebellum, whereas a reduction was observed in the amygdala when compared to the control groups. Hippocampus uptake was not significantly altered when analyzed as whole. However, when segmenting it into dorsal and ventral hippocampus, it was possible to observe reduced tracer uptake in the dorsal hippocampus. These results suggest possible alterations in early stages of DM regarding cognition and brain glucose metabolism induced. Further studies are necessary to evaluate possible long-term alterations after DM induction in this animal model.

Keywords: small animal PET; ^{18}F-fluorodeoxyglucose; Wistar rats; glucose brain metabolism; diabetic rats; Diabete mellitus

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent diseases characterized by chronic complications related to hyperglycemia, which progressive damage to the peripheral nerves. Additionally, DM related deleterious effects on central nervous system (CNS) may impair brain functions, such as memory and cognition (Ahtiluoto et al., 2010; Sims-Robinson, Kim, Rosko, & Feldman, 2010). In the last decade, studies with both experimental and human models have focused on brain alterations related to diabetes mellitus type 1, showing morphological, electrophysiological and behavioral changes promoted by disease (P. B Bagatini et al., 2017; Geert Jan Biessels, 2013; de Senna et al., 2017; Van Elderen et al., 2010). Nonetheless, few studies investigated the early manifestations of neurological deficits induced by DM, which raises the question of when the DM-related deleterious effects in the brain starts.

Several studies evaluated brain glucose metabolism and gross anatomical changes produced by DM through imaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging and magnetic resonance spectroscopy (Bednarik et al., 2017b; Perantie et al., 2011; Zhou et al., 2018). Results obtained by functional and metabolic neuroimaging during DM demonstrate a decrease in whole brain volume in experimental diabetic animals with or without cognitive impairment (Zhou et al., 2018). When investigating young children with early-onset type-1 DM , a high prevalence of structural abnormalities in the brain were observed. Alterations such as white matter volume reduction within the parietal cortex were associated with the presence of high hyperglycemia and high variability in glucose levels (Ho et al., 2008; Mauras et al., 2015; Perantie et al., 2011).

Although cognitive changes in the diabetic brain are subtle, diabetic rats with cognitive impairment showed alterations primarily in the caudate putamen and hippocampus. Hippocampal

impairment is related to potential learning and memory dysfunction observed in diabetic rats in hypoglycemic states (Sang et al., 2005; Yamada et al., 2004; Zhou et al., 2018). However, it is still unknown when these cognitive alterations start after DM onset. Therefore, the aim of this study was to evaluate behavioral and glucose brain metabolism alterations during in the early stages of a DM rat experimental model

2. MATERIALS AND METHODS

2.1 Animal procedures

Eighteen 12-weeks old male Wistar rats were obtained from the Centro de Modelos Biológicos Experimentais (CEMBE) of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). They were kept in standard laboratory conditions at the University facilities, with food and water ad libitum and a 12 h light/dark cycle. Twelve hours prior to the small animal PET procedure, the animals were maintained under food restriction to avoid glucose levels fluctuations. All experiments involving animals were approved by the University's ethical committee (CEUA 7660/PUCRS) and were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institute of Health (USA). All efforts were made to minimize animal suffering and the number of animals needed.

2.2 Experimental Design

The study design was divided into two parts: baseline and Diabetes period, which is depicted in Figure 1. After basal period evaluation, animals were assigned to DM group (n=10) or control group (n=8). All experimental procedures and analysis were performed on both periods, i.e. baseline and after induction.

Daily food and water intake was quantified during the whole experiment, as well as urinary output and feces values for each cage with until 3 animals. Also, body weight and glucose levels were monitored during the experiment.

2.3 Diabetes mellitus induction

Diabetes Mellitus (DM) was induced by streptozotocin injection(Akbarzadeh et al., 2007). Briefly, after an overnight fasting period, animals from DM group received a single intraperitoneal (i.p.) injection of streptozotocin (STZ - Sigma-Aldrich Brasil Ltda; 60 mg/kg of body weight) diluted in 0.1 M sodium-citrate buffer, pH 4.5. Control animals received an equivalent amount of sodium-citrate buffer i.p. Blood glucose levels were measured in blood collected from the tail vein 72h after i.p. injections using a portable glucometer (On Call Plus, ACON Laboratories, USA). A positive control was used to assess test validity. Only animals with blood glucose levels >300 mg/dL and symptoms of polyuria and polydipsia were considered diabetic and used in the present study (DM group, n=10). Blood glucose levels of all animals were assessed in different moments along the experiment.

2.4 Physiological Parameters

In order to confirm DM induction, physiological parameters as urinary and feces output and body weight were monitored along the experimental procedure. Daily food and water intake during whole experiment were also verified.

2.4.1 Body weight

Baseline body weight was measured before DM induction. Body weight gain was calculated 32 days after DM induction, subtracting final values from baseline body weight. For body weight control purposes, measures on days 16, 27 and 40 after DM induction were also performed.

2.4.2 Food Intake

Food intake was assessed through all experiment until euthanasia. Every cage until 3 animals received, daily, 500 g of food. Food intake was calculated with values obtained at each two days. Briefly, on the first day, 500 g of food were offered and two days later the remaining amount of food was measured. Food intake was obtained by subtracting the weight of remaining food from initial weight of food offered.

2.4.3 Water intake

Water intake assessment was similar to food intake. All cages received daily a total of 600 mL of water. On the first day, two bottles of 300 mL were offered to each cage, and the next day, the remaining amount of water was measured. Water intake was obtained by subtracting the final water volume from initial value of water offered (600 mL in total).

2.4.4 Urinary and fecal output

Urinary output as well as fecal output were measured after DM induction until the end of the experiment, with values obtained every two days. Daily values of mean bowel movements mixed to urine were calculated by subtracting the dry sawdust weight (150 grams) from wet sawdust weight (sawdust plus bowel movements mixed with urine).

2.5 Behavioral Analysis

2.5.1 Open field test

Open field (OF) test was carried out on experimental days 1 and 34. The test was performed as described in literature (Do Nascimento et al., 2011). Briefly, rats were placed in a round wooden box with linoleum floor, measuring 30 cm in radius and 40 cm in height. Rats were allowed to explore the box during 5 minutes while their behavior was recorded (Sony Handycam DCR PJ5). Locomotor parameters evaluated were: 1- Distance traveled (meters); 2- Speed (meters/min); 3-Total time immobile (seconds or minutes); 4-Number of immobility episodes. All parameters were evaluated using the ANY-MAZE software (Stoelting Co., Wood Dale, U.S.A.).

2.5.2 Novel object recognition test

In order to evaluate short-term memory and spatial memory as a proxy for cognition before and after DM induction, the novel object recognition test (NOR) was performed on experimental days 2 and 35. For this purpose, rats were placed inside the same apparatus used in OF. They were allowed to explore two equal (familiar) iron objects for 5 min (trial 1), then objects were removed. After 50 min, one familiar and one new object were presented to the animal for 5

min (trial 2), changing the location of one of the objects. The preference index (PI), which can be used as a measured of cognitive function (Antunes 2012) was calculated as the ratio between time spent on exploring the new object and total time spent on object exploration. The Discrimination Index (DI), which allows to evaluate the discrimination between novel and familiar objects, was also calculated as the ratio between the difference on exploration times (novel and familiar) and the total amount of exploration ($Di=(T_N-T_F)/(T_N+T_F)$) (Antunes & Biala, 2012; Silvers, Harrod, Mactutus, & Booze, 2007). Behavioral tests were recorded on video (Sony Handycam DCR PJ5) for further analysis using ANY-MAZE software (Stoelting Co., Wood Dale, U.S.A.)

2.6 PET imaging

2.6.1 ^{18}F-FDG production

The ^{18}F-fluoride ions were produced using an on-site cyclotron (PET Trace 16 MeV; GE Medical System) and transferred to an automated synthesis system (Fastlab GE). The ^{18}F-FDG synthesis was performed according to the Hamacher method, under sterile conditions, and following the guidelines on current Good Radiopharmacy Practice (cGRPP) in the preparation of radiopharmaceuticals. ^{18}F-FDG was tested for sterility, pyrogenicity, pH, radionuclidic identity and purity, radiochemical identity and purity, residual solvents and membrane filter integrity on each production run (Hamacher, Coenen, & Stocklin, 1986).

2.6.2 ^{18}F-FDG PET Scan

Animals were individually anesthetized using a mixture of isoflurane and medical oxygen (3-4%) and 1 mCi of ^{18}F-FDG was administered through the tail vein. Afterwards, animals were returned to their home cage for a 30-min period for conscious tracer uptake. This step was performed to ensure best ^{18}F-FDG uptake in freely moving rats and to avoid the problem of confounding anesthetic effects (Lancelot & Zimmer, 2010). After the uptake period, rats were anesthetized, placed in a headfirst prone position and scanned with the TriumphTM MicroPET (LabPET-4, TriFoil Imaging, Northridge, CA, USA). Animals were kept under anesthesia using isoflurane (2-3%). During the scan, animals were kept on a heating pads to avoid body temperature decrease. For imaging acquisition, a 60-minute listmode static scan was acquired

with the field of vision (FOV; 3.75 cm) centered on the rat's head (Baptista et al., 2015; Mirrione et al., 2014).

2.6.3 Image acquisition and quantification

All data were reconstructed using a 3D ordered subsets expectation-maximization (3D-OSEM) algorithm with 20 iterations and no attenuation correction. Each reconstructed PET image was spatially normalized to a ^{18}F-FDG PET template already in MNI space using the Fusion Toolbox of PMOD v3.5 (PMOD Technologies, Zurich, Switzerland). The standard uptake value (SUV) was calculated (Baptista et al., 2015; Mirrione et al., 2014). Changes in ^{18}F-FDG tracer uptake in predefined regions of interest (ROIs) were obtained using the whole brain normalized SUV. Each ROI SUV value was divided by whole brain SUV value, being expressed as SUV ratio (SUVr). The selected brain regions were analyzed bilaterally and were selected due to its relation to cognition, memory and locomotor function: hippocampus, frontal cortex, motor cortex, amygdala and cerebellum.

2.7 Statistical Analysis

All statistical analyses were performed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The normality of the variables was tested using the Kolmogorov-Smirnov test.

Comparisons were made between diabetic rats and normal rats regarding changes in body weight, food and water intake, urine output throughout the experiment using 2-way repeated measures ANOVA model. The difference in brain tracer uptake expressed as SUVr in the selected areas between groups was also calculated using the two-way repeated measures ANOVA model. The effects of STZ-induced type-1 diabetes laterality were analyzed using two-sample Student's t test and paired sample t-test. The effects of STZ-induced type-1 diabetes on ambulation (open field), memory performance (NOR) were analyzed using two-sample Student's t test and paired sample t-test. Continuous variables are expressed as the mean ± standard deviation or as the median (25th and 75th percentile) and a level of 5% was considered statistically significant.

3 RESULTS

3.1 Physiological Parameters

Diabetic induction was confirmed in the DM group by the presence of characteristic symptoms such as polydipsia, polyuria and polyphagia, observed 1 week after induction. A percentage of disease incidence in the experimental model of approximately 80%.

At baseline, blood glucose levels did not differ between experimental groups ($t_{(16)}$ =1.65, p=0.302 –Fig 2A). After DM induction, glucose levels were significantly increased only in DM group, indicating that induction was successful ($t_{(16)}$ = 14.30; p<0.000001 – Fig 2A).

Weight gain was also different between groups, as DM group lost weight while the opposite occurred for control animals ($t_{(16)}$ =12,59, p<0.001 –Fig 2B). Interestingly, while control animals constantly increased weight, DM group showed a more stable curve, losing weight in some points (Fig 2B, insert).

Water and food intake were significantly higher in DM group compared to Control group ($t_{(60)}$ =11.68; p<0.001 and $t_{(26)}$ =5.79; p<0.001, respectively – Fig 2C and 2D) as well as urinary and fecal output ($t_{(20)}$ =21.60; p<0.001 – Fig 2E). Regarding the relationship between intake and excretion, DM group presented both parameters higher than control group ($F_{(1,20)}$ =462.8; p<0.001 – Fig 2F). However, DM group presented increased excretion compared to intake, while control group presented the opposite ($F_{(1,20)}$ =139.5; p<0.001 – Fig 2F).

3.2 Behavior analysis

No significant differences were observed in the OF when analyzing the time versus group interactions. All parameters evaluated showed a statistically significant effect of “time “variable for both groups. Distance traveled was decreased for DM and control groups after induction ($F_{(1,16)}$ = 136.8; p<0.01 – Fig 3A). Time spent immobile, immobile episodes and rearing, also decreased after DM induction for both groups ($F_{(1,16)}$ = 166.1; p<0.01; $F_{(1,16)}$ = 240.7; p<0.01 and $F_{(1,16)}$ = 101.8; p<0.01– respectively; Fig 4B, 3C and 3D).

Regarding NOR, exploration time in trial 1 increased post-induction independent of experimental group ($F_{(1,97;31,47)}$ = 10,87; p=0.0003 – Fig 4A), while in trial 2 no significant

alterations were observed. Since no differences were observes for trial 2, no significant differences were observed for time exploring novel object (Fig 4B). The total number of investigations (for both objects) presented a time versus experimental group interaction only in trial 2 ($F_{(3,48)}$ =3.71; p=0.017 – Fig 4C). While control animals increased number of investigations after vehicle injections, DM animals presented a decrease. Percentage of novel objects investigation presented a significant increase in trial 2 in the post- vehicle injection for control animals ($F_{(1,16)}$ =7.75; p=0.015 – Fig4D). Discrimination index post DM induction was significant smaller for both experimental groups (DM and controls, $F_{(1,16)}$ =8.7; p=0.009 – Fig4E). The preference index reduced post DM induction in DM group ($F_{(1,16)}$ =8.5; p=0.001 – Fig4F), while no significant differences were observed in control groups.

3.3 Brain glucose metabolism

Statistical analysis from SUVr data shows no general alterations in regions as frontal and motor cortex as well as hippocampus (Fig 5A to C). Analyzing hippocampus into antero-dorsal hippocampus, a time effect was observed only in DM group in dorsal hippocampus region ($F_{(1,16)}$ =6.83; p<0.005; Fig 5D), while no significant alterations were observed in ventral hippocampus (Fig 5E). A time versus group interaction could be observed in cerebellum when analyzing as whole. While controls showed increase in SUVr, DM had no alterations ($F_{(1,16)}$ =14.26; p<0.01). Dividing cerebellum into grey and white areas, both areas showed a group versus time interaction, were increased SUVr after DM induction was observed only in control group ($F_{(1,16)}$ =9.14; p<0.05 and $F_{(1,16)}$ =9.96; p<0.05)

A time versus group interaction was also observed for SUVr in amygdala ($F_{(1,16)}$ =8.628; p<0.005). The DM group had increased SUVr after induction while no alteration was observed for control group ($F_{(1,16)}$ =30.87; p<0.01). Consequently, DM group had a significantly higher SUVr when compared to control after induction ($F_{(1,16)}$ =10.63; p<0.05). No significant differences were observed in SUVr for both frontal cortex and motor cortex.

Statistical analysis from SUVr data shows in regions as auditory cortex, orbitofrontal cortex nucleus accumbens core as well as hippocampus differences in laterality (p<0.05, table

1). Analyzing posterior hippocampus was observed in DM group significant alteration. This area showed increased SUVr after DM induction, while control group no alteration.

The DM group had decrease SUVr in auditory cortex in both sides after induction while no alteration was observed for control group. Consequently, control group had a significantly decrease SUVr in both sides in orbitofrontal cortex. No significant differences were observed in SUVr for both nucleus accumbes core in groups control and DM (table 2).

FIGURE LEGENDS

Figure 1.

Experimental Design. At the beginning, animals were submitted to the open field test (OF), novel object recognition (NOR) and MicroPET for basal period evaluation. Following, animals were randomly assigned to Diabetes Mellitus (DM) group (n=10) or control group (n=8). Animals assigned to DM group received an intra-peritoneal (i.p.) injection of streptozotocin (60 mg/kg) , while animals assigned to control group received vehicle i.p. injection. Twenty days after streptozotocin injection, behavior tests and microPET analysis were repeated.

Figure 2.

Physiological parameters. To confirm Diabetes Mellitus (DM) induction, Glucose Blood levels (A) in day 1 and day 12 experiment, weight gain (B), water and food intake (C and D, respectively) as well as excretion and intake/excretion ratio (E) were evaluated during all time experiment and result are depicted here.*$p<0.05$; **$p<0.01$; DM = Diabetes Mellitus. Variables are expressed as the mean ± standard deviation (A,C,D,E) and as the median (25th and 75th percentile) (B).

Figure 3.

Open Field Test (OF). Both group showed similar behavior in the Open Field Test. Reduced distance traveled (A); time spent immobile (B); immobile episodes (C) and rearing scores (D) were observed for both groups (DM and control) following injections with STZ and vehicle, respectively. No differences were observed in speed (E). **$p<0.01$; DM = Diabetes Mellitus; STZ = Streptozotocin. Variables are expressed as the mean ± standard deviation.

