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ALTERAÇÕES SINÁPTICAS NA DOENÇA DE ALZHEIMER

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Orientador: Prof. Dr. Eduardo Rigon Zimmer
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Aos pacientes que sofrem da Doença de Alzheimer e a todos ao redor.

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APRESENTAÇÃO

A seguinte tese está organizada em conformidade com as normas do Programa de Pós-Graduação em Ciências Biológicas: Bioquímica da Universidade Federal do Rio Grande do Sul, artigo 31° do regimento de 2023 seguindo a opção 2:

"Parágrafo único- A forma de redação da Dissertação ou Tese poderá ser: 1) a usualmente empregada, com Abstract, Resumo, Introdução, Material e Métodos, Resultados, Discussão e Bibliografia ou **2) uma forma alternativa, composta de Abstract, Resumo, Introdução, Trabalhos publicados, aceitos para publicação e submetidos à publicação, Conclusões e Bibliografia"**.

Assim, a tese está dividida nas seguintes partes:

Parte I

Abstract/Resumo

Introdução: introdução abordando os temas importantes para o entendimento dos trabalhos de pesquisa a serem apresentados na Parte II.

Objetivos: Objetivos gerais e específicos

Parte II

Artigos, os quais estão divididos em três capítulos, sendo que cada capítulo contém um breve prefácio seguido do um artigo científico;

Parte III

Conclusão

Referências

PARTE I

RESUMO

A doença de Alzheimer (DA) é definida pelo acúmulo de proteínas insolúveis β-amiloide e tau no cérebro, levando à degeneração neuronal e comprometimento cognitivo. Além disso, a disfunção sináptica é um elemento fundamental da DA, intimamente associada aos sintomas de demência, porém ainda pouco entendida. Marcadores sinápticos, como neurogranina (Ng), SNAP25 e SV2A, têm sido reconhecidos como indicadores de degeneração sináptica, mesmo antes da evidência de neurodegeneração axonal. Modelos de animais transgênicos, como o TgF344-AD, representam uma ferramenta valiosa para explorar esses processos. Assim, este estudo teve como objetivo aprofundar a compreensão da sinaptopatia na DA por meio de três metodologias: i. Condução de revisão sistemática com metanálise do sistema glutamatérgico; ii. Análise de dados de biomarcadores sinápticos e de neurodegeneração axonal de indivíduos no espectro da DA; iii. Análise de densidade sináptica em ratos TgF344-AD por meio da quantificação de SV2A por captação do radioligante [^{18}F]SDM8-microtomografia por emissão de pósitrons (PET). Na primeira parte deste estudo, demonstramos que ocorre uma depleção generalizada de componentes do sistema glutamatérgico na DA. Tais como redução nos níveis de glutamato e aspartato, de recaptação de glutamato, na densidade dos receptores AMPAR-GluA2/3, NMDAR-GluN2B e na atividade de NMDAR no tecido cerebral humano. Na segunda parte deste estudo, mostramos que a tau total (t-tau), atualmente postulado como biomarcador de neurodegeneração avançada, está mais fortemente associado com alterações nos biomarcadores sinápticos Ng e SNAP25 do que com os biomarcadores canônicos de neurodegeneração, atrofia hipocampal e proteína neurofilamentar de cadeia leve (NfL), em indivíduos com e sem comprometimento cognitivo. Ademais, na terceira parte deste estudo, mostramos redução na densidade sináptica cerebral em ratos TgF344-AD envelhecidos, empregando análise de [^{18}F]SDM8-PET. Portanto, adicionamos evidência de sinaptopatia na DA, especialmente no sistema glutamatérgico, sugerimos a utilização do biomarcador t-tau como indicador de disfunção sináptica e uma reinterpretação do mesmo em ensaios clínicos e, finalmente, que o modelo TgF344-AD demonstra grande potencial translacional para estudo de densidade sináptica na DA.

Palavras-chave: doença de Alzheimer; disfunção sináptica; sistema glutamatérgico; TgF344-AD