Figure 4.

Novel Object Recognition Test (NOR). Both experimental groups (DM and control) showed a similar increase in exploration time in seconds after induction, in trial 1 (A) while no differences were observed for trial 2 (A). Time exploring the novel object showed no differences between experimental groups and times assessed (B). Regarding total investigations in trial 2, an opposite effect could be seen, where control animals increased the investigation of both objects after DM induction (vehicle injection) while DM animals decreased (after STZ injection - C). Control animals demonstrated increased novel object investigation after vehicle injection, while DM animals showed no difference (D). Variations in discrimination index were similar between groups, both reduced after DM induction (E) and the preference index, indicating the preference for investigating the novel object, was reduced in DM group after induction, while no differences could be observed in control animals (F). *$p<0.05$; **$p<0.01$; DM=Diabetes

Mellitus; STZ=Streptozotocin; Basal= before induction; Pos= after induction STZ or vehicle; T1= trial with two equal familiar objects; T2= after 50min of the trial 1, repeated trial with two different objects (one familiar and one new). Variables are expressed as the mean ± standard deviation.

Figure 5.

^{18}F-FDG SUVr microPET. Graphs depicting ^{18}F-FDG SUVr absorption in different Regions of Interest (ROIs) analyzed. Increased ^{18}F-FDG SUVr absorption indicates reduced glucose metabolism, while reduced ^{18}F-FDG SUVr absorption indicates the opposite. Regions as Frontal and motor cortex (A and B respectively); hippocampus and ventral hippocampus (C and E respectively) demonstrated no differences in ^{18}F-FDG SUVr absorption, and, consequently, in glucose metabolism. Analysis of Dorsal hippocampus indicated increased ^{18}F-FDG SUVr absorption in DM group after induction, with no alterations in control group (D). Cerebellum analyzed as a whole and subdivided in grey and white areas, demonstrated increased ^{18}F-FDG SUVr absorption only in control group, after induction, with no alterations in DM group (F, G and H respectively). Amygdala presented increase ^{18}F-FDG SUVr absorption only in DM group, after induction (I). DM=Diabetes Mellitus; *$p<0.05$; **$p<0.01$. MicroPET imaging. J) Indication of Regions Of Interest (ROIs) used for ^{18}F-FDG SUVr absorption analysis in microPET images. K) representative image of DM and control animals brains in microPET analysis in 42 day experiment. DM=Diabetes Mellitus. All values are mean ± SD.

Figure 1.

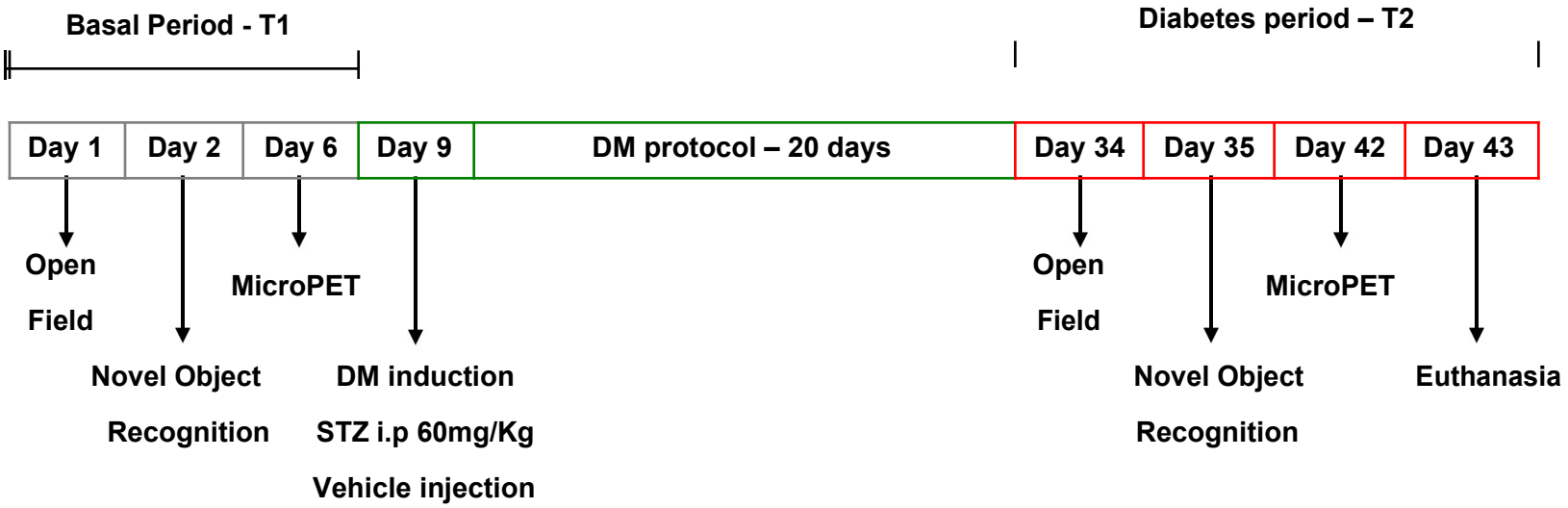
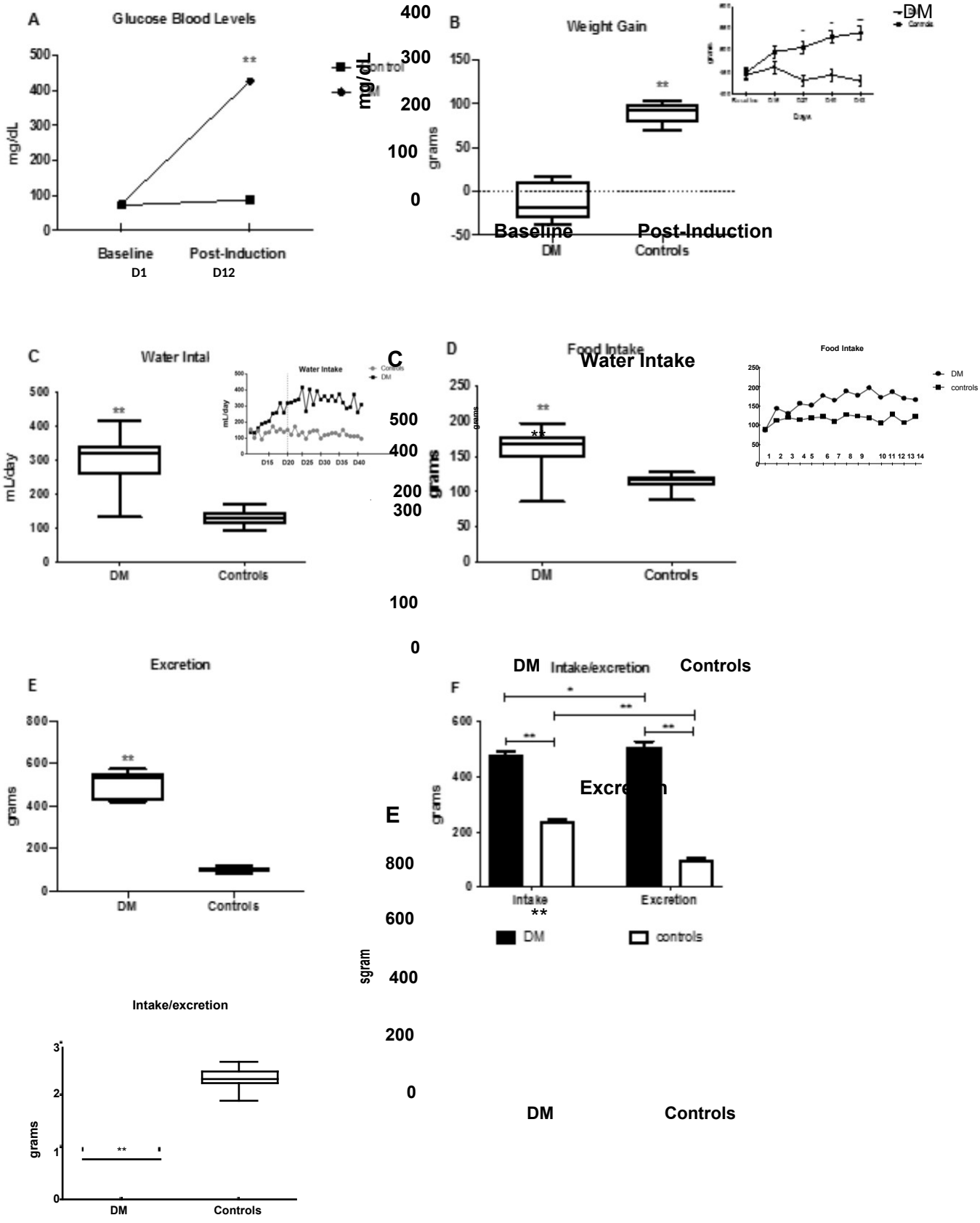

Basal Period - T1
Diabetes period – T2
Day 1
Day 2
Day 6
Day 9
DM protocol – 20 days
Day 34
Day 35
Day 42
Day 43
Open Field
Novel Object Recognition
MicroPET
DM induction STZ i.p 60mg/Kg Vehicle injection
Open Field
Novel Object Recognition
MicroPET
Euthanasia

Figure 2.



A
Glucose Blood Levels
B
Weight Gain
Baseline
Post-Induction
DM
Controls
C
Water Intake
D
Food Intake
E
Excretion
F
Intake/excretion
DM
Controls

Figure 3.

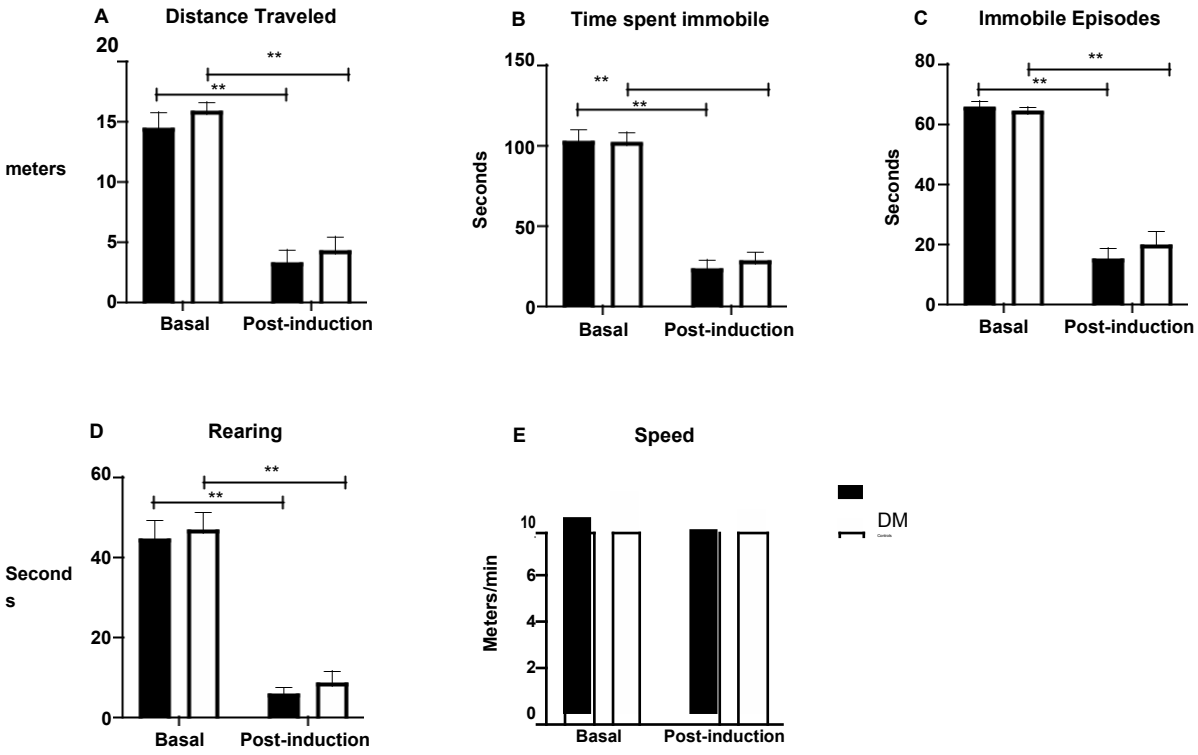

A
Distance Traveled
meters
20
15
10
5
0
**
**
Basal
Post-induction
B
Time spent immobile
Seconds
150
100
50
0
**
**
Basal
Post-induction
C
Immobile Episodes
Seconds
80
60
40
20
0
**
**
Basal
Post-induction
D
Rearing
Seconds
60
40
20
0
**
**
Basal
Post-induction
E
Speed
Meters/min
10
6
4
2
0
Basal
Post-induction
DM

Figure 4.

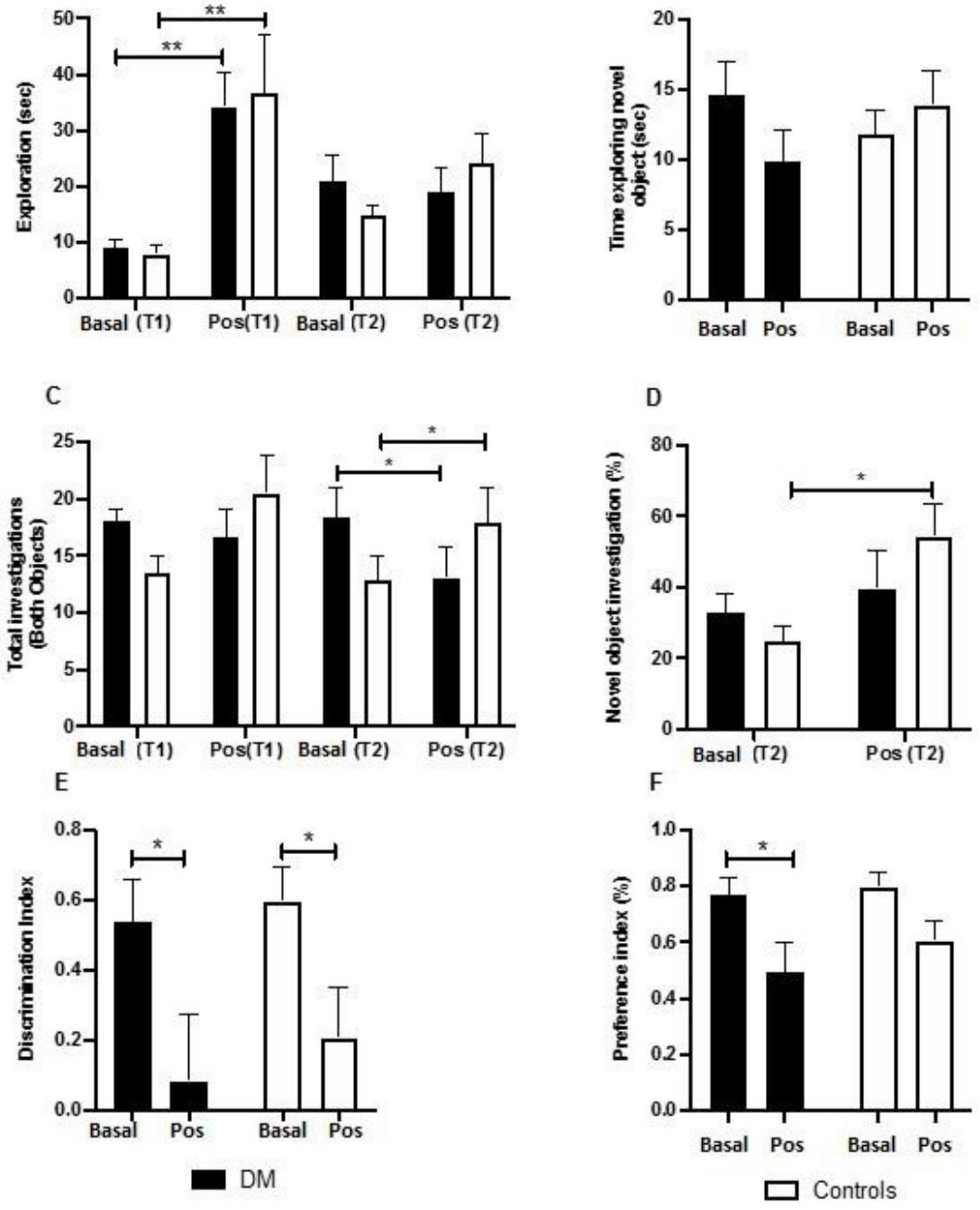
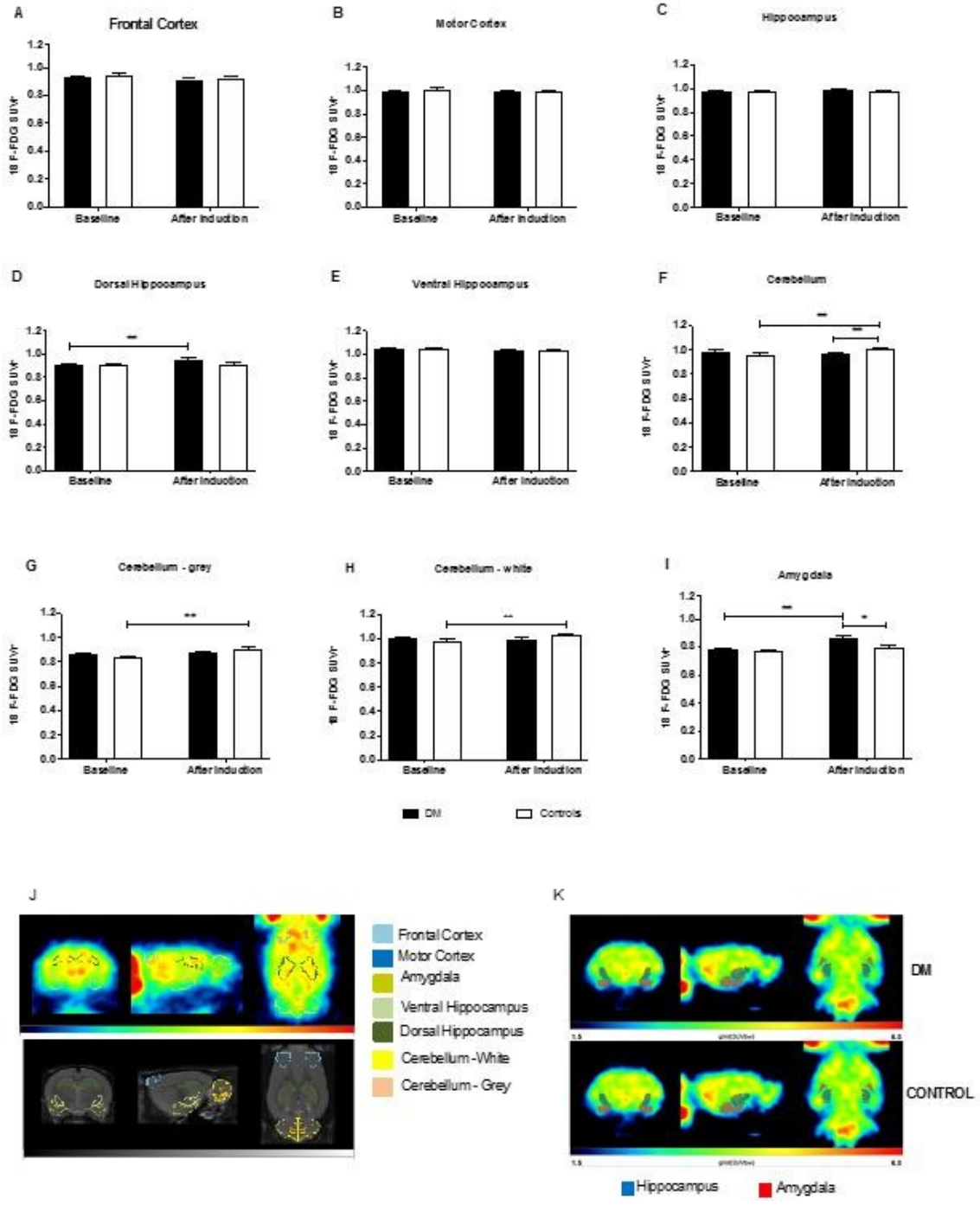