ABSTRACT

Alzheimer's disease (AD) is characterized by the accumulation of insoluble amyloid-β (Aβ) and tau proteins in the brain, leading to neuronal degeneration and cognitive impairment. Additionally, synaptic dysfunction is a fundamental element of AD, closely associated with dementia symptoms, yet still not well understood. Synaptic markers such as neurogranin (Ng), SNAP25, and SV2A have been recognized as indicators of synaptic degeneration, even before evidence of axonal neurodegeneration. Transgenic animal models, such as the TgF344-AD, represent a valuable tool for exploring these processes. Thus, this study aimed to deepen the understanding of synaptopathy in AD through three methodologies: i. Conducting a systematic review with meta-analysis of the glutamatergic system; ii. Analyzing data from synaptic and axonal neurodegeneration biomarkers of individuals on the AD spectrum; iii. Analyzing synaptic density in TgF344-AD rats through SV2A quantification by uptake of the radioligand [^{18}F]SDM8-positron emission tomography (PET). In the first part of this study, we demonstrated a widespread depletion of components of the glutamatergic system in AD, such as reductions in glutamate and aspartate levels, glutamate reuptake, density of AMPAR-GluA2/3 and NMDAR-GluN2B receptors, and NMDAR activity in human brain tissue. In the second part of this study, we showed that total tau (t-tau), currently postulated as a biomarker of advanced neurodegeneration, is more strongly associated with changes in synaptic biomarkers Ng and SNAP25 than with canonical neurodegeneration biomarkers, hippocampal atrophy, and neurofilament light chain protein (NfL), in individuals with and without cognitive impairment. Furthermore, in the third part of this study, we showed a reduction in brain synaptic density in aged TgF344-AD rats, employing [^{18}F]SDM8-PET analysis. Therefore, we add evidence of synaptopathy in AD, particularly in the glutamatergic system, suggesting the use of t-tau as an indicator of synaptic dysfunction and a reinterpretation of it in clinical trials, and finally, that the TgF344-AD model demonstrates great translational potential for studying synaptic density in AD.

Keywords: Alzheimer’s disease; synaptic dysfunction; glutamatergic system; TgF344-AD

LISTA DE ABREVIATURAS

Aβ	Beta-amiloide
DA	Doença de Alzheimer
AMPAR	Receptor de Ácido α-amino-3-hidroxi-5-metil-4-isoxazolpropiónico
APP	Proteína Precursora Amiloide
APPs-β	Fragmento N-terminal da APP
cAMP	Monofosfato de Adenosina Cíclico
DAG	Diacilglicerol
EAAT	Transportador de Aminoácidos Excitatórios
FDA	Food and Drug Administration
FYN	Proteína Tirosina-quinase
GAP43	Proteína Associada a Crescimento-43
GFAP	Proteína Ácida Fibrilar Glial
GS	Glutamina Sintetase
KAR	Receptor de Cainato
LCR	Líquido Cefalorraquidiano
LTD	Depressão de Longo Prazo
LTP	Potenciação de Longo Prazo
MAPK	Proteína Quinase Ativada por Mitógeno
mGluR	Receptor Metabotrópico de Glutamato
MRI	Imagem por Ressonância Magnética
Ng	Neurogranina
NMDAR	Receptor N-metil-D-aspartato
NfL	Proteína Filamentar de Cadeia Leve
PET	Tomografia por Emissão de Pósitrons
PSD95	Proteína de Densidade Pós-sináptica 95
SNAP25	Proteína Associada ao Sinaptossomo de 25kDa
SNC	Sistema Nervoso Central
SV2A	Glicoproteína de Vesícula Sináptica 2A
sPDGF	Fator Solúvel de Crescimento Derivado de Plaquetas
t-tau	Tau-total
TREM2	Receptor Expressado em Células Mieloides 2
YKL40	Proteína Chitinase-3-like 1

SUMÁRIO

1 INTRODUÇÃO

1.1 O SISTEMA GLUTAMATÉRGICO

O sistema glutamatérgico é uma rede complexa e expansiva dentro do sistema nervoso central (SNC) que desempenha um papel crítico na função cerebral. Os elementos funcionais da neurotransmissão glutamatérgica incluem neurônios pré e pós-sinápticos, bem como células gliais, coletivamente referidos como sinapse tripartite (**Figura 1**) (HALASSA; FELLIN; HAYDON, 2007; LALO et al., 2021). Porém, o papel do glutamato vai além da neurotransmissão; ele é um hub metabólico que conecta o metabolismo da glicose e dos aminoácidos com a atividade sináptica (ANDERSEN et al., 2021).

O glutamato é o neurotransmissor mais abundante do SNC de vertebrados, com receptores presentes em mais de 90% dos neurônios e 40% das sinapses (BUKKE et al., 2020; CONWAY, 2020; GASIOROWSKA et al., 2021). Essa presença extensa ressalta sua importância no SNC, onde atua como o principal neurotransmissor excitatório (COX et al., 2022; ORREGO; VILLANUEVA, 1993). Os processos de sinalização do cérebro dependem tanto do glutamato que o SNC é frequentemente referido como uma máquina glutamato/GABA, com o GABA servindo como o principal neurotransmissor inibitório (NICIU; KELMENDI; SANACORA, 2012).

O glutamato é um aminoácido não-essencial e sua síntese está ligada ao metabolismo energético celular. A glicose é o principal substrato para sua síntese e doadores de grupos aminos como outros aminoácidos (i.e aspartato), amônia ou nucleotídeos participam da síntese, com o ciclo do ácido tricarboxílico (TCA) desempenhando um papel central. Nos astrócitos, a piruvato carboxilase converte piruvato em oxaloacetato, essencial para a síntese de glutamato, enquanto nos neurônios, o piruvato entra no ciclo TCA via piruvato desidrogenase (ANDERSEN et al., 2021).