Exploration (sec)
50
40
30
20
10
0
**
**
Basal (T1)
Pos(T1)
Basal (T2)
Pos (T2)
Time exploring novel object (sec)
20
15
10
5
0
Basal
Pos
Basal
Pos
C
Total investigations (Both Objects)
25
20
15
10
5
0
*
*
Basal (T1)
Pos(T1)
Basal (T2)
Pos (T2)
D
Novel object investigation (%)
80
60
40
20
0
*
Basal (T2)
Pos (T2)
E
Discrimination Index
0.8
0.6
0.4
0.2
0.0
*
*
Basal
Pos
Basal
Pos
DM
F
Preference index (%)
1.0
0.8
0.6
0.4
0.2
0.0
*
Basal
Pos
Basal
Pos
Controls

Figure 5.


A
Frontal Cortex
B
Motor Cortex
C
Hippocampus
D
Dorsal Hippocampus
E
Ventral Hippocampus
F
Cerebellum
G
Cerebellum - grey
H
Cerebellum - white
I
Amygdala
18 F-FDG SUVr
Baseline
After Induction
DM
Controls
J
Frontal Cortex
Motor Cortex
Amygdala
Ventral Hippocampus
Dorsal Hippocampus
Cerebellum - White
Cerebellum - Grey
K
DM
CONTROL
Hippocampus
Amygdala

Tables Legends

Tables 1

Laterality microPET. Both experimental groups (DM and control) showed a increase in glucose metabolism unequal for both sides DM=Diabetes Mellitus; Control=sodium- citrate buffer(vehicle) injection. Values of SUVr are expressed as the mean ± standard deviation. L= left side of region brain. R = left side of region brain. $p<0.05$.

Table 1. Differences of laterality of the ^{18}F-FDG SUV values of the short-term follow-up for individual brain regions of control (n 8) and diabetic (n=10) rats at day 42.

Brain regions	Side	Control	Diabetes	*p*
		Mean±SE	Mean±SE	
Orbitofrontal Cortex	R	1,10±0,03	1,09±0,05	
Orbitofrontal Cortex	L	1,14±0,04	1,12±0,04	0,024
Auditory Cortex	R	0,91±0,03	0,87±0,04	
Auditory Cortex	L	0,94±0,03	0,90±0.04	0,015
Hipocamppus Posterior	R	0,91± 0,03	0.95±0.05	
Hipocamppus Posterior	L	0,91±0,04	0.94±0.04	0,034
Nucleus Accumbes	R	1,04±0,03	1,04±0,02	
Nucleus Accumbes	L	1,02±0,05	1,03±0.02	**0.005**

Table 2

Laterality microPET. In analyses after induction STZ or vehicle, groups (DM and control) showed results different in glucose metabolism for both sides. DM=Diabetes Mellitus; Control= vehicle injection. Values of SUVr are expressed as the mean ± standard deviation. L= left side of region brain. R = left side of region brain. $p<0.05$.

Table 2. Brain regions of glucose metabolism induced by STZ or vehicle.

Analysis of regions	Group	Activation glucose metabolism	Side	*p*
Auditory Cortex	DM	Decrease	L	**0,021 ***
			R	**0,008 ***
Auditory Cortex	CTRL	No change	L	0,148
			R	0,486
Orbito frontal Cortex	DM	No change	L	0,148
			R	0,486
Orbito frontal Cortex	CTRL	Decrease	L	0,643
			R	**0,007***
Posterior	DM	Increase	L	**0.032***
Hippocampus			R	**0.024***
Posterior	CTRL	No change	L	0.229
Hippocampus			R	0.942
Nucleus Accumbens	DM	No change	L	0.633
Core			R	0.735
NucleAccumbens	CTRL	No change	L	0.952
Core			R	**0.046***

4. DISCUSSION

In the present study we evaluated behavioral and brain glucose metabolism through 18F-FDG PET, in order to better understand possible alterations in these parameters in early stage DM. The data presented affects mainly brain glucose metabolism, whereas small behavioral alterations are induced. To the best of our knowledge, this is one study assessing possible short-term memory impairment and changes in brain glucose metabolism of STZ diabetic rats through 18F-FDG PET. Our data predict the first changes in diabetes that in other study found by Guan and colleagues, the long-term diabetes in elderly diabetic mice cause cognitive disturbances, impairments in spatial memory and in the regions of the amygdala and hippocampus (Guan et al., 2016).

The protocol used in this study successfully induced DM in animals as observed by the presence classical DM symptoms, such as increased water intake (polydipsia), increased excretion (polyuria and bowel movements), variation in food intake (polyphagia) and, more important, hyperglycemia. Only 2 animals were excluded from the study because they did not induce diabetes. These findings are in agreement with previous studies, confirming the validity of the model (Ates et al., 2007; P. B Bagatini et al., 2017; Venturini et al., 2010b).

The effect of diabetes on ambulatory activity was evaluated by the OF test focusing on activity levels, in order to understand how early stage diabetes can affect locomotion in rat animal model. In our results, we found that both groups equally maintained their exploratory capacity following DM induction (STZ or vehicle injection). This was surprising since according to the literature a decrease in locomotor activity of diabetic rats is expected when compared to controls (Andersen, 2012; Kalyani, Corriere, & Ferrucci, 2014; Reagan, 2012). The variability of experimental protocols may be the key to the differences found in the literature, such as different rodent strains, rodent age, protocol used for diabetes induction, duration of disease, post-induction evaluation and types of behavioral tests.

The absence of locomotor impairment observed by us agrees with ^{18}F-FDG uptake in the motor cortex (Fig. 5B), which was not altered. When analyzing tracer uptake in the cerebellum,

the control group showed increase in brain glucose metabolism while DM had no alterations. Overall, brain glucose metabolism in the brain is mainly determined by two factors: the regional complexity of functional networks and the energy efficiency of such networks for wiring (Tomasi, Wang, & Volkow, 2013). High functional areas and areas with high connectivity will show increased metabolism, while less active areas will show the opposite. Thus, alterations observed by us in cerebellum glucose metabolism in control animals might be a normal modification during development, and the absence of alterations observe in DM group can be interpreted as an initial impairment in this region due to diabetes development, supporting the hypothesis that the early effects of DM are first seen in the brain, leading to behavioral alterations along with DM chronicity. Movement control is coordinated by several – motor and cognitive - brain areas. Cerebellum is a subcortical region that connects to other brain regions, as motor cortex, involved in regulation of motor coordination, cognition and attention, receiving input from a variety of regions in cerebral cortex (Allali et al., 2018; Koziol et al., 2014). Studies with humans show that cerebellar hypermetabolism is associated with a compensatory activity to maintain cognitive functions in neurodegenerative diseases, such as Parkinson disease, Alzheimer's disease, Dementia with Lewy bodies and social anxiety among others (Blum et al., 2018; Su et al., 2019; Ye et al., 2019).

Previous studies involving experimental rat models and humans are still controversial in relation to the onset of cognitive alterations, such as memory deficit in DM (Biala, 2012; Haider, Ahmed, & Tabassum, 2013; Nunes et al., 2017; Zhou et al., 2018). However elderly diabetic rats were predisposed to complications with cognitive and affective disorders, caused by atrophy and alteration of glucose metabolism of the hippocampus in long term diabetes. In this research compared to normal-aged mice, middle-aged diabetics are likely to complicate small vessel brain disease with affected and cognitive disorders related with Beclin1-mediated autophagy (Guan et al., 2016) In our study, both groups, healthy control and DM animals, demonstrated a similar behavior regarding total investigation of objects in NOR, in trial 1, either before or after DM induction. The first trial is considered a training phase, were both objects are equal. However, in

trial 2, where there is one familiar object and one novel object and, moreover, one object was placed in a different location, DM animal behaved in opposite direction of control animals: while control animals increased the number of total investigations in trial 2, DM animals reduced (Fig. 4C). In order to recognize a new object, it is necessary that the animal: 1) identify these two identical objects and 2) retain these two identical objects in working memory, indicating that more cognitive skills are necessary for novelty recognition. Once the object is replaced by a novel one, in case the animal was able to recognize the novelty, it will behave differentially, showing more interest by the novel object. The same can be extrapolated for spatial memory. This suggests that DM animals present an impairment on short-term memory and spatial memory, but can also allow us to infer that working memory might also be altered (Antunes & Biala, 2012; Silvers et al., 2007).

Hippocampus is associated, among other functions, with cognition, specially memory, and this area can be segmented in anterior and posterior portions (dorso-ventral in rodents) which are structurally and functionally distinct (Collin et al., 2015; Dalton et al., 2017; Fanselow & Dong, 2010). This anatomical and functional division implies a differential connectivity from hippocampus to hypothalamic areas related to different behavior and cognitive functions (Strange, Witter, Lein, & Moser, 2014). Despite of no differences in brain glucose metabolism of hippocampus as whole, analysis into dorso-ventral hippocampus demonstrated a increased in glucose metabolism in dorsal hippocampus, after DM induction, when compared to healthy control animals, where no alterations were observed. According to the Moser theory (Moser and Moser 1998), dorsal hippocampus is directly related to memory function while ventral area is involved in modulating emotional and affective processes, specifically anxiety and frustration (Fanselow & Dong, 2010; Moser & Moser, 1998). In this way, the increase in brain glucose metabolism observed in DM group is in agreement with behavior alterations observed in NOR, were DM animals present impaired short-term memory and special memory. Since no alterations were observed regarding glucose metabolism in dorsal hippocampus in control animals, it is

supposed that this is the normal course of development, and the slightly increase observed in DM suggested an effect of early stage diabetes.

One of the ways found to analyze the SUV of the regions was to correct it by means of normalization of data using whole-brain activity SUV image analyzes, generating the SUVr. It is a widely used analysis technique in rodents, and its was used in all our datasets. In our study, the hyperglycemia present decreased the arterial flow of ^{18}F-FDG due to a faster blood clearance, and that there is a different ^{18}F-FDG flow gradient compared to arterial with the same injected dose. In order to minimize this limitation, normalization of data to whole-brain activity outperformed SUV or% ID / g image analyzes in all our datasets and is a widely used analysis technique in rodents can be normalized. In this case, normalization will be based on the general activity of the brain and not just the restricted selection of voxels (Feltes et al., 2017; Welch et al, 2013). Differences in laterality present in diabetic animals may be related to cell losses and / or selective changes in the blood-brain barrier induced by DM. Hippocampus anterior increase is related with concept of neural (or cognitive) reserve, whereby plasticity and compensatory mechanisms can maintain cognitive performance in the face of insult in the mature brain (Barulli & Stern, 2013).

While hippocampus is considered the central brain area related to memory, amygdala is the emotional center of our brain. However, studies show that our emotional center also plays an important role in memory consolidation regarding emotional contexts, the also called emotional memories (Janak & Tye, 2015; Roozendaal et al., 2009). It is important to remind that amygdala's connectivity expands to several brain regions, as frontal cortex and hippocampus regulating the effects of emotional arousal and stress exposure to these brain areas (Roozendaal et al., 2009). It is known that stress impairs memory retrieval and working memory, mainly via amygdala modulation of hippocampal stress exposure to stress hormones as noradrenaline and glucocorticoids (Roozendaal et al., 2003, 2004). We observed a increase in glucose metabolism in amygdala in DM animals, while control animals did not show any alterations.

Although animals have a greater propensity for novelty, it is important to consider that after, prolonged exposure, a reduced preference for novelty occurs, and this means that the objects have become familiar, which might also explain the differences observed in NOR. When we repeat NOR and DM animals have decreased their time investigating both objects, it may suggest that animals are habituated (Biala, 2012; Branchi, Ricceri, Cellulare, & Superiore, 2004).

On the other hand, we cannot rule out anxiety behavior, since DM animals presented reduced exploratory behavior when facing novelty (NOR trial 2). This is also supported by percent of time spent investigating the novel object. Even though no differences in time in seconds spent exploring novel objects could be observed between groups and trials, percent of time spent investigating novel object was increased in control group, while no alterations were observed in DM group after induction (STZ or vehicle – Fig. 4D). According to the literature, DM patients – both type 1 and 2 - presents symptoms of depression and anxiety, which was also shown in animal models (Buchberger et al., 2016; Naicker, Johnson, et al., 2017; Naicker, Øverland, et al., 2017; Rechenberg, Whittemore, & Grey, 2017). However, more experiments are necessary to confirm this hypothesis.

The mechanisms involved in diabetes-induced cognitive decline are related to glucose homeostasis, hypothalamic-pituitary-adrenal axis dysregulation (HPA), and decreased insulin activity (Wrighten, Piroli, Grillo, & Reagan, 2009a). However, brain cells are not totally dependent on insulin for glucose supply, since they have independent means of obtaining glucose from blood, although a small percentage of insulin crosses the blood-brain barrier and some studies evidence the local production of insulin in the blood SNC (Blázquez, Velázquez, & Hurtado-carneiro, 2014). Despite of glucose obtainment in the brain is insulin independent, insulin receptors (IR) and their subtrees are present in large quantities in CNS. The IR are present in regions such as the olfactory bulb, cerebral cortex, hippocampus, cerebellum and choroid plexus (Ghasemi, Haeri, Dargahi, Mohamed, & Ahmadiani, 2013; Rhea, Salameh, & Banks, 2019). Moreover, the decrease in IR activity may be a triggering factor, producing deficits in hippocampal plasticity and, finally, in cognitive decline (Wrighten et al., 2009a).

This study presents some caveats. Despite of several studies indicating STZ injection as an Type-1 Diabetes induction model, we cannot affirm that the model has total insulin ablation. We also cannot affirm that insulin receptors are hypo-responsive. Thus, it is possible that animals present a mixed model between type 1 and type 2 Diabetes Mellitus. This might have an important role for identifying the early impairments of DM evaluated by us. Moreover, it is possible that the time since induction and diabetes mellitus establishment may not have had enough to overcome the intrinsic compensatory mechanisms, maintaining intact the cerebral glucose metabolism in brain regions as motor cortex, frontal cortex, and hippocampus when analyzed globally. This premise is related to the well-described concept of neural (or cognitive) reserve, whereby plasticity and compensatory mechanisms can maintain cognitive performance in the face of insult in the mature brain (Barulli & Stern, 2013). However, in other brain areas, as cerebellum, dorsal hippocampus and amygdala, this might not be true, being the first sign of DM effects on brain glucose metabolism. Also, our study evaluated brain glucose metabolism in a more generalized, globally analysis. More detailed, close analysis should be performed in order to better understand the effects of DM on brain glucose metabolism.

5. CONCLUSION

Results presented here provide data on the variability of diabetes progression, which may induce low-level cognitive impairment, and no alterations on locomotor activity. Importantly, some tenuous alterations could be observed in brain glucose metabolism areas related to both locomotion and cognition, suggesting that DM affects brain metabolism first, without marked behavioral alterations.

AUTHOR CONTRIBUTIONS

SS, LLX, PBB, MAE and AW – study design; data analysis and interpretation, manuscript elaboration. SS, PFRN, LVP, BM, GZL and LTN conducted the experimental procedures. SS, CL, PKF - data collection and interpretation. SS, PKF, GTV, SG and JCC conducted the microPET-FDG scans and analysis. LLX and AW – Final approval of submitted version.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

All applicable international, national, and institutional guidelines for the care and use of animals were followed. The University Ethical Committee (CEUA 7660/PUCRS) approved all experiments involving animals

FUNDING

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001; Conselho Nacional de Pesquisa e Desenvolvimento (CNPq); Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). Léder Leal Xavier and Jaderson Costa are CNPq researchers.

REFERENCE

Aguilar, M., Bhuket, T., Torres, S., Liu, B., & Wong, R. J. (2015). Prevalence of the metabolic syndrome in the United States, 2003-2012. *Jama*, *313*(19), 1973–1974. https://doi.org/10.1001/jama.2015.4260

Ahlqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., … Groop, L. (2018). Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *The Lancet Diabetes and Endocrinology*, *6*(5), 361–369. https://doi.org/10.1016/S2213-8587(18)30051-2

Ahtiluoto, S., Polvikoski, T., Peltonen, M., Solomon, A., Tuomilehto, J., Winblad, B., … Kivipelto, M. (2010). Diabetes, Alzheimer disease, and vascular dementia: A population-based neuropathologic study. *Neurology*. https://doi.org/10.1212/WNL.0b013e3181f4d7f8

Akbarzadeh, A., Norouzian, D., Mehrabi, M., Jamshidi, S., Farhangi, A., Allah Verdi, A., …

Akbarzadeh, A. (2007). *INDUCTION OF DIABETES BY STREPTOZOTOCIN IN RATS*. *Indian Journal of Clinical Biochemistry* (Vol. 22).

Allali, G., Blumen, H. M., Devanne, H., Pirondini, E., Delval, A., & Van De Ville, D. (2018). Brain imaging of locomotion in neurological conditions. *Neurophysiologie Clinique*. https://doi.org/10.1016/j.neucli.2018.10.004

American Diabetes Association. (2012). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *35 Suppl 1*, S64-71. https://doi.org/10.2337/dc12-s064

American Diabetes Association. (2016). Classification and diagnosis of diabetes. *Diabetes Care*, *39*, S13–S22.

Andersen, H. (2012). Motor dysfunction in diabetes. *Diabetes Metab Res Rev*, *28*(October 2011), 89–92. https://doi.org/10.1002/dmrr

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing*. https://doi.org/10.1007/s10339-011-0430-z

Ates, O., Cayli, S. R., Yucel, N., Altinoz, E., Kocak, A., Durak, M. A., … Yologlu, S. (2007). Central nervous system protection by resveratrol in streptozotocin-induced diabetic rats. *Journal of Clinical Neuroscience*. https://doi.org/10.1016/j.jocn.2005.12.010

Atilgan, H., & Kwan, A. C. (2018). Same lesson, varied choices by frontal cortex. *Nature Neuroscience*, *21*(12), 1648–1650. https://doi.org/10.1038/s41593-018-0282-2

Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. *The Lancet*, *383*(9911), 69–82. https://doi.org/10.1016/S0140-6736(13)60591-7

Bach, J. F., & Chatenoud, L. (2012). The hygiene hypothesis: An explanation for the increased frequency of insulin-dependent diabetes. *Cold Spring Harbor Perspectives in Medicine*, *2*(2), 1–10. https://doi.org/10.1101/cshperspect.a007799

Bagatini, P. B, Xavier, L. L., Bertoldi, K., Moysés, F., Lovatel, G., Tartari, L., … Rodrigues, I.

(2017). An evaluation of aversive memory and hippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol. *Neuroscience Letters*, *636*, 184–189. https://doi.org/10.1016/j.neulet.2016.10.059

Bagatini, Pamela Brambilla, Xavier, L. L., Bertoldi, K., Moysés, F., Lovatel, G., Neves, L. T., … Achaval, M. (2017). An evaluation of aversive memory and hippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol. *Neuroscience Letters*, *636*, 184–189. https://doi.org/10.1016/j.neulet.2016.10.059

Bagatini, Pamela Brambilla, Xavier, L. L., Neves, L. T., Saur, L., Barbosa, S., Baptista, P. P. A., … Achaval, M. (2014). Resveratrol prevents akinesia and restores neuronal tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta of diabetic rats. *Brain Research*, *1592*, 101–112. https://doi.org/10.1016/j.brainres.2014.10.007

Banks, W. A., Owen, J. B., & Erickson, M. A. (2012). Insulin in the brain: There and back again. *Pharmacology and Therapeutics*, *136*(1), 82–93. https://doi.org/10.1016/j.pharmthera.2012.07.006

Bansal, R., AHMAD, N., & KIDWAI, J. R. (2017). 済無 No Title No Title. *Journal of Chemical Information and Modeling*, *8*(9), 1–58. https://doi.org/10.1017/CBO9781107415324.004

Baptista, P. P. A., Saur, L., Bambrilla, P., Greggio, S., Teribele, G., Sabrina, V., … Moriguchi, M. (2015). Antidepressant Effects of Ketamine Are Not Related to 18 F-FDG Metabolism or Tyrosine Hydroxylase Immunoreactivity in the Ventral Tegmental Area of Wistar Rats. *Neurochemical Research*, *40*, 1153–1164. https://doi.org/10.1007/s11064-015-1576-3

Barulli, D., & Stern, Y. (2013). Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts. *Trends in Cognitive Sciences*, *17*(10), 502–509. https://doi.org/10.1016/j.tics.2013.08.012

Bednarik, P., Moheet, A. A., Grohn, H., Kumar, A. F., Eberly, L. E., Seaquist, E. R., & Mangia, S. (2017a). Type 1 diabetes and impaired awareness of hypoglycemia are associated with

reduced brain gray matter volumes. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2017.00529

Bednarik, P., Moheet, A. A., Grohn, H., Kumar, A. F., Eberly, L. E., Seaquist, E. R., & Mangia, S. (2017b). Type 1 diabetes and impaired awareness of hypoglycemia are associated with reduced brain gray matter volumes. *Frontiers in Neuroscience*, *11*(SEP). https://doi.org/10.3389/fnins.2017.00529

Biala, M. A. G. (2012). The novel object recognition memory : neurobiology , test procedure , and its modifications. *Cogn Process*, *13*, 93–110. https://doi.org/10.1007/s10339-011-0430-z

Biessels, G., Strachan, M., Visseren, F., Kappelle, L., & Whitmer, R. (2014). Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *The Lancet Diabetes & Endocrinology*, *2*(3), 246–255.

Biessels, G J. (2013). Sweet memories: 20 years of progress in research on cognitive functioning in diabetes. *European Journal of Pharmacology*, *719*(1–3), 153–160. https://doi.org/10.1016/j.ejphar.2013.04.055

Biessels, Geert Jan. (2013). Sweet memories: 20 years of progress in research on cognitive functioning in diabetes. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2013.04.055

Biessels, Geert Jan, van der Heide, L. P., Kamal, A., Bleys, R. L. A. ., & Gispen, W. H. (2002). Ageing and diabetes: implications for brain function. *European Journal of Pharmacology*, *441*(1), 1–14. https://doi.org/10.1016/S0014-2999(02)01486-3

Blázquez, E., Velázquez, E., & Hurtado-carneiro, V. (2014). Insulin in the brain : its pathophysiological implications for states related with central insulin resistance , type 2 diabetes and alzheimer ' s disease. *Frontiers in Endocrinology*, *5*(October), 1–21. https://doi.org/10.3389/fendo.2014.00161

Blázquez, E., Velázquez, E., Hurtado-Carneiro, V., & Ruiz-Albusac, J. M. (2014). Insulin in the brain: Its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and alzheimer's disease. *Frontiers in Endocrinology*. Frontiers Research Foundation. https://doi.org/10.3389/fendo.2014.00161

Blum, D., la Fougère, C., Pilotto, A., Maetzler, W., Berg, D., Reimold, M., & Liepelt-Scarfone, I. (2018). Hypermetabolism in the cerebellum and brainstem and cortical hypometabolism are independently associated with cognitive impairment in Parkinson's disease. *European Journal of Nuclear Medicine and Molecular Imaging*. https://doi.org/10.1007/s00259-018-4085-1

Branchi, I., Ricceri, L., Cellulare, B., & Superiore, I. (2004). Refining learning and memory assessment in laboratory rodents . An ethological perspective. *Ann Ist Super Sanità 2004;40(2):231-236*, *40*(2), 231–236.

Bruehl, H., Sweat, V., Tirsi, A., Shah, B., & Convit, A. (2011). Obese Adolescents with Type 2 Diabetes Mellitus Have Hippocampal and Frontal Lobe Volume Reductions. *Neuroscience and Medicine*, *2*(1), 34–42. https://doi.org/10.4236/nm.2011.21005.Obese

Buchberger, B., Huppertz, H., Krabbe, L., Lux, B., Mattivi, J. T., & Siafarikas, A. (2016). Symptoms of depression and anxiety in youth with type 1 diabetes: A systematic review and meta-analysis. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2016.04.019

Cabrera, S. M., Henschel, A. M., & Hessner, M. J. (2016). Innate inflammation in type 1 diabetes. *Translational Research*, *167*(1), 214–227. https://doi.org/10.1016/j.trsl.2015.04.011

Callaghan, B. C., Cheng, H. T., Stables, C. L., Smith, A. L., & Feldman, E. L. (2012). Diabetic neuropathy : clinical manifestations and current treatments. *The Lancet Neurology*, *11*(6), 521–534. https://doi.org/10.1016/S1474-4422(12)70065-0

Cobas, R. A., Bosi Ferraz, M., de Mattos Matheus, A. S., Monteiro Tannus, L. R., Antonio Negrato, C., de Araujo, L. A., … Brito Gomes, M. (2013). The cost of type 1 diabetes: A nationwide multicentre study in Brazil. *Bulletin of the World Health Organization*, *91*(6), 434–440. https://doi.org/10.2471/BLT.12.110387

Collin, S. H. P., Milivojevic, B., & Doeller, C. F. (2015). Memory hierarchies map onto the hippocampal long axis in humans. *Nature Neuroscience*. https://doi.org/10.1038/nn.4138

Dalton, M. A., Zeidman, P., Barry, D. N., Williams, E., & Maguire, E. A. (2017). Segmenting subregions of the human hippocampus on structural magnetic resonance image scans: An illustrated tutorial. *Brain and Neuroscience Advances*. https://doi.org/10.1177/2398212817701448

Damasceno, D. C., Netto, A. O., Iessi, I. L., Gallego, F. Q., Corvino, S. B., Dallaqua, B., … Rudge, M. V. C. (2014). Streptozotocin-induced diabetes models: Pathophysiological mechanisms and fetal outcomes. *BioMed Research International*, *2014*, 1–11. https://doi.org/10.1155/2014/819065

de Almeida-Pititto, B., Dias, M. L., de Moraes, A. C. F., Ferreira, S. R. G., Franco, D. R., & Eliaschewitz, F. G. (2015). Type 2 diabetes in Brazil: Epidemiology and management. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *8*, 17–28. https://doi.org/10.2147/DMSO.S72542

De Beeck, A. O., & Eizirik, D. L. (2016). Viral infections in type 1 diabetes mellitus-why the β cells? *Nature Reviews Endocrinology*, *12*(5), 263–273. https://doi.org/10.1038/nrendo.2016.30

de Senna, P. N., Bagatini, P. B., Galland, F., Bobermin, L., do Nascimento, P. S., Nardin, P., … Xavier, L. L. (2017). Physical exercise reverses spatial memory deficit and induces hippocampal astrocyte plasticity in diabetic rats. *Brain Research*. https://doi.org/10.1016/j.brainres.2016.10.024

De Senna, P. N., Ilha, J., Baptista, P. P. A., Do Nascimento, P. S., Leite, M. C., Paim, M. F., … Xavier, L. L. (2011). Effects of physical exercise on spatial memory and astroglial alterations in the hippocampus of diabetic rats. *Metabolic Brain Disease*, *26*(4), 269–279. https://doi.org/10.1007/s11011-011-9262-x

De Senna, P. N., Xavier, L. L., Bagatini, P. B., Saur, L., Galland, F., Zanotto, C., … Achaval, M. (2015). Physical training improves non-spatial memory, locomotor skills and the blood brain barrier in diabetic rats. *Brain Research*, *1618*, 75–82. https://doi.org/10.1016/j.brainres.2015.05.026

Do Nascimento, P. S., Lovatel, G. A., Barbosa, S., Ilha, J., Centenaro, L. A., Malysz, T., … Achaval, M. (2011). Treadmill training improves motor skills and increases tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta in diabetic rats. *Brain Research*, *1382*, 173–180. https://doi.org/10.1016/j.brainres.2011.01.063

Dufrane, D., Van Steenberghe, M., Guiot, Y., Goebbels, R. M., Saliez, A., & Gianello, P. (2006). Streptozotocin-induced diabetes in large animals (pigs/primates): Role of GLUT2 transporter and β-cell plasticity. *Transplantation*, *81*(1), 36–45. https://doi.org/10.1097/01.tp.0000189712.74495.82

Egede, L. E., Bishu, K. G., Walker, R. J., & Dismuke, C. E. (2016). Impact of diagnosed depression on healthcare costs in adults with and without diabetes: United States, 2004-2011. *Journal of Affective Disorders*, *195*, 119–126. https://doi.org/10.1016/j.jad.2016.02.011

Elsner, M., Guldbakke, B., Tiedge, M., Munday, R., & Lenzen, S. (2000). Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. *Diabetologia*, *43*(12), 1528–1533. https://doi.org/10.1007/s001250051564

Fanselow, M. S., & Dong, H. W. (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron*. https://doi.org/10.1016/j.neuron.2009.11.031

Fava, A. A. S. (2013). Association of incidence of type 1 diabetes with mortality from infectious disease and with antibiotic susceptibility at a country level, 859–865. https://doi.org/10.1007/s00592-013-0464-z

Funk, J. (2007). Distúrbios do pâncreas endócrino. In S. McPhee & W. Ganong (Eds.), *Fisiopatologia da doença: uma introdução à medicina clínica* (5th ed., p. 642). São Paulo: McGraw-Hill Interamericana do Brasil.

Gaspar, J., Baptista, F., Macedo, M., & Ambrósio, A. (2016). Inside the Diabetic Brain: Role of Different Players Involved in Cognitive Decline. *ACS Chemical Neuroscience*, *7*, 131–142. https://doi.org/10.1021/acschemneuro.5b00240

GBD. (2015). Global , regional , and national comparative risk assessment of 79 behavioural , environmental and occupational , and metabolic risks or clusters of risks in 188 countries , 1990 – 2013 : a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *386*, 1990–2013. https://doi.org/10.1016/S0140-6736(15)00128-2

Ghasemi, R., Haeri, A., Dargahi, L., Mohamed, Z., & Ahmadiani, A. (2013). Insulin in the brain: Sources, localization and functions. *Molecular Neurobiology*. https://doi.org/10.1007/s12035-012-8339-9

Gispen, W. H., & Biessels, G.-J. (2000). Cognition and synaptic plasticity in diabetes mellitus. *Trends in Neurosciences*, *23*(11), 542–549. https://doi.org/10.1016/S0166-2236(00)01656-8

Guan, Z. F., Zhou, X. L., Zhang, X. M., Zhang, Y., Wang, Y. M., Guo, Q. L., … Liu, Y. C. (2016). Beclin-1- mediated autophagy may be involved in the elderly cognitive and affective disorders in streptozotocin-induced diabetic mice. *Translational Neurodegeneration*, *5*(1), 1–10. https://doi.org/10.1186/s40035-016-0070-4

Haider, S., Ahmed, S., & Tabassum, S. (2013). Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. *Acta Neurol Belg*, *113*, 35–41.

https://doi.org/10.1007/s13760-012-0121-2

Haider, S., Ahmed, S., Tabassum, S., Memon, Z., Ikram, M., & Haleem, D. J. (2013). Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. *Acta Neurologica Belgica*, *113*(1), 35–41. https://doi.org/10.1007/s13760-012-0121-2

Hamacher, K., Coenen, H. H., & Stocklin, G. (1986). Efficient Stereospecific Synthesis of No-Carrier-Added2@[18F]-Fluoro-2-Deoxy D-Glucose Using Aminopolyether Supported Nucleophilic Substitution. *J Nucl Med*, *27*(February), 235–239.