Após a liberação, o glutamato se liga a receptores nos neurônios pré e pós-sinápticos ou é captado por transportadores em células gliais e neuronais. Os receptores de glutamato são diversos, com mais de 20 identificados no SNC, cada um com múltiplos subtipos (PANKEVICH; DAVIS; ALTEVOGT, 2011). Esses receptores são divididos em duas classes principais: ionotrópicos e metabotrópicos. Os receptores ionotrópicos incluem N-metil-D-aspartato (NMDA), ácido α-amino-3-hidroxi-5-metil-4-isoxazolpropiónico (AMPA) e receptor de cainato (KAR), enquanto os receptores metabotrópicos de glutamato (mGluR) estão envolvidos em mecanismos de sinalização mais complexos. O funcionamento adequado desses

receptores é essencial para manter a estabilidade e plasticidade sináptica, que são cruciais para processos como memória, aprendizado, cognição e comportamento motor (BLISS; COLLINGRIDGE, 1993; HANSEN et al., 2021).

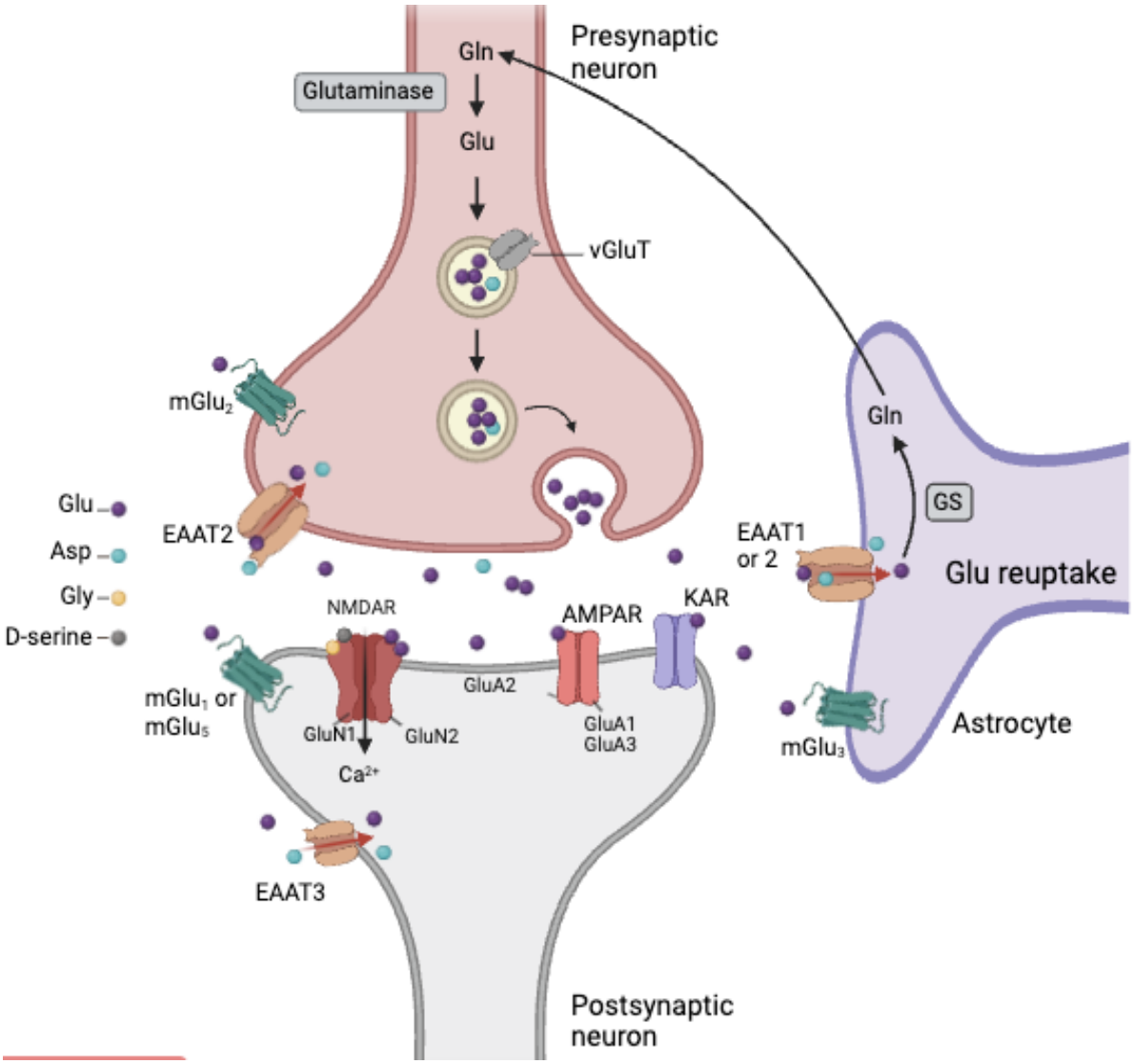

Figura 1. Neurotransmissão glutamatérgica. O glutamato (glu) é sintetizado através de múltiplas vias metabólicas, armazenado em vesículas (transportadores vesiculares de glutamato, VGluTs) e liberado durante a transmissão sináptica. Após interagir com receptores metabotrópicos (mGluRs) e ionotrópicos (NMDAR, KAR e AMPAR), o glutamato residual é então removido por transportadores específicos de glutamato (EAATs). Após a recaptação, o glutamato é convertido em glutamina (gln) pela enzima glutamina sintetase (GS), que é expressa exclusivamente em astrócitos. No ciclo glutamato-glutamina, a glutamina é liberada do astrócito e captada pelo neurônio, onde é convertida em glutamato pela glutaminase. Glicina (Gly). Aspartato (Asp).

Os mGluRs são receptores acoplados à proteína G que desempenham um papel significativo na modulação da transmissão sináptica e plasticidade. Ao contrário dos receptores ionotrópicos, que dependem do fluxo de íons, os mGluRs exercem seus efeitos indiretamente através de cascatas de sinalização intracelular. Quando o glutamato se liga a esses receptores,

ele ativa uma proteína G, que então desencadeia um sistema de segundo mensageiro, levando a mudanças funcionais no citoplasma e, em última análise, afetando a expressão gênica e a síntese de proteínas (LESAGE; STECKLER, 2010). Oito subtipos de mGluR (mGluR1-8) foram identificados e são categorizados em três grupos com base em sua sequência de aminoácidos, propriedades farmacológicas e vias de transdução de sinal (DANBOLT, 2001; KIM et al., 2008). Os receptores do Grupo I (mGluR1 e mGluR5) tipicamente aumentam a excitabilidade através da ativação da fosfolipase C, resultando em aumento do cálcio intracelular via inositol-trifosfato e estimulação da proteína quinase C via diacilglicerol. Os receptores do Grupo II (mGluR2 e mGluR3) e do Grupo III (mGluR4, 6, 7, 8) estão ligados a proteínas G inibitórias que diminuem os níveis de monofosfato de adenosina cíclico (cAMP) através da inibição da adenilil ciclase (HANSEN et al., 2021).