Hawkins, B. T., Lundeen, T. F., Norwood, K. M., Brooks, H. L., & Egleton, R. D. (2007). Increased blood-brain barrier permeability and altered tight junctions in experimental diabetes in the rat: Contribution of hyperglycaemia and matrix metalloproteinases. *Diabetologia*, *50*(1), 202–211. https://doi.org/10.1007/s00125-006-0485-z

Hernández-Fonseca, J. P., Rincón, J., Pedreañez, A., Viera, N., Arcaya, J. L., Carrizo, E., & Mosquera, J. (2009). Structural and ultrastructural analysis of cerebral cortex, cerebellum, and hypothalamus from diabetic rats. *Experimental Diabetes Research*, *2009*, 329632. https://doi.org/10.1155/2009/329632

Ho, M. S., Weller, N. J., Ives, F. J., Carne, C. L., Murray, K., vanden Driesen, R. I., … Jones, T. W. (2008). Prevalence of Structural Central Nervous System Abnormalities in Early-Onset Type 1 Diabetes Mellitus. *Journal of Pediatrics*. https://doi.org/10.1016/j.jpeds.2008.03.005

Huang, M., Gao, L., Yang, L., Lin, F., & Lei, H. (2012). Abnormalities in the brain of streptozotocin-induced type 1 diabetic rats revealed by diffusion tensor imaging. *NeuroImage: Clinical*, *1*(1), 57–65. https://doi.org/10.1016/j.nicl.2012.09.004

Huber, J. D., VanGilder, R. L., & Houser, K. A. (2006). Streptozotocin-induced diabetes progressively increases blood-brain barrier permeability in specific brain regions in rats. *American Journal of Physiology - Heart and Circulatory Physiology*, *291*(6), 2660–2668.

https://doi.org/10.1152/ajpheart.00489.2006

IDF. (2019). Idf diabetes atlas 9, Prevalence of Diabetes.

Jakobsen, J., Sidenius, P., Gundersen, H., & Østerby, R. (1987). Quantitative Changes of Cerebral Neocortical Structure in Insulin-Treated Long-Term Streptozocin-lnduced Diabetes in Rats. *Diabetes*, *36*(5), 597–601.

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature*. https://doi.org/10.1038/nature14188

Jing, Y.-H., Chen, K.-H., Kuo, P.-C., Pao, C.-C., & Chen, J.-K. (2013). Neurodegeneration in streptozotocin-induced diabetic rats is attenuated by treatment with resveratrol. *Neuroendocrinology*, *98*(2), 116–127. https://doi.org/10.1159/000350435

Kalyani, R. R., Corriere, M., & Ferrucci, L. (2014). Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol*, *2*(10), 819– 829. https://doi.org/10.1016/S2213-8587(14)70034-8.Age-related

Kang, L., Routh, V. H., Kuzhikandathil, E. V, Gaspers, L. D., & Levin, B. E. (2004). Physiological and Molecular Characteristics of Rat Hypothalamic Ventromedial Nucleus Glucosensing Neurons To evaluate potential mechanisms for neuronal glu-cosensing, fura-2 Ca 2 imaging and single-cell RT-PCR were carried out in dissociated ventromedial , (10), 549–559.

Koekkoek, P. S., Kappelle, L. J., Berg, E. Van Den, Rutten, G. E. H. M., & Biessels, G. J. (2015). Cognitive function in patients with diabetes mellitus : guidance for daily care. *The Lancet Neurology*, *14*(3), 329–340. https://doi.org/10.1016/S1474-4422(14)70249-2

Kopschina Feltes, P., de Vries, E. F. J., Juarez-Orozco, L. E., Kurtys, E., Dierckx, R. A. J. O., Moriguchi-Jeckel, C. M., & Doorduin, J. (2019). Repeated social defeat induces transient glial activation and brain hypometabolism: A positron emission tomography imaging study. *Journal of Cerebral Blood Flow and Metabolism*, *39*(3), 439–453.

https://doi.org/10.1177/0271678X17747189

Koziol, L. F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., … Yamazaki, T. (2014). Consensus paper: The cerebellum's role in movement and cognition. *Cerebellum*. https://doi.org/10.1007/s12311-013-0511-x

Kumagai, A. K. (1999). Glucose transport in brain and retina: Implications in the management and complications of diabetes. *Diabetes/Metabolism Research and Reviews*, *15*(4), 261–273. https://doi.org/10.1002/(SICI)1520-7560(199907/08)15:4<261::AID-DMRR43>3.0.CO;2-Z

Lancelot, S., & Zimmer, L. (2010). Small-animal positron emission tomography as a tool for neuropharmacology. *Trends in Pharmacological Sciences*, *31*(9), 411–417. https://doi.org/10.1016/j.tips.2010.06.002

Ledoux, S. P., & Wilson, G. L. (1984). Effects of streptozotocin on a clonal isolate of rat insulinoma cells. *BBA - Molecular Cell Research*, *804*(4), 387–392. https://doi.org/10.1016/0167-4889(84)90064-8

Levin, B. E., Routh, V. H., Kang, L., Sanders, N. M., & Dunn-Meynell, A. A. (2004). Perspectives in Diabetes Neuronal Glucosensing What Do We Know After 50 Years? *Diabetes*, *53*(8), 2521–2528. Retrieved from http://diabetes.diabetesjournals.org/content/diabetes/53/10/2521.full.pdf

Malone, J. I., Hanna, S. K., & Saporta, S. (2006). Hyperglycemic brain injury in the rat. *Brain Research*, *1076*(1), 9–15. https://doi.org/10.1016/j.brainres.2005.12.072

Martínez-Tellez, R., Gómez-Villalobos, M. D. J., & Flores, G. (2005). Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. *Brain Research*, *1048*(1–2), 108–115. https://doi.org/10.1016/j.brainres.2005.04.048

Mauras, N., Mazaika, P., Buckingham, B., Weinzimer, S., White, N. H., Tsalikian, E., … Reiss, A. L. (2015). Longitudinal assessment of neuroanatomical and cognitive differences in

young children with type 1 diabetes: Association with hyperglycemia. *Diabetes*, *64*(5), 1770–1779. https://doi.org/10.2337/db14-1445

McCrimmon, R. J., Ryan, C. M., & Frier, B. M. (2012). Diabetes and cognitive dysfunction. *The Lancet*, *379*(9833), 2291–2299. https://doi.org/10.1016/S0140-6736(12)60360-2

Mergenthaler, P., Lindauer, U., Dienel, G., & Meisel, A. (2013). Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends in Neuroscience*, *36*(10), 587–597. https://doi.org/10.1016/j.tins.2013.07.001.Sugar

Mirrione, M. M., Schulz, D., Lapidus, K. A. B., Zhang, S., Goodman, W., & Henn, F. A. (2014). Increased metabolic activity in the septum and habenula during stress is linked to subsequent expression of learned helplessness behavior. *Frontiers in Human Neuroscience*, *8*(1 FEB). https://doi.org/10.3389/fnhum.2014.00029

Moheet, A., Mangia, S., & Seaquist, E. R. (2015). Impact of diabetes on cognitive function and brain structure. *Annals of the New York Academy of Sciences*, *1353*, 60–71. https://doi.org/10.1111/nyas.12807

Mooradian, A. D. (1997). Central nervous system complications of diabetes mellitus — a perspective from the blood–brain barrier. *Brain Research Reviews*, *23*(3), 210–218. https://doi.org/10.1016/S0165-0173(97)00003-9

Moser, M. B., & Moser, E. I. (1998). Functional differentiation in the hippocampus. *Hippocampus*. https://doi.org/10.1002/(SICI)1098-1063(1998)8:6<608::AID-HIPO3>3.0.CO;2-7

Mueller, K., Sacher, J., Arelin, K., Holiga, S., Kratzsch, J., Villringer, A., & Schroeter, M. (2012). Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI , serum marker and gene expression study. *Translational Psychiatry*, (October), 1–7. https://doi.org/10.1038/tp.2012.121

Muramatsu, K., Ikutomo, M., Tamaki, T., Shimo, S., & Niwa, M. (2018). Effect of streptozotocin-induced diabetes on motor representations in the motor cortex and corticospinal tract in rats. *Brain Research*, *1680*, 115–126. https://doi.org/10.1016/j.brainres.2017.12.016

Musen, G., In, K. L., Sparks, C. R., Weinger, K., Hwang, J., Ryan, C. M., … Jacobson, A. M. (2006). Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes*, *55*(2), 326–333. https://doi.org/10.2337/diabetes.55.02.06.db05-0520

Naicker, K., Johnson, J. A., Skogen, J. C., Manuel, D., Øverland, S., Sivertsen, B., & Colman, I. (2017). Type 2 diabetes and comorbid symptoms of depression and anxiety: Longitudinal associations with mortality risk. *Diabetes Care*. https://doi.org/10.2337/dc16-2018

Naicker, K., Øverland, S., Johnson, J. A., Manuel, D., Skogen, J. C., Sivertsen, B., & Colman, I. (2017). Symptoms of anxiety and depression in type 2 diabetes: Associations with clinical diabetes measures and self-management outcomes in the Norwegian HUNT study. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2017.07.002

NCD-RisC. (2016). Worldwide trends in diabetes since 1980 : a pooled analysis of 751 population-based studies with 4 · 4 million participants. *The Lancet*, *6736*(16), 1–18. https://doi.org/10.1016/S0140-6736(16)00618-8

Noble, J. A., Valdes, A. M., Varney, M. D., Carlson, J. A., Moonsamy, P., Fear, A. L., … Erlich, H. A. (2010). HLA class I and genetic susceptibility to type 1 diabetes: Results from the type 1 diabetes genetics consortium. *Diabetes*, *59*(11), 2972–2979. https://doi.org/10.2337/db10-0699

Northam, E. A., Rankins, D., Lin, A., Wellard, R. M., Pell, G. S., Finch, S. J., … Cameron, F. J. (2009). Central nervous system function in youth with type 1 diabetes 12 years after disease onset. *Diabetes Care*, *32*(3), 445–450. https://doi.org/10.2337/dc08-1657

Northman E., Anderson P, Rani J, Hughes M, W. G. et al. (2001). Neuropsychological Profiles of Children Disease Onset. *Diabetes Care*, *24*(9), 1541–1546.

Nunes, P., Senna, D., Brambilla, P., Galland, F., Bobermin, L., Severo, P., … Leal, L. (2017). Physical exercise reverses spatial memory de fi cit and induces hippocampal astrocyte plasticity in diabetic rats. *Brain Research*, *1655*(July 2016), 242–251. https://doi.org/10.1016/j.brainres.2016.10.024

Okamoto, M. M., Anhê, G. F., Sabino-Silva, R., Marques, M. F. D. S. F., Freitas, H. S., Mori, R. C. T., … Machado, U. F. (2011). Intensive insulin treatment induces insulin resistance in diabetic rats by impairing glucose metabolism-related mechanisms in muscle and liver. *Journal of Endocrinology*, *211*(1), 55–64. https://doi.org/10.1530/JOE-11-0105

Özdemir, N. G., Akbaş, F., Kotil, T., & Yilmaz, A. (2016). Analysis of diabetes-related cerebellar changes in streptozotocin-induced diabetic rats. *Turkish Journal of Medical Sciences*, *46*(5), 1579–1592. https://doi.org/10.3906/sag-1412-125

Parashar, A., Mehta, V., & Malairaman, U. (2018). Type 2 Diabetes Mellitus Is Associated with Social Recognition Memory Deficit and Altered Dopaminergic Neurotransmission in the Amygdala. *Annals of Neurosciences*, *24*(4), 212–220. https://doi.org/10.1159/000479637

Perantie, D. C., Koller, J. M., Weaver, P. M., Lugar, H. M., Black, K. J., White, N. H., & Hershey, T. (2011). Prospectively determined impact of type 1 diabetes on brain volume during development. *Diabetes*. https://doi.org/10.2337/db11-0589

Petty, S., & Mitchell, P. (2004). Images in neuroscience. *Journal of Clinical Neuroscience*, *11*(2), 166–167. https://doi.org/10.1016/j.jocn.2003.07.003

Piazza, F. V., Pinto, G. V., Trott, G., Marcuzzo, S., Gomez, R., & Fernandes, M. da C. (2011). Enriched environment prevents memory deficits in type 1 diabetic rats. *Behavioural Brain Research*, *217*(1), 16–20. https://doi.org/10.1016/j.bbr.2010.09.017

Pirot, P., Cardozo, A., & Eizirik, D. (2008). Mediators and Mechanisms of Pancreatic Beta-cell

Death in Type 1 Diabetes. *Arquivos Brasileiros de Endocrinologia e Metabologia*, *52*(1), 156–165.

Pugazhenthi, S., Qin, L., & Reddy, P. H. (2016). Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. https://doi.org/10.1016/j.bbadis.2016.04.017

Radenković, M., Stojanović, M., & Prostran, M. (2016). Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. *Journal of Pharmacological and Toxicological Methods*, *78*, 13–31. https://doi.org/10.1016/j.vascn.2015.11.004

Reagan, L. P. (2012). DIABETES AS A CHRONIC METABOLIC STRESSOR: CAUSES, CONSEQUENCES AND CLINICAL COMPLICATIONS. *Exp Neurol*, *233*(1), 68–78. https://doi.org/10.1016/j.expneurol.2011.02.004.DIABETES

Reagan, L. P., Grillo, C. A., & Piroli, G. G. (2008). The As and Ds of stress : Metabolic , morphological and behavioral consequences. *European Journal of Pharmacology*, *585*, 64–75. https://doi.org/10.1016/j.ejphar.2008.02.050

Rechenberg, K., Whittemore, R., & Grey, M. (2017). Anxiety in Youth With Type 1 Diabetes. *Journal of Pediatric Nursing*. https://doi.org/10.1016/j.pedn.2016.08.007

Redondo, M. J., Steck, A. K., & Pugliese, A. (2018). Genetics of type 1 diabetes. *Pediatric Diabetes*, *19*(3), 346–353. https://doi.org/10.1111/pedi.12597

Revsin, Y., Rekers, N. V., Louwe, M. C., Saravia, F. E., De Nicola, A. F., De Kloet, E. R., & Oitzl, M. S. (2009). Glucocorticoid receptor blockade normalizes hippocampal alterations and cognitive impairment in streptozotocin-induced type 1 diabetes mice. *Neuropsychopharmacology*, *34*(3), 747–758. https://doi.org/10.1038/npp.2008.136

Rhea, E. M., Salameh, T. S., & Banks, W. A. (2019). Routes for the delivery of insulin to the central nervous system : A comparative review. *Experimental Neurology*, *313*(October 2018), 10–15. https://doi.org/10.1016/j.expneurol.2018.11.007

Roglic, G., & World Health Organization. (n.d.). *Global report on diabetes*.

Rooijackers, H. M. M., Wiegers, E. C., Tack, C. J., Van Der Graaf, M., & De Galan, B. E. (2016, February 1). Brain glucose metabolism during hypoglycemia in type 1 diabetes: Insights from functional and metabolic neuroimaging studies. *Cellular and Molecular Life Sciences*. Birkhauser Verlag AG. https://doi.org/10.1007/s00018-015-2079-8

Roozendaal, B., Griffith, Q. K., Buranday, J., De Quervain, D. J. F., & McGaugh, J. L. (2003). The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: Dependence on the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0337480100

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2651

Roozendaal, B., McReynolds, J. R., & McGaugh, J. L. (2004). The Basolateral Amygdala Interacts with the Medial Prefrontal Cortex in Regulating Glucocorticoid Effects on Working Memory Impairment. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.4664-03.2004

Sang, W. S., Fan, Y., Hong, S. M., Liu, Z., Matsumori, Y., Weinstein, P. R., … Liu, J. (2005). Hypoglycemia induces transient neurogenesis and subsequent progenitor cell loss in the rat hippocampus. *Diabetes*. https://doi.org/10.2337/diabetes.54.2.500

SBD. (2020). *Sociedade Brasileira de Diabetes*. *Diabetes Mellitus Tipo 1 e Tipo2* (Vol. 5).