Os KAR são compostos por duas famílias de subunidades relacionadas: GluK5–7 e KA-1 e 2. Os receptores nativos provavelmente formam combinações tetraméricas, que podem ser tanto homoméricas quanto heteroméricas. Embora as subunidades KA-1 e KA-2 não formem receptores homoméricos funcionais, elas criam locais de ligação de alta afinidade para o kainato, permitindo a ligação de ligantes agonistas. Essas subunidades se combinam com a subfamília GluR5–7 para formar receptores funcionais que se assemelham aos receptores kainato nativos (BLEAKMAN et al., 2002). Quando expressos em sistemas heterólogos, os receptores homoméricos contendo KA1 e/ou KA2 são praticamente inativos, indicando uma função moduladora, em contraste com as subunidades GluR5-7, que formam receptores funcionais ativados por ligantes (HERB et al., 1992).

Os receptores NMDA e AMPA desempenham papéis interdependentes na despolarização da membrana durante a sinalização sináptica, mas possuem propriedades e distribuições anatômicas distintas. Eles são fundamentais na mediação da plasticidade sináptica, aprimorando a neurotransmissão glutamatérgica e a expressão gênica (BARCO; BAILEY; KANDEL, 2006).

Os receptores NMDA são canais ionotrópicos permeáveis a íons de cálcio, sódio e potássio, e desempenham um papel crucial na sinalização neuronal, sendo altamente expressos em neurônios e astrócitos, especialmente no hipocampo (BABAEI, 2021). A peculiaridade dos receptores NMDA reside no seu funcionamento como detectores de coincidência: para que o canal se abra, é necessário que o glutamato se ligue ao receptor e que a célula pós-sináptica esteja despolarizada, pois o canal é bloqueado por magnésio em níveis fisiológicos e só se abre com a despolarização da célula. A ativação rápida dos receptores AMPA, que também interagem com o glutamato de forma independente, facilita a ativação dos receptores NMDA

ao se abrir e permitir influxo de sódio, despolarizando a membrana e subsequentemente superando o bloqueio de magnésio. Consequentemente, há um influxo de cálcio pelo canal NMDA que promove a propagação do potencial de ação e iniciação de cascatas de sinalização intracelulares e expressão gênica, eventos que marcam os potenciais de longo prazo (LTP). A ocorrência de LTP é um dos mecanismos que descrevem a plasticidade sináptica e que estão subjacentes a processos cognitivos, como formação de memória e aprendizagem (BARCO; BAILEY; KANDEL, 2006).