Schnedl, W. J., Ferber, S., Johnson, J. H., & Newgard, C. B. (1994). STZ transport and cytotoxicity: Specific enhancement in GLUT2-expressing cells. *Diabetes*, *43*(11), 1326– 1333. https://doi.org/10.2337/diabetes.43.11.1326

Schoenle, E. J., Schoenle, D., Molinari, L., & Largo, R. H. (2002). Impaired intellectual development in children with type I diabetes: Association with hba1c, age at diagnosis and sex. *Diabetologia*, *45*(1), 108–114. https://doi.org/10.1007/s125-002-8250-6

Silvers, J. M., Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Journal of Neuroscience Methods*. https://doi.org/10.1016/j.jneumeth.2007.06.032

Sima, A. A. F. (2010, December). Encephalopathies: The emerging diabetic complications. *Acta Diabetologica*. https://doi.org/10.1007/s00592-010-0218-0

Simpson, I. A., Appel, N. M., Hokari, M., Oki, J., Holman, G. D., Maher, F., … Smith, Q. R. (1999). Blood-brain barrier glucose transporter: Effects of hypo- and hyperglycemia revisited. *Journal of Neurochemistry*, *72*(1), 238–247. https://doi.org/10.1046/j.1471-4159.1999.0720238.x

Sims-Robinson, C., Kim, B., Rosko, A., & Feldman, E. L. (2010, October). How does diabetes accelerate Alzheimer disease pathology? *Nature Reviews Neurology*. https://doi.org/10.1038/nrneurol.2010.130

Siniscalchi, M. J., Phoumthipphavong, V., Ali, F., Lozano, M., & Kwan, A. C. (2016). Fast and slow transitions in frontal ensemble activity during flexible sensorimotor behavior. *Nature Neuroscience*, *19*(9), 1234–1242. https://doi.org/10.1038/nn.4342

Sonneville, R., Den Hertog, H. M., Güiza, F., Gunst, J., Derese, I., Wouters, P. J., … Vanhorebeek, I. (2012). Impact of hyperglycemia on neuropathological alterations during critical illness. *Journal of Clinical Endocrinology and Metabolism*, *97*(6), 2113–2123. https://doi.org/10.1210/jc.2011-2971

Strange, B. A., Witter, M. P., Lein, E. S., & Moser, E. I. (2014). Functional organization of the hippocampal longitudinal axis. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn3785

Su, J., Huang, Q., Ren, S., Xie, F., Zhai, Y., Guan, Y., … Hua, F. (2019). Altered Brain Glucose Metabolism Assessed by 18F-FDG PET Imaging Is Associated with the Cognitive Impairment of CADASIL. *Neuroscience*.

https://doi.org/10.1016/j.neuroscience.2019.07.048

Sul, J. H., Jo, S., Lee, D., & Jung, M. W. (2011). Role of rodent secondary motor cortex in value-based action selection. *Nature Neuroscience*, *14*(9), 1202–1210. https://doi.org/10.1038/nn.2881

Teka, W. W., Hamade, K. C., Barnett, W. H., Kim, T., Markin, S. N., Rybak, I. A., & Molkov, Y. I. (2017). *From the motor cortex to the movement and back again*. *PLoS ONE* (Vol. 12). https://doi.org/10.1371/journal.pone.0179288

Tesfaye, S., Boulton, A. J. M., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., … Jones, T. (2010). Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*, *33*(10), 2285–2293. https://doi.org/10.2337/dc10-1303

Tomasi, D., Wang, G. J., & Volkow, N. D. (2013). Energetic cost of brain functional connectivity. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1303346110

Tomlinson, D. R., & Gardiner, N. J. (2008). Glucose neurotoxicity. *Nature Reviews. Neuroscience*, *9*, 36–45. https://doi.org/10.1038/nrn2294

Van Duinkerken, E., Klein, M., Schoonenboom, N. S. M., Hoogma, R. P. L. M., Moll, A. C., Snoek, F. J., … Diamant, M. (2009). Functional brain connectivity and neurocognitive functioning in patients with long-standing type 1 diabetes with and without microvascular complications: A magnetoencephalography study. *Diabetes*, *58*(10), 2335–2343. https://doi.org/10.2337/db09-0425

Van Elderen, S. G. C., De Roos, A., De Craen, A. J. M., Westendorp, R. G. J., Blauw, G. J., Jukema, J. W., … Van Der Grond, J. (2010). Progression of brain atrophy and cognitive decline in diabetes mellitus: A 3-year follow-up. *Neurology*. https://doi.org/10.1212/WNL.0b013e3181f25f06

Venturini, C. D., Merlo, S., Souto, A. A., Fernandes, M. D. C., Gomez, R., & Rhoden, C. R. (2010a). Resveratrol and red wine function as antioxidants in the central nervous system without cellular proliferative effects during experimental diabetes. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.4161/oxim.3.6.14741

Venturini, C. D., Merlo, S., Souto, A. A., Fernandes, M. D. C., Gomez, R., & Rhoden, C. R. (2010b). Resveratrol and red wine function as antioxidants in the central nervous system without cellular proliferative effects during experimental diabetes. *Oxidative Medicine and Cellular Longevity*, *3*(6). https://doi.org/10.4161/oxim.3.6.14741

Wrighten, S. A., Piroli, G. G., Grillo, C. A., & Reagan, L. P. (2009a). A look inside the diabetic brain: Contributors to diabetes- induced brain aging. *Biochim Biophys Acta*, *1792*(5), 444– 453. https://doi.org/10.1016/j.bbadis.2008.10.013.A

Wrighten, S. A., Piroli, G. G., Grillo, C. A., & Reagan, L. P. (2009b). A look inside the diabetic brain: Contributors to diabetes-induced brain aging. *Biochimica et Biophysica Acta*, *1792*(5), 444–453. https://doi.org/10.1016/j.bbadis.2008.10.013

Yamada, K. A., Rensing, N., Izumi, Y., De Erausquin, G. A., Gazit, V., Dorsey, D. A., & Herrera, D. G. (2004). Repetitive Hypoglycemia in Young Rats Impairs Hippocampal Long-Term Potentiation. *Pediatric Research*. https://doi.org/10.1203/01.PDR.0000110523.07240.C1

Ye, B. S., Lee, S., Yoo, H., Chung, S. J., Lee, Y. H., Choi, Y., … Yun, M. (2019). Distinguishing between dementia with Lewy bodies and Alzheimer's disease using metabolic patterns. *Neurobiology of Aging*. https://doi.org/10.1016/j.neurobiolaging.2019.10.020

Zhou, Y., Li, X., Xie, H., Cao, D., Nie, B., & Zhao, Y. (2018). Neuroscience Letters Voxel-based morphology analysis of STZ-induced type 1 diabetes mellitus rats with and without cognitive impairment. *Neuroscience Letters*, *684*(July), 210–217. https://doi.org/ 10.1016/j.neulet.2018.08.017

5. CONSIDERAÇÕES

FINAIS 5.1 CONCLUSÕES

Os principais resultados obtidos neste estudo foram:

1- A indução diabética foi confirmada no grupo DM pela presença de sinais característicos como polidipsia, poliúria e polifagia, observados 1 semana após a indução.

2- A indução da diabetes mellitus com o uso de estreptozotocina, em ratos Wistar, diminuiu o peso corpóreo dos animais e aumentou a glicemia, enquanto os animais controles aumentaram de peso.

3- A ingesta de água foi maior no grupo diabético bem como as excretas urinárias e fecais em relação aos controles

4- Os grupos diferiram ao longo do tempo na análise do OF.

5- No OF, a distância percorrida foi diminuída para os grupos DM e controle após a indução.

6- No OF, o tempo gasto imóvel, episódios imóveis e criação, também diminuiu após a indução do DM nos dois grupos.

7- No NOR, os animais diabéticos diminuíram o número de investigações nos objetos.

8- No NOR, os animais diabéticos e controles diminuíram o índice de discriminação mas somente os diabéticos reduziram o índice de preferência.

9- O metabolismo de glicose encefálica não apresentou alterações quando analisados globalmente no córtex frontal e motor, bem como hipocampo.

10- A região do hipocampo no hipocampo ântero-dorsal, observou-se efeito no tempo apenas no grupo DM na região do hipocampo dorsal

11- Os animais controles apresentam um hipometabolismo de glicose na região do cerebelo, enquanto em ratos diabéticos o metabolismo não se altera.

12- Os animais diabéticos apresentam um hipometabolismo de glicose na região da amigdala.

13- O metabolismo de glicose das regiões do córtex frontal e região do córtex motor não são afetadas na diabetes inicial.

14- Nas regiões encefálicas existe uma diferença de lateralidade e de distribuição metabólica de glicose no córtex auditivo, córtex orbitofrontal, hipocampo posterior e núcleo accumbens core entre ratos diabéticos e controles.

6. PERSPECTIVAS

De acordo com os resultados desta tese é possível perceber a sua contribuição vital em estudos futuros que envolvam a diabetes e o efeito dela nas regiões encefálicas e suas possíveis consequências. Como perspectivas futuras, vislumbramos estudos que possibilitem trazer novas informações científicas sobre o mapeamento da diabetes em estágios iniciais. Entretanto, novos projetos dererão seguir o mesmo racional com a utilização de critérios específicos como a utilização de ratos Wistar, induzidos por STZ, com 3 meses de idade, para corroborar com esses conhecimentos compartilhados nesta tese. Os autores deverão projetar um desenho experimental que mantenha os animais diabéticos durante um período médio de 30 dias e de 90 dias para observação das alterações neurometabólitas, histológicas e comportamentais. Com isso, propõem-se a utilização das seguintes técnicas:

1. Avaliação das alterações comportamentais/locomotoras com o teste do campo aberto e o teste de reconhecimento do novo objeto.
2. Avaliação do metabolismo encefálico com uso da técnica de microPET-CT, com 18F-FDG como marcador metabólico associada a técnica de imunoistoquímica para detecção de citocromo c oxidase

a) Avaliação da resposta imune via microglia com a detecção de isoquinolina marcada com carbono 11, a 11C-(R)-PK11195 analisada por microPET-CT.
b) Avaliação das alterações na morfologia e polaridade astrocitária, com o uso da técnica imunoistoquímica para proteína glial fibrilar ácida (GFAP) analisada por morfometria planar, densitometria óptica regional, densitometria óptica celular e círculos concêntricos de Sholl.
c) Estimativa do volume das estruturas encefálicas, com o método de Cavalieri, e a densidade neuronal e glial nestas diferentes regiões utilizando a técnica de Nissl associada ao dissector óptico e ao fracionador óptico
d) Analisar a imunorreatividade para tirosina hidroxilase, por morfometria planar, densitometria óptica regional e densitometria óptica celular no estriado, na substância nigra pars compacta, no campo retrorubral e na área tegmental ventral.
e) Correlacionar os achados neurometabólicos, neuroimunes e histofisiológicos com as alterações comportamentais detectadas nos diferentes grupos experimentais.

REFERÊNCIAS

Aguilar, M., Bhuket, T., Torres, S., Liu, B., & Wong, R. J. (2015). Prevalence of the metabolic syndrome in the United States, 2003-2012. *Jama*, *313*(19), 1973–1974. https://doi.org/10.1001/jama.2015.4260

Ahlqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., … Groop, L. (2018). Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *The Lancet Diabetes and Endocrinology*, *6*(5), 361–369. https://doi.org/10.1016/S2213-8587(18)30051-2

Ahtiluoto, S., Polvikoski, T., Peltonen, M., Solomon, A., Tuomilehto, J., Winblad, B., … Kivipelto, M. (2010). Diabetes, Alzheimer disease, and vascular dementia: A population-based neuropathologic study. *Neurology*. https://doi.org/10.1212/WNL.0b013e3181f4d7f8

Akbarzadeh, A., Norouzian, D., Mehrabi, M., Jamshidi, S., Farhangi, A., Allah Verdi, A., … Akbarzadeh, A. (2007). *INDUCTION OF DIABETES BY STREPTOZOTOCIN IN RATS*. *Indian Journal of Clinical Biochemistry* (Vol. 22).

Allali, G., Blumen, H. M., Devanne, H., Pirondini, E., Delval, A., & Van De Ville, D. (2018). Brain imaging of locomotion in neurological conditions. *Neurophysiologie Clinique*. https://doi.org/10.1016/j.neucli.2018.10.004

American Diabetes Association. (2012). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *35 Suppl 1*, S64-71. https://doi.org/10.2337/dc12-s064

American Diabetes Association. (2016). Classification and diagnosis of diabetes.

Diabetes Care, *39*, S13–S22.

Andersen, H. (2012). Motor dysfunction in diabetes. *Diabetes Metab Res Rev*, *28*(October 2011), 89–92. https://doi.org/10.1002/dmrr

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing*. https://doi.org/10.1007/s10339-011-0430-z

Ates, O., Cayli, S. R., Yucel, N., Altinoz, E., Kocak, A., Durak, M. A., … Yologlu, S. (2007). Central nervous system protection by resveratrol in streptozotocin-induced diabetic rats. *Journal of Clinical Neuroscience*. https://doi.org/10.1016/j.jocn.2005.12.010

Atilgan, H., & Kwan, A. C. (2018). Same lesson, varied choices by frontal cortex. *Nature Neuroscience*, *21*(12), 1648–1650. https://doi.org/10.1038/s41593-018-0282-2

Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. *The Lancet*, *383*(9911), 69–82. https://doi.org/10.1016/S0140-6736(13)60591-7

Bach, J. F., & Chatenoud, L. (2012). The hygiene hypothesis: An explanation for the increased frequency of insulin-dependent diabetes. *Cold Spring Harbor Perspectives in Medicine*, *2*(2), 1–10. https://doi.org/10.1101/cshperspect.a007799

Bagatini, P. B, Xavier, L. L., Bertoldi, K., Moysés, F., Lovatel, G., Tartari, L., … Rodrigues, I. (2017). An evaluation of aversive memory and hippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol. *Neuroscience Letters*, *636*, 184–189. https://doi.org/10.1016/j.neulet.2016.10.059

Bagatini, Pamela Brambilla, Xavier, L. L., Bertoldi, K., Moysés, F., Lovatel, G., Neves, L. T., … Achaval, M. (2017). An evaluation of aversive memory and hippocampal

oxidative status in streptozotocin-induced diabetic rats treated with resveratrol. *Neuroscience Letters*, *636*, 184–189. https://doi.org/10.1016/j.neulet.2016.10.059

Bagatini, Pamela Brambilla, Xavier, L. L., Neves, L. T., Saur, L., Barbosa, S., Baptista, P. P. A., … Achaval, M. (2014). Resveratrol prevents akinesia and restores neuronal tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta of diabetic rats. *Brain Research*, *1592*, 101–112. https://doi.org/10.1016/j.brainres.2014.10.007

Banks, W. A., Owen, J. B., & Erickson, M. A. (2012). Insulin in the brain: There and back again. *Pharmacology and Therapeutics*, *136*(1), 82–93. https://doi.org/10.1016/j.pharmthera.2012.07.006

Bansal, R., AHMAD, N., & KIDWAI, J. R. (2017). 済無 No Title No Title. *Journal of Chemical Information and Modeling*, *8*(9), 1–58. https://doi.org/10.1017/CBO9781107415324.004

Baptista, P. P. A., Saur, L., Bambrilla, P., Greggio, S., Teribele, G., Sabrina, V., … Moriguchi, M. (2015). Antidepressant Effects of Ketamine Are Not Related to 18 F-FDG Metabolism or Tyrosine Hydroxylase Immunoreactivity in the Ventral Tegmental Area of Wistar Rats. *Neurochemical Research*, *40*, 1153–1164. https://doi.org/10.1007/s11064-015-1576-3

Barulli, D., & Stern, Y. (2013). Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts. *Trends in Cognitive Sciences*, *17*(10), 502–509. https://doi.org/10.1016/j.tics.2013.08.012

Bednarik, P., Moheet, A. A., Grohn, H., Kumar, A. F., Eberly, L. E., Seaquist, E. R., & Mangia, S. (2017a). Type 1 diabetes and impaired awareness of hypoglycemia are associated with reduced brain gray matter volumes. *Frontiers in Neuroscience*.

https://doi.org/10.3389/fnins.2017.00529

Bednarik, P., Moheet, A. A., Grohn, H., Kumar, A. F., Eberly, L. E., Seaquist, E. R., & Mangia, S. (2017b). Type 1 diabetes and impaired awareness of hypoglycemia are associated with reduced brain gray matter volumes. *Frontiers in Neuroscience*, *11*(SEP). https://doi.org/10.3389/fnins.2017.00529

Biala, M. A. G. (2012). The novel object recognition memory : neurobiology , test procedure , and its modifications. *Cogn Process*, *13*, 93–110. https://doi.org/ 10.1007/s10339-011-0430-z

Biessels, G., Strachan, M., Visseren, F., Kappelle, L., & Whitmer, R. (2014). Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *The Lancet Diabetes & Endocrinology*, *2*(3), 246–255.

Biessels, G J. (2013). Sweet memories: 20 years of progress in research on cognitive functioning in diabetes. *European Journal of Pharmacology*, *719*(1–3), 153–160. https://doi.org/10.1016/j.ejphar.2013.04.055

Biessels, Geert Jan. (2013). Sweet memories: 20 years of progress in research on cognitive functioning in diabetes. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2013.04.055

Biessels, Geert Jan, van der Heide, L. P., Kamal, A., Bleys, R. L. A. ., & Gispen, W. H. (2002). Ageing and diabetes: implications for brain function. *European Journal of Pharmacology*, *441*(1), 1–14. https://doi.org/10.1016/S0014-2999(02)01486-3

Blázquez, E., Velázquez, E., & Hurtado-carneiro, V. (2014). Insulin in the brain : its pathophysiological implications for states related with central insulin resistance , type 2 diabetes and alzheimer ' s disease. *Frontiers in Endocrinology*, *5*(October), 1–21. https://doi.org/10.3389/fendo.2014.00161

Blázquez, E., Velázquez, E., Hurtado-Carneiro, V., & Ruiz-Albusac, J. M. (2014). Insulin in the brain: Its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and alzheimer's disease. *Frontiers in Endocrinology*. Frontiers Research Foundation. https://doi.org/10.3389/fendo.2014.00161

Blum, D., la Fougère, C., Pilotto, A., Maetzler, W., Berg, D., Reimold, M., & Liepelt-Scarfone, I. (2018). Hypermetabolism in the cerebellum and brainstem and cortical hypometabolism are independently associated with cognitive impairment in Parkinson's disease. *European Journal of Nuclear Medicine and Molecular Imaging*. https://doi.org/10.1007/s00259-018-4085-1

Branchi, I., Ricceri, L., Cellulare, B., & Superiore, I. (2004). Refining learning and memory assessment in laboratory rodents . An ethological perspective. *Ann Ist Super Sanità 2004;40(2):231-236*, *40*(2), 231–236.

Bruehl, H., Sweat, V., Tirsi, A., Shah, B., & Convit, A. (2011). Obese Adolescents with Type 2 Diabetes Mellitus Have Hippocampal and Frontal Lobe Volume Reductions. *Neuroscience and Medicine*, *2*(1), 34–42. https://doi.org/10.4236/nm.2011.21005.Obese

Buchberger, B., Huppertz, H., Krabbe, L., Lux, B., Mattivi, J. T., & Siafarikas, A. (2016). Symptoms of depression and anxiety in youth with type 1 diabetes: A systematic review and meta-analysis. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2016.04.019

Cabrera, S. M., Henschel, A. M., & Hessner, M. J. (2016). Innate inflammation in type 1 diabetes. *Translational Research*, *167*(1), 214–227. https://doi.org/10.1016/j.trsl.2015.04.011

Callaghan, B. C., Cheng, H. T., Stables, C. L., Smith, A. L., & Feldman, E. L. (2012). Diabetic neuropathy : clinical manifestations and current treatments. *The Lancet Neurology*, *11*(6), 521–534. https://doi.org/10.1016/S1474-4422(12)70065-0

Cobas, R. A., Bosi Ferraz, M., de Mattos Matheus, A. S., Monteiro Tannus, L. R., Antonio Negrato, C., de Araujo, L. A., … Brito Gomes, M. (2013). The cost of type 1 diabetes: A nationwide multicentre study in Brazil. *Bulletin of the World Health Organization*, *91*(6), 434–440. https://doi.org/10.2471/BLT.12.110387

Collin, S. H. P., Milivojevic, B., & Doeller, C. F. (2015). Memory hierarchies map onto the hippocampal long axis in humans. *Nature Neuroscience*. https://doi.org/10.1038/nn.4138

Dalton, M. A., Zeidman, P., Barry, D. N., Williams, E., & Maguire, E. A. (2017). Segmenting subregions of the human hippocampus on structural magnetic resonance image scans: An illustrated tutorial. *Brain and Neuroscience Advances*. https://doi.org/10.1177/2398212817701448

Damasceno, D. C., Netto, A. O., Iessi, I. L., Gallego, F. Q., Corvino, S. B., Dallaqua, B., … Rudge, M. V. C. (2014). Streptozotocin-induced diabetes models: Pathophysiological mechanisms and fetal outcomes. *BioMed Research International*, *2014*, 1–11. https://doi.org/10.1155/2014/819065

de Almeida-Pititto, B., Dias, M. L., de Moraes, A. C. F., Ferreira, S. R. G., Franco, D. R., & Eliaschewitz, F. G. (2015). Type 2 diabetes in Brazil: Epidemiology and management. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *8*, 17–28. https://doi.org/10.2147/DMSO.S72542

De Beeck, A. O., & Eizirik, D. L. (2016). Viral infections in type 1 diabetes mellitus-why the β cells? *Nature Reviews Endocrinology*, *12*(5), 263–273.

https://doi.org/10.1038/nrendo.2016.30

de Senna, P. N., Bagatini, P. B., Galland, F., Bobermin, L., do Nascimento, P. S., Nardin, P., … Xavier, L. L. (2017). Physical exercise reverses spatial memory deficit and induces hippocampal astrocyte plasticity in diabetic rats. *Brain Research*. https://doi.org/10.1016/j.brainres.2016.10.024

De Senna, P. N., Ilha, J., Baptista, P. P. A., Do Nascimento, P. S., Leite, M. C., Paim, M. F., … Xavier, L. L. (2011). Effects of physical exercise on spatial memory and astroglial alterations in the hippocampus of diabetic rats. *Metabolic Brain Disease*, *26*(4), 269–279. https://doi.org/10.1007/s11011-011-9262-x

De Senna, P. N., Xavier, L. L., Bagatini, P. B., Saur, L., Galland, F., Zanotto, C., … Achaval, M. (2015). Physical training improves non-spatial memory, locomotor skills and the blood brain barrier in diabetic rats. *Brain Research*, *1618*, 75–82. https://doi.org/10.1016/j.brainres.2015.05.026

Do Nascimento, P. S., Lovatel, G. A., Barbosa, S., Ilha, J., Centenaro, L. A., Malysz, T., … Achaval, M. (2011). Treadmill training improves motor skills and increases tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta in diabetic rats. *Brain Research*, *1382*, 173–180. https://doi.org/10.1016/j.brainres.2011.01.063

Dufrane, D., Van Steenberghe, M., Guiot, Y., Goebbels, R. M., Saliez, A., & Gianello, P. (2006). Streptozotocin-induced diabetes in large animals (pigs/primates): Role of GLUT2 transporter and β-cell plasticity. *Transplantation*, *81*(1), 36–45. https://doi.org/10.1097/01.tp.0000189712.74495.82

Egede, L. E., Bishu, K. G., Walker, R. J., & Dismuke, C. E. (2016). Impact of diagnosed depression on healthcare costs in adults with and without diabetes:

United States, 2004-2011. *Journal of Affective Disorders*, *195*, 119–126. https://doi.org/10.1016/j.jad.2016.02.011

Elsner, M., Guldbakke, B., Tiedge, M., Munday, R., & Lenzen, S. (2000). Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. *Diabetologia*, *43*(12), 1528–1533. https://doi.org/10.1007/s001250051564

Fanselow, M. S., & Dong, H. W. (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron*. https://doi.org/10.1016/j.neuron.2009.11.031

Fava, A. A. S. (2013). Association of incidence of type 1 diabetes with mortality from infectious disease and with antibiotic susceptibility at a country level, 859–865. https://doi.org/10.1007/s00592-013-0464-z

Funk, J. (2007). Distúrbios do pâncreas endócrino. In S. McPhee & W. Ganong (Eds.), *Fisiopatologia da doença: uma introdução à medicina clínica* (5th ed., p. 642). São Paulo: McGraw-Hill Interamericana do Brasil.

Gaspar, J., Baptista, F., Macedo, M., & Ambrósio, A. (2016). Inside the Diabetic Brain: Role of Different Players Involved in Cognitive Decline. *ACS Chemical Neuroscience*, *7*, 131–142. https://doi.org/10.1021/acschemneuro.5b00240

GBD. (2015). Global , regional , and national comparative risk assessment of 79 behavioural , environmental and occupational , and metabolic risks or clusters of risks in 188 countries , 1990 – 2013 : a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *386*, 1990–2013. https://doi.org/10.1016/S0140-6736(15)00128-2

Ghasemi, R., Haeri, A., Dargahi, L., Mohamed, Z., & Ahmadiani, A. (2013). Insulin in

the brain: Sources, localization and functions. *Molecular Neurobiology*. https://doi.org/10.1007/s12035-012-8339-9

Gispen, W. H., & Biessels, G.-J. (2000). Cognition and synaptic plasticity in diabetes mellitus. *Trends in Neurosciences*, *23*(11), 542–549. https://doi.org/10.1016/S0166-2236(00)01656-8

Guan, Z. F., Zhou, X. L., Zhang, X. M., Zhang, Y., Wang, Y. M., Guo, Q. L., … Liu, Y. C. (2016). Beclin-1- mediated autophagy may be involved in the elderly cognitive and affective disorders in streptozotocin-induced diabetic mice. *Translational Neurodegeneration*, *5*(1), 1–10. https://doi.org/10.1186/s40035-016-0070-4

Haider, S., Ahmed, S., & Tabassum, S. (2013). Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. *Acta Neurol Belg*, *113*, 35–41. https://doi.org/10.1007/s13760-012-0121-2

Haider, S., Ahmed, S., Tabassum, S., Memon, Z., Ikram, M., & Haleem, D. J. (2013). Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. *Acta Neurologica Belgica*, *113*(1), 35–41. https://doi.org/10.1007/s13760-012-0121-2

Hamacher, K., Coenen, H. H., & Stocklin, G. (1986). Efficient Stereospecific Synthesis of No-Carrier-Added2@[18F]-Fluoro-2-Deoxy D-Glucose Using Aminopolyether Supported Nucleophilic Substitution. *J Nucl Med*, *27*(February), 235–239.

Hawkins, B. T., Lundeen, T. F., Norwood, K. M., Brooks, H. L., & Egleton, R. D. (2007). Increased blood-brain barrier permeability and altered tight junctions in experimental diabetes in the rat: Contribution of hyperglycaemia and matrix metalloproteinases. *Diabetologia*, *50*(1), 202–211. https://doi.org/10.1007/s00125-006-0485-z

Hernández-Fonseca, J. P., Rincón, J., Pedreañez, A., Viera, N., Arcaya, J. L., Carrizo, E., & Mosquera, J. (2009). Structural and ultrastructural analysis of cerebral cortex, cerebellum, and hypothalamus from diabetic rats. *Experimental Diabetes Research*, *2009*, 329632. https://doi.org/10.1155/2009/329632

Ho, M. S., Weller, N. J., Ives, F. J., Carne, C. L., Murray, K., vanden Driesen, R. I., … Jones, T. W. (2008). Prevalence of Structural Central Nervous System Abnormalities in Early-Onset Type 1 Diabetes Mellitus. *Journal of Pediatrics*. https://doi.org/10.1016/j.jpeds.2008.03.005

Huang, M., Gao, L., Yang, L., Lin, F., & Lei, H. (2012). Abnormalities in the brain of streptozotocin-induced type 1 diabetic rats revealed by diffusion tensor imaging. *NeuroImage: Clinical*, *1*(1), 57–65. https://doi.org/10.1016/j.nicl.2012.09.004

Huber, J. D., VanGilder, R. L., & Houser, K. A. (2006). Streptozotocin-induced diabetes progressively increases blood-brain barrier permeability in specific brain regions in rats. *American Journal of Physiology - Heart and Circulatory Physiology*, *291*(6), 2660–2668. https://doi.org/10.1152/ajpheart.00489.2006

IDF. (2019). Idf diabetes atlas 9, Prevalence of Diabetes.

Jakobsen, J., Sidenius, P., Gundersen, H., & Østerby, R. (1987). Quantitative Changes of Cerebral Neocortical Structure in Insulin-Treated Long-Term Streptozocin-lnduced Diabetes in Rats. *Diabetes*, *36*(5), 597–601.

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature*. https://doi.org/10.1038/nature14188

Jing, Y.-H., Chen, K.-H., Kuo, P.-C., Pao, C.-C., & Chen, J.-K. (2013). Neurodegeneration in streptozotocin-induced diabetic rats is attenuated by treatment with resveratrol. *Neuroendocrinology*, *98*(2), 116–127.

https://doi.org/10.1159/000350435

Kalyani, R. R., Corriere, M., & Ferrucci, L. (2014). Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol*, *2*(10), 819–829. https://doi.org/10.1016/S2213-8587(14)70034-8.Age-related

Kang, L., Routh, V. H., Kuzhikandathil, E. V, Gaspers, L. D., & Levin, B. E. (2004). Physiological and Molecular Characteristics of Rat Hypothalamic Ventromedial Nucleus Glucosensing Neurons To evaluate potential mechanisms for neuronal glu-cosensing, fura-2 Ca 2 imaging and single-cell RT-PCR were carried out in dissociated ventromedial , (10), 549–559.

Koekkoek, P. S., Kappelle, L. J., Berg, E. Van Den, Rutten, G. E. H. M., & Biessels, G. J. (2015). Cognitive function in patients with diabetes mellitus : guidance for daily care. *The Lancet Neurology*, *14*(3), 329–340. https://doi.org/10.1016/S1474-4422(14)70249-2

Kopschina Feltes, P., de Vries, E. F. J., Juarez-Orozco, L. E., Kurtys, E., Dierckx, R. A. J. O., Moriguchi-Jeckel, C. M., & Doorduin, J. (2019). Repeated social defeat induces transient glial activation and brain hypometabolism: A positron emission tomography imaging study. *Journal of Cerebral Blood Flow and Metabolism*, *39*(3), 439–453. https://doi.org/10.1177/0271678X17747189

Koziol, L. F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., … Yamazaki, T. (2014). Consensus paper: The cerebellum's role in movement and cognition. *Cerebellum*. https://doi.org/10.1007/s12311-013-0511-x

Kumagai, A. K. (1999). Glucose transport in brain and retina: Implications in the management and complications of diabetes. *Diabetes/Metabolism Research and*

Reviews, *15*(4), 261–273. https://doi.org/10.1002/(SICI)1520-7560(199907/08)15:4<261::AID-DMRR43>3.0.CO;2-Z

Lancelot, S., & Zimmer, L. (2010). Small-animal positron emission tomography as a tool for neuropharmacology. *Trends in Pharmacological Sciences*, *31*(9), 411–417. https://doi.org/10.1016/j.tips.2010.06.002

Ledoux, S. P., & Wilson, G. L. (1984). Effects of streptozotocin on a clonal isolate of rat insulinoma cells. *BBA - Molecular Cell Research*, *804*(4), 387–392. https://doi.org/10.1016/0167-4889(84)90064-8

Levin, B. E., Routh, V. H., Kang, L., Sanders, N. M., & Dunn-Meynell, A. A. (2004). Perspectives in Diabetes Neuronal Glucosensing What Do We Know After 50 Years? *Diabetes*, *53*(8), 2521–2528. Retrieved from http://diabetes.diabetesjournals.org/content/diabetes/53/10/2521.full.pdf

Malone, J. I., Hanna, S. K., & Saporta, S. (2006). Hyperglycemic brain injury in the rat. *Brain Research*, *1076*(1), 9–15. https://doi.org/10.1016/j.brainres.2005.12.072

Martínez-Tellez, R., Gómez-Villalobos, M. D. J., & Flores, G. (2005). Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. *Brain Research*, *1048*(1–2), 108–115. https://doi.org/10.1016/j.brainres.2005.04.048

Mauras, N., Mazaika, P., Buckingham, B., Weinzimer, S., White, N. H., Tsalikian, E., … Reiss, A. L. (2015). Longitudinal assessment of neuroanatomical and cognitive differences in young children with type 1 diabetes: Association with hyperglycemia. *Diabetes*, *64*(5), 1770–1779. https://doi.org/10.2337/db14-1445

McCrimmon, R. J., Ryan, C. M., & Frier, B. M. (2012). Diabetes and cognitive dysfunction. *The Lancet*, *379*(9833), 2291–2299. https://doi.org/10.1016/S0140-

6736(12)60360-2

Mergenthaler, P., Lindauer, U., Dienel, G., & Meisel, A. (2013). Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends in Neuroscience*, *36*(10), 587–597. https://doi.org/10.1016/j.tins.2013.07.001.Sugar

Mirrione, M. M., Schulz, D., Lapidus, K. A. B., Zhang, S., Goodman, W., & Henn, F. A. (2014). Increased metabolic activity in the septum and habenula during stress is linked to subsequent expression of learned helplessness behavior. *Frontiers in Human Neuroscience*, *8*(1 FEB). https://doi.org/10.3389/fnhum.2014.00029