Os receptores NMDA são formados por uma combinação de diferentes subunidades: sete subunidades de GluN1, quatro variantes de GluN2 (GluN2A-D) e duas de GluN3 (GluN3A-B) (PAOLETTI; BELLONE; ZHOU, 2013). Os receptores NMDA são únicos entre os canais iônicos ativados por ligantes devido à sua necessidade de dois co-agonistas obrigatórios, que se ligam nos sítios de ligação de glicina e glutamato localizados nas subunidades GluN1 (Kuryatov et al. 1994; Wafford et al. 1995; Hirai et al. 1996; Kew et al. 2000) e GluN2 (Laube et al. 1997; Anson et al. 1998), respectivamente. Estudos eletrofisiológicos demonstraram que a ativação do receptor NMDA requer a ocupação de dois sítios independentes de glicina e dois sítios independentes de glutamato (Benveniste and Mayer 1991; Clements and Westbrook 1991). Portanto, a configuração mínima necessária para um receptor NMDA funcional é provavelmente um tetrâmero composto por duas subunidades GluN1 e duas subunidades GluN2. Nesse sentido, as subunidades GluN2A e GluN2B são particularmente importantes, pois se ligam ao glutamato e mediam a excitotoxicidade em neurônios corticais (BABAEI, 2021)

Já os receptores AMPA são compostos por diferentes combinações de subunidades GluA1–GluA4. Os receptores AMPA sinápticos, frequentemente apresentam uma combinação de GluA1 e GluA2 (HANSEN et al., 2021), crucial para a plasticidade neural. Eles são rapidamente reciclados na área pós-sináptica, com seu número na membrana plasmática refletindo o equilíbrio entre exocitose e endocitose (HANSEN et al., 2021).

A alta densidade de receptores NMDA contribui para a aprendizagem, memória e plasticidade sináptica (BARCO; BAILEY; KANDEL, 2006; LEE; YASUDA; EHLERS, 2010). No entanto, a superativação desses receptores pode levar à perda neuronal devido à excitotoxicidade, o que é uma dicotomia central na função desses receptores. A ativação dos receptores NMDA sinápticos promove mudanças transcricionais mediadas por cálcio que fortalecem a saúde neuronal e a resistência a danos celulares, enquanto a superativação pode resultar em efeitos tóxicos.

A concentração extracelular de glutamato é rigidamente regulada em condições saudáveis. Em repouso, essa concentração é em torno de 0.6 μM (BOUVIER et al., 1992), embora exista debate sobre o valor exato (FEATHERSTONE; SHIPPY, 2008). Durante a excitação, o nível de glutamato aumenta para 10 μM (CLEMENTS et al., 1992). Contudo, a superexcitação consiste em um aumento anormal de glutamato na fenda sináptica, que superativa o NMDAR, permitindo uma sobrecarga de influxo de cálcio que, em última análise, leva à morte celular (LUCAS; NEWHOUSE, 1957) e está implicada em várias doenças neurológicas, como epilepsia, dependência, esclerose lateral amiotrófica, doença de Parkinson e DA (ALCOREZA et al., 2021; DONG; WANG; QIN, 2009). Por essa razão a recaptação é crucial para prevenir a excitotoxicidade. Na sinapse tripartite, neurônio e astrócitos realizam a recaptação rápida do glutamato por meio da expressão de altos níveis de transportadores de aminoácidos excitatórios (EAATs): GLAST (transportador de glutamato-aspartato, também conhecido como EAAT1), GLT-1 (transportador de glutamato 1, também conhecido como EAAT2), EAAT3, EAAT4 e EAAT5 (TANAKA, 2000). Os transportadores EAAT3-5 são encontrados apenas em neurônios, enquanto GLAST/EAAT1 e GLT1/EAAT2, são predominantemente ou exclusivamente expressos em astrócitos, respectivamente (DANBOLT; FURNESS; ZHOU, 2016).

A síntese e reciclagem do glutamato são ainda gerenciadas pelo ciclo glutamato-glutamina (DANBOLT, 2001). Após a recaptação pelas células gliais, o glutamato é convertido em glutamina pela glutamina sintetase (GS). Esta glutamina é então transportada de volta aos neurônios pré-sinápticos, onde é convertida novamente em glutamato pela glutaminase e armazenada em vesículas sinápticas pelos transportadores vesiculares de glutamato (vGluTs) para futura liberação (BIRNBAUMER et al., 1994; DANBOLT; FURNESS; ZHOU, 2016).