Moheet, A., Mangia, S., & Seaquist, E. R. (2015). Impact of diabetes on cognitive function and brain structure. *Annals of the New York Academy of Sciences*, *1353*, 60–71. https://doi.org/10.1111/nyas.12807

Mooradian, A. D. (1997). Central nervous system complications of diabetes mellitus — a perspective from the blood–brain barrier. *Brain Research Reviews*, *23*(3), 210–218. https://doi.org/10.1016/S0165-0173(97)00003-9

Moser, M. B., & Moser, E. I. (1998). Functional differentiation in the hippocampus. *Hippocampus*. https://doi.org/10.1002/(SICI)1098-1063(1998)8:6<608::AID-HIPO3>3.0.CO;2-7

Mueller, K., Sacher, J., Arelin, K., Holiga, S., Kratzsch, J., Villringer, A., & Schroeter, M. (2012). Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI , serum marker and gene expression study. *Translational Psychiatry*, (October), 1–7. https://doi.org/10.1038/tp.2012.121

Muramatsu, K., Ikutomo, M., Tamaki, T., Shimo, S., & Niwa, M. (2018). Effect of streptozotocin-induced diabetes on motor representations in the motor cortex and

corticospinal tract in rats. *Brain Research*, *1680*, 115–126. https://doi.org/10.1016/j.brainres.2017.12.016

Musen, G., In, K. L., Sparks, C. R., Weinger, K., Hwang, J., Ryan, C. M., … Jacobson, A. M. (2006). Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes*, *55*(2), 326–333. https://doi.org/10.2337/diabetes.55.02.06.db05-0520

Naicker, K., Johnson, J. A., Skogen, J. C., Manuel, D., Øverland, S., Sivertsen, B., & Colman, I. (2017). Type 2 diabetes and comorbid symptoms of depression and anxiety: Longitudinal associations with mortality risk. *Diabetes Care*. https://doi.org/10.2337/dc16-2018

Naicker, K., Øverland, S., Johnson, J. A., Manuel, D., Skogen, J. C., Sivertsen, B., & Colman, I. (2017). Symptoms of anxiety and depression in type 2 diabetes: Associations with clinical diabetes measures and self-management outcomes in the Norwegian HUNT study. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2017.07.002

NCD-RisC. (2016). Worldwide trends in diabetes since 1980 : a pooled analysis of 751 population-based studies with 4 · 4 million participants. *The Lancet*, *6736*(16), 1–18. https://doi.org/10.1016/S0140-6736(16)00618-8

Noble, J. A., Valdes, A. M., Varney, M. D., Carlson, J. A., Moonsamy, P., Fear, A. L., … Erlich, H. A. (2010). HLA class I and genetic susceptibility to type 1 diabetes: Results from the type 1 diabetes genetics consortium. *Diabetes*, *59*(11), 2972–2979. https://doi.org/10.2337/db10-0699

Northam, E. A., Rankins, D., Lin, A., Wellard, R. M., Pell, G. S., Finch, S. J., … Cameron, F. J. (2009). Central nervous system function in youth with type 1

diabetes 12 years after disease onset. *Diabetes Care*, *32*(3), 445–450. https://doi.org/10.2337/dc08-1657

Northman E., Anderson P, Rani J, Hughes M, W. G. et al. (2001). Neuropsychological Profiles of Children Disease Onset. *Diabetes Care*, *24*(9), 1541–1546.

Nunes, P., Senna, D., Brambilla, P., Galland, F., Bobermin, L., Severo, P., … Leal, L. (2017). Physical exercise reverses spatial memory de fi cit and induces hippocampal astrocyte plasticity in diabetic rats. *Brain Research*, *1655*(July 2016), 242–251. https://doi.org/10.1016/j.brainres.2016.10.024

Okamoto, M. M., Anhê, G. F., Sabino-Silva, R., Marques, M. F. D. S. F., Freitas, H. S., Mori, R. C. T., … Machado, U. F. (2011). Intensive insulin treatment induces insulin resistance in diabetic rats by impairing glucose metabolism-related mechanisms in muscle and liver. *Journal of Endocrinology*, *211*(1), 55–64. https://doi.org/10.1530/JOE-11-0105

Özdemir, N. G., Akbaş, F., Kotil, T., & Yilmaz, A. (2016). Analysis of diabetes-related cerebellar changes in streptozotocin-induced diabetic rats. *Turkish Journal of Medical Sciences*, *46*(5), 1579–1592. https://doi.org/10.3906/sag-1412-125

Parashar, A., Mehta, V., & Malairaman, U. (2018). Type 2 Diabetes Mellitus Is Associated with Social Recognition Memory Deficit and Altered Dopaminergic Neurotransmission in the Amygdala. *Annals of Neurosciences*, *24*(4), 212–220. https://doi.org/10.1159/000479637

Perantie, D. C., Koller, J. M., Weaver, P. M., Lugar, H. M., Black, K. J., White, N. H., & Hershey, T. (2011). Prospectively determined impact of type 1 diabetes on brain volume during development. *Diabetes*. https://doi.org/10.2337/db11-0589

Petty, S., & Mitchell, P. (2004). Images in neuroscience. *Journal of Clinical*

Neuroscience, *11*(2), 166–167. https://doi.org/10.1016/j.jocn.2003.07.003

Piazza, F. V., Pinto, G. V., Trott, G., Marcuzzo, S., Gomez, R., & Fernandes, M. da C. (2011). Enriched environment prevents memory deficits in type 1 diabetic rats. *Behavioural Brain Research*, *217*(1), 16–20. https://doi.org/10.1016/j.bbr.2010.09.017

Pirot, P., Cardozo, A., & Eizirik, D. (2008). Mediators and Mechanisms of Pancreatic Beta-cell Death in Type 1 Diabetes. *Arquivos Brasileiros de Endocrinologia e Metabologia*, *52*(1), 156–165.

Pugazhenthi, S., Qin, L., & Reddy, P. H. (2016). Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. https://doi.org/10.1016/j.bbadis.2016.04.017

Radenković, M., Stojanović, M., & Prostran, M. (2016). Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. *Journal of Pharmacological and Toxicological Methods*, *78*, 13–31. https://doi.org/10.1016/j.vascn.2015.11.004

Reagan, L. P. (2012). DIABETES AS A CHRONIC METABOLIC STRESSOR: CAUSES, CONSEQUENCES AND CLINICAL COMPLICATIONS. *Exp Neurol*, *233*(1), 68–78. https://doi.org/10.1016/j.expneurol.2011.02.004.DIABETES

Reagan, L. P., Grillo, C. A., & Piroli, G. G. (2008). The As and Ds of stress : Metabolic , morphological and behavioral consequences. *European Journal of Pharmacology*, *585*, 64–75. https://doi.org/10.1016/j.ejphar.2008.02.050

Rechenberg, K., Whittemore, R., & Grey, M. (2017). Anxiety in Youth With Type 1 Diabetes. *Journal of Pediatric Nursing*. https://doi.org/10.1016/j.pedn.2016.08.007

Redondo, M. J., Steck, A. K., & Pugliese, A. (2018). Genetics of type 1 diabetes.

Pediatric Diabetes, *19*(3), 346–353. https://doi.org/10.1111/pedi.12597

Revsin, Y., Rekers, N. V., Louwe, M. C., Saravia, F. E., De Nicola, A. F., De Kloet, E. R., & Oitzl, M. S. (2009). Glucocorticoid receptor blockade normalizes hippocampal alterations and cognitive impairment in streptozotocin-induced type 1 diabetes mice. *Neuropsychopharmacology*, *34*(3), 747–758. https://doi.org/10.1038/npp.2008.136

Rhea, E. M., Salameh, T. S., & Banks, W. A. (2019). Routes for the delivery of insulin to the central nervous system : A comparative review. *Experimental Neurology*, *313*(October 2018), 10–15. https://doi.org/10.1016/j.expneurol.2018.11.007

Roglic, G., & World Health Organization. (n.d.). *Global report on diabetes*.

Rooijackers, H. M. M., Wiegers, E. C., Tack, C. J., Van Der Graaf, M., & De Galan, B. E. (2016, February 1). Brain glucose metabolism during hypoglycemia in type 1 diabetes: Insights from functional and metabolic neuroimaging studies. *Cellular and Molecular Life Sciences*. Birkhauser Verlag AG. https://doi.org/10.1007/s00018-015-2079-8

Roozendaal, B., Griffith, Q. K., Buranday, J., De Quervain, D. J. F., & McGaugh, J. L. (2003). The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: Dependence on the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0337480100

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2651

Roozendaal, B., McReynolds, J. R., & McGaugh, J. L. (2004). The Basolateral Amygdala Interacts with the Medial Prefrontal Cortex in Regulating

Glucocorticoid Effects on Working Memory Impairment. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.4664-03.2004

Sang, W. S., Fan, Y., Hong, S. M., Liu, Z., Matsumori, Y., Weinstein, P. R., … Liu, J. (2005). Hypoglycemia induces transient neurogenesis and subsequent progenitor cell loss in the rat hippocampus. *Diabetes*. https://doi.org/10.2337/diabetes.54.2.500

SBD. (2020). *Sociedade Brasileira de Diabetes*. *Diabetes Mellitus Tipo 1 e Tipo2* (Vol. 5).

Schnedl, W. J., Ferber, S., Johnson, J. H., & Newgard, C. B. (1994). STZ transport and cytotoxicity: Specific enhancement in GLUT2-expressing cells. *Diabetes*, *43*(11), 1326–1333. https://doi.org/10.2337/diabetes.43.11.1326

Schoenle, E. J., Schoenle, D., Molinari, L., & Largo, R. H. (2002). Impaired intellectual development in children with type I diabetes: Association with hba1c, age at diagnosis and sex. *Diabetologia*, *45*(1), 108–114. https://doi.org/10.1007/s125-002-8250-6

Silvers, J. M., Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Journal of Neuroscience Methods*. https://doi.org/10.1016/j.jneumeth.2007.06.032

Sima, A. A. F. (2010, December). Encephalopathies: The emerging diabetic complications. *Acta Diabetologica*. https://doi.org/10.1007/s00592-010-0218-0

Simpson, I. A., Appel, N. M., Hokari, M., Oki, J., Holman, G. D., Maher, F., … Smith, Q. R. (1999). Blood-brain barrier glucose transporter: Effects of hypo- and hyperglycemia revisited. *Journal of Neurochemistry*, *72*(1), 238–247. https://doi.org/10.1046/j.1471-4159.1999.0720238.x

Sims-Robinson, C., Kim, B., Rosko, A., & Feldman, E. L. (2010, October). How does diabetes accelerate Alzheimer disease pathology? *Nature Reviews Neurology*. https://doi.org/10.1038/nrneurol.2010.130

Siniscalchi, M. J., Phoumthipphavong, V., Ali, F., Lozano, M., & Kwan, A. C. (2016). Fast and slow transitions in frontal ensemble activity during flexible sensorimotor behavior. *Nature Neuroscience*, *19*(9), 1234–1242. https://doi.org/10.1038/nn.4342

Sonneville, R., Den Hertog, H. M., Güiza, F., Gunst, J., Derese, I., Wouters, P. J., … Vanhorebeek, I. (2012). Impact of hyperglycemia on neuropathological alterations during critical illness. *Journal of Clinical Endocrinology and Metabolism*, *97*(6), 2113–2123. https://doi.org/10.1210/jc.2011-2971

Strange, B. A., Witter, M. P., Lein, E. S., & Moser, E. I. (2014). Functional organization of the hippocampal longitudinal axis. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn3785

Su, J., Huang, Q., Ren, S., Xie, F., Zhai, Y., Guan, Y., … Hua, F. (2019). Altered Brain Glucose Metabolism Assessed by 18F-FDG PET Imaging Is Associated with the Cognitive Impairment of CADASIL. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2019.07.048

Sul, J. H., Jo, S., Lee, D., & Jung, M. W. (2011). Role of rodent secondary motor cortex in value-based action selection. *Nature Neuroscience*, *14*(9), 1202–1210. https://doi.org/10.1038/nn.2881

Teka, W. W., Hamade, K. C., Barnett, W. H., Kim, T., Markin, S. N., Rybak, I. A., & Molkov, Y. I. (2017). *From the motor cortex to the movement and back again*. *PLoS ONE* (Vol. 12). https://doi.org/10.1371/journal.pone.0179288

Tesfaye, S., Boulton, A. J. M., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., …

Jones, T. (2010). Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*, *33*(10), 2285–2293. https://doi.org/10.2337/dc10-1303

Tomasi, D., Wang, G. J., & Volkow, N. D. (2013). Energetic cost of brain functional connectivity. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1303346110

Tomlinson, D. R., & Gardiner, N. J. (2008). Glucose neurotoxicity. *Nature Reviews. Neuroscience*, *9*, 36–45. https://doi.org/10.1038/nrn2294

Van Duinkerken, E., Klein, M., Schoonenboom, N. S. M., Hoogma, R. P. L. M., Moll, A. C., Snoek, F. J., … Diamant, M. (2009). Functional brain connectivity and neurocognitive functioning in patients with long-standing type 1 diabetes with and without microvascular complications: A magnetoencephalography study. *Diabetes*, *58*(10), 2335–2343. https://doi.org/10.2337/db09-0425

Van Elderen, S. G. C., De Roos, A., De Craen, A. J. M., Westendorp, R. G. J., Blauw, G. J., Jukema, J. W., … Van Der Grond, J. (2010). Progression of brain atrophy and cognitive decline in diabetes mellitus: A 3-year follow-up. *Neurology*. https://doi.org/10.1212/WNL.0b013e3181f25f06

Venturini, C. D., Merlo, S., Souto, A. A., Fernandes, M. D. C., Gomez, R., & Rhoden, C. R. (2010a). Resveratrol and red wine function as antioxidants in the central nervous system without cellular proliferative effects during experimental diabetes. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.4161/oxim.3.6.14741

Venturini, C. D., Merlo, S., Souto, A. A., Fernandes, M. D. C., Gomez, R., & Rhoden, C. R. (2010b). Resveratrol and red wine function as antioxidants in the central

nervous system without cellular proliferative effects during experimental diabetes. *Oxidative Medicine and Cellular Longevity*, *3*(6). https://doi.org/10.4161/oxim.3.6.14741

Wrighten, S. A., Piroli, G. G., Grillo, C. A., & Reagan, L. P. (2009a). A look inside the diabetic brain: Contributors to diabetes- induced brain aging. *Biochim Biophys Acta*, *1792*(5), 444–453. https://doi.org/10.1016/j.bbadis.2008.10.013.A

Wrighten, S. A., Piroli, G. G., Grillo, C. A., & Reagan, L. P. (2009b). A look inside the diabetic brain: Contributors to diabetes-induced brain aging. *Biochimica et Biophysica Acta*, *1792*(5), 444–453. https://doi.org/10.1016/j.bbadis.2008.10.013

Yamada, K. A., Rensing, N., Izumi, Y., De Erausquin, G. A., Gazit, V., Dorsey, D. A., & Herrera, D. G. (2004). Repetitive Hypoglycemia in Young Rats Impairs Hippocampal Long-Term Potentiation. *Pediatric Research*. https://doi.org/10.1203/01.PDR.0000110523.07240.C1

Ye, B. S., Lee, S., Yoo, H., Chung, S. J., Lee, Y. H., Choi, Y., … Yun, M. (2019). Distinguishing between dementia with Lewy bodies and Alzheimer's disease using metabolic patterns. *Neurobiology of Aging*. https://doi.org/10.1016/j.neurobiolaging.2019.10.020

Zhou, Y., Li, X., Xie, H., Cao, D., Nie, B., & Zhao, Y. (2018). Neuroscience Letters Voxel-based morphology analysis of STZ-induced type 1 diabetes mellitus rats with and without cognitive impairment. *Neuroscience Letters*, *684*(July), 210–217. https://doi.org/10.1016/j.neulet.2018.08.017

Anexo A

Carta de Aprovação da Comissão de Ética para o Uso de Animais

CEUA/PUCRS 7660

SIPESQ

Sistema de Pesquisas da PUCRS

Código SIPESQ: 7660 Porto Alegre, 22 de novembro de 2016.

Prezado(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou o Projeto de Pesquisa "Análise comportamental, neurometabólica e histofisiológica de ratos Wistar com diabetes mellitus induzido por estreptozotocina." coordenado por LEDER LEAL XAVIER.

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está autorizada a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Nº de Animais	Espécie	Duração do Projeto
210	Ratos	22/11/2016 - 22/06/2017

Atenciosamente,

Comissão de Ética no Uso de Animais (CEUA)

Pontifícia Universidade Católica do Rio Grande do Sul
Pró-Reitoria de Graduação
Av. Ipiranga, 6681 - Prédio 1 - 3º. andar
Porto Alegre - RS - Brasil
Fone: (51) 3320-3500 - Fax: (51) 3339-1564
E-mail: prograd@pucrs.br
Site: www.pucrs.br