Dessa forma, fica evidente a complexa rede do sistema glutamatérgico e a importância de manter seus níveis controlados para manutenção da homeostasia cerebral e para o bom funcionamento de processos cognitivos. Entretanto, anormalidades do sistema estão associadas com doenças neurodegenerativas, como na doença de Alzheimer (DA).

1.2 A DOENÇA DE ALZHEIMER

A DA foi descrita pela primeira vez pelo médico alemão Alois Alzheimer em 1906, durante o Encontro de Psiquiatras do Sudoeste da Alemanha. O caso clínico apresentado foi o de sua paciente Auguste D., de 51 anos, que apresentava sintomas que não se encaixavam em

nenhuma doença conhecida na época. A paciente, que residia em um asilo, demonstrava desorientação, grave prejuízo de memória, perda de noção de tempo e espaço, pensamentos persecutórios, fala alterada e mudanças de humor (STELZMANN; SCHNITZLEIN; MURTAGH, 1995).

Auguste D. faleceu quatro anos e meio após o início dos sintomas, permitindo que Alzheimer analisasse a histopatologia de seu sistema nervoso. Ele observou atrofia cerebral e a presença de placas senis ("miliary foci"). Além disso, o psiquiatra descreveu, pela primeira vez, a presença de emaranhados neurofibrilares nos neurônios corticais, especialmente nas camadas superiores, utilizando o método recente – na época – de impregnação por prata (STELZMANN; SCHNITZLEIN; MURTAGH, 1995). Em seu artigo de 1907, Alzheimer enfatizou a importância de não forçar o enquadramento dos sintomas de um paciente psiquiátrico em uma doença previamente conhecida (STELZMANN; SCHNITZLEIN; MURTAGH, 1995).

A DA é uma doença neurodegenerativa progressiva, responsável por cerca de 60 a 80% dos casos de demência (ALZHEIMER; ASSOCIATION, 2024). Embora tida como rara quando descoberta, estima-se que existam aproximadamente 57 milhões de pessoas vivendo com demência no mundo atualmente (NICHOLS et al., 2022). Além disso, com o aumento da expectativa de vida, observa-se uma maior incidência de doenças neurodegenerativas prevendo-se que esse número triplique até 2050 (ALZHEIMER; ASSOCIATION, 2024).

É estimado que apenas 1% dos casos de DA ocorram antes dos 60 anos, sendo diretamente associados com mutações genéticas. Nesses casos, a herança da mutação dos genes para proteína precursora amiloide (APP), para preselinina 1 (PSEN1) ou para presenilina 2 (PSEN2) garantem o desenvolvimento da forma familiar da DA (BEKRIS et al., 2010). Contudo, a grande maioria dos casos de DA ocorre, de fato, na senioridade, afetando 1 a cada 9 idosos com 65 anos ou mais nos EUA (ALZHEIMER; ASSOCIATION, 2024) e cerca de 960 mil de idosos nessa faixa etária no Brasil (BERTOLA et al., 2023). Esses casos são chamados de DA esporádica. Embora sua causa exata não esteja elucidada, intensa investigação científica revelou que a idade é o maior fator de risco, seguido da presença da forma e4 do gene da apolipoproteina E (APOE) (FARRER, 1997) e histórico familiar de DA (MAYEUX et al., 1991).

Clinicamente, a DA se manifesta por perda de memória inicial, seguida de declínio cognitivo progressivo, desorientação, dificuldades na linguagem, perda funcional global, além de alterações de personalidade e de humor, incluindo depressão (HARDY, 2006). Esses sintomas culminam em total dependência de cuidadores antes do paciente vir a óbito (ALZHEIMER; ASSOCIATION, 2024). Apesar do diagnóstico ser majoritariamente clínico, a confirmação da DA pode ser realizada por análise *post-mortem* ou *in vivo* utilizando

biomarcadores. Tais análises consistem na identificação dos marcos histopatológicos da doença: placas amiloides, emaranhados neurofibrilares e neurodegeneração (CLIFFORD R.J. JR. et al., 2018).

1.2.1 Beta-amiloide

As placas amiloides são depósitos extracelulares insolúveis de fibrilas amiloides, compostas majoritariamente pelo peptídeo β-amiloide (Aβ) (SELKOE, D.J., 1991). Em 1984, esse peptídeo foi isolado, sequenciado e demonstrado pela primeira vez como um constituinte primário dos depósitos polimórficos meningovasculares em pacientes com Síndrome de Down (GLENNER; WONG, 1984). Um ano depois, Master et al mostrou que a sequência completa das placas amiloides parenquimais eram idênticas aquelas encontradas no ambiente perivascular descrito anteriormente, com o diferencial de que nas placas a extensão se estendia até o resíduo 42 (MASTERS et al., 1985).

O Aβ é um fragmento de 4kDa originado do processamento da APP, uma molécula precursora amplamente presente em sinapses e produzida no cérebro por neurônios, células vasculares e sanguíneas, incluindo plaquetas, e em menor escala, por astrócitos (HAMPEL et al., 2021a). Primeiramente, a APP sofre processamento sequencial por proteases integrais, chamadas secretases (**Figura 2a**). β-secretases clivam APP no domínio extracelular, gerando a secreção de um fragmento N-terminal (APPs-β) e a manutenção de um fragmento C-terminal (CTF-β) na membrana. Em seguida, γ-secretases clivam diversas vezes o CTF-β em sítios intramembranares, liberando o peptídeo Aβ e um fragmento intracelular. É interessante lembrar que a APP também sofre outro processamento em que não há formação de peptídeo Aβ, realizado por α-secretases em vez de β-secretases, nomeadamente via não-amiloidogênica (UDDIN et al., 2020).

Ademais, diferentes peptídeos Aβ podem ser gerados dependendo do sítio de clivagem da γ-secretase. Já foram identificados os produtos $A\beta_{1-38}$, $A\beta_{1-40}$ e $A\beta_{1-42}$, cujos números representam a posição do aminoácido em que ocorre a clivagem (HAASS; SELKOE, 2007). Os sítios catalíticos da γ-secretase responsáveis por esses produtos são a presenilina-1 e presenilina-2, cujas mutações estão associadas com o aumento da agressividade da DA (SELKOE, 2011). Nesse sentido, o sítio exato onde ocorre a clivagem está diretamente relacionado com a toxicidade, sendo que o peptídeo $A\beta_{1-42}$ tem maior tendência de agregar e maior capacidade sinaptotóxica (JARRETT; BERGER; LANSBURY, 1993; SELKOE, D.J;

HARDY, 2016). Além dos monômeros e das placas de Aβ, também já foram descritos agregados de dímeros, oligômeros, protofibrilas e fibrilas (**Figura 2b**).

Embora se saiba que a secreção de Aβ no líquido cefalorraquidiano (LCR) e no plasma seja fisiológica (HAASS et al., 1992), a causa para o acúmulo de tal proteína ainda não está clara. Esforço de diversos grupos de pesquisa gerou diferentes hipóteses que tentam explicar os mecanismos subjacentes à patologia. A hipótese da cascata amiloide, proposta em 1992 por Hardy e Higgins propõe que as placas amiloides são neurotóxicas e a causa primária e direta da formação de emaranhados neurofibrilares e da neurodegeneração vistos na DA (HARDY; HIGGINS, 1992). Tal hipótese foi postulada com base em contextos de aumento na expressão de APP ou de Aβ, incluindo trauma craniano e demência pugilística. Consequentemente, haveria um acúmulo de Aβ em placas em diversas regiões do cérebro que desencadearia neurodegeneração e aparição dos sintomas precocemente nesses pacientes (HARDY, J. A.; HIGGINS, 1992; SELKOE, D.J., 1991).

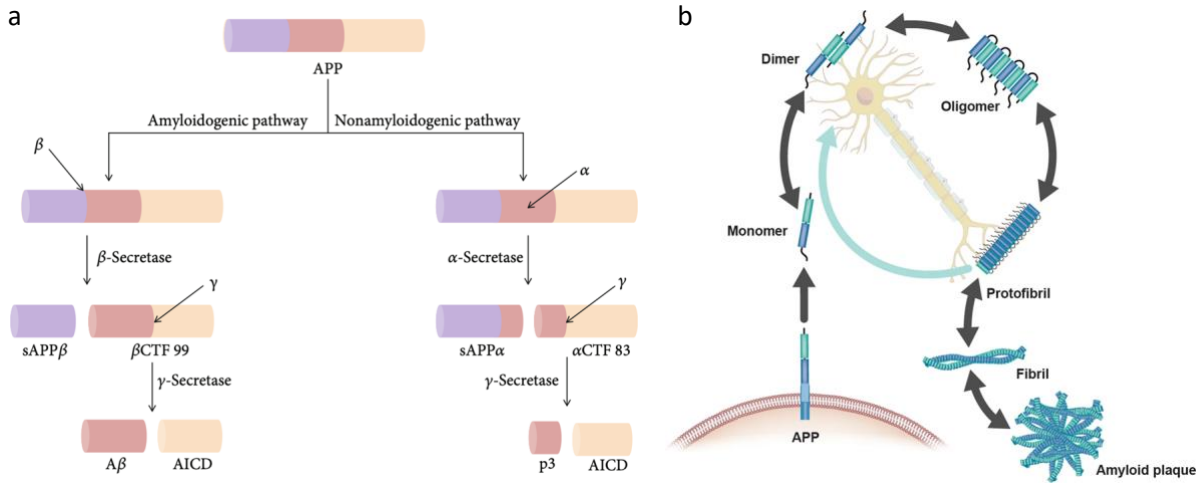

Figura 2. Esquema da clivagem da APP e formação do peptídeo Aβ. **a.** Processamento da proteína transmembrana APP. Na via amiloidogênica (seta para a esquerda), a APP sofre clivagem pela β-secretase (β) e pela γ-secretase (γ), liberando no meio extracelular APPs-β, o peptídeo Aβ e um domínio intracelular (AICD). Na via não amiloidogênica (seta para direita) o processamento se dá pela α-secretase (α) e pela γ-secretase, liberando APPs-α no meio extracelular, peptídeo p3 e AICD. **b.** Formação de espécies de Aβ: monômeros, dímeros, oligômeros, protofibrilas, fibrilas e placas amiloides, os quais são caracterizados pelo tamanho, conformação e solubilidade, e podem ser convertidos uns nos outros bidirecionalmente. Modificado de UDDIN et al., 2020 e HAMPEL et al., 2021.

Outra evidência de suporte para a hipótese da cascata amiloide seria a Síndrome de Down, cujos pacientes apresentam uma cópia extra do cromossomo 21, onde o gene para a APP está contido (GOLDGABER et al., 1987). Dessa forma, há uma superexpressão da proteína Aβ

e, de fato, os pacientes portadores da trissomia do 21 apresentam maior incidência de DA (ALZHEIMER; ASSOCIATION, 2024).

Contudo, muitas evidências sugerem que as placas amiloides, isoladamente, não são suficientes para causar prejuízo de memória, enfraquecendo a hipótese da cascata amiloide. Surpreendentemente, um estudo da década de 1990 foi um dos primeiros a mostrar falhas nessa hipótese, demonstrando uma correlação insignificante entre o número de placas amiloides e o e os resultados de testes cognitivos de 70 pacientes com a DA (TERRY, 1994). Além disso, o estudo revelou que o prejuízo cognitivo não estava correlacionado com a perda neuronal, mas sim com a perda sináptica. Ademais, 10 % dos indivíduos com 50 anos de idade possuem placas senis, mas são cognitivamente saudáveis (JANSEN et al., 2015).

O aducanumabe, um anticorpo monoclonal direcionado contra o peptídeo Aβ, foi aprovado pelo órgão americano FDA em 2021 para o tratamento da DA. Estudos mostraram que o aducanumabe foi capaz de reduzir as placas amiloides no cérebro (SEVIGNY et al., 2016), mas os dados sobre a melhoria cognitiva foram ambíguos. Dois grandes ensaios clínicos, ENGAGE e EMERGE, produziram resultados conflitantes, o que levou a debates sobre a eficácia do medicamento (WALSH et al., 2021), destacando a necessidade de mais pesquisas para entender a relação entre a redução das placas e a melhoria dos sintomas clínicos.

Essa contradição entre declínio cognitivo e toxicidade do Aβ destacou outros produtos formados a partir de Aβ, como os oligômeros solúveis de Aβ (AβOs). Estes apresentam neurotoxicidade mesmo na ausência das fibrilas de Aβ (LAMBERT et al., 1998). Nesse sentido, foi proposto que os AβOs são as toxinas primárias presentes no cérebro dos pacientes portadores de DA e a causa genuína do prejuízo sináptico (CLINE et al., 2018; HERRUP, 2015).

1.2.2 Tau

Os emaranhados neurofibrilares são agregados intracelulares de polímeros fibrosos da proteína tau (filamentos helicoidais duplos, ‘PHF’). Os PHFs são encontrados no citosol de neurônios e sua quantidade é mais bem correlacionada com a gravidade da DA comparada a placas amiloides (MASTERS et al., 2015). A primeira observação de PHFs em cérebro de indivíduos com a DA foi relatada em 1963 por Michael Kidd (KIDD, 1963). Em sua carta, o autor relatou a presença de emaranhados densos de fibrilas argirofílicas em neurônios corticais, que apareciam como formas espirais ou “raquetes de squash”. Ademais, ele observou que tais emaranhados coravam com *congo red* e então exibiam birrefringência, sugerindo a presença de

micelas organizadas longitudinalmente, as quais denominou como PHF. Mais de uma década depois, Weingarten et al isolaram a tau pela primeira vez em um estudo que utilizou porcos para investigar os componentes necessários para formação de microtúbulos no cérebro. Eles descobriram que o "fator intercambiável" essencial para a estabilização dos microtúbulos consistia em um uma proteína dissociável, a qual eles propuseram nomear como tau (WEINGARTEN et al., 1975).

Existe uma grande heterogeneidade nas formas de tau encontradas no sistema nervoso central (CNS) humano. A tau é codificada pelo gene MAPT localizado no cromossomo 17 (NEVE et al., 1986) (**Figura 3a**). Por meio de splicing alternativo dos exons 3, 6 e 10, são produzidas seis isoformas de tau: 0N3R, 1N3R, 2N3R, 0N4R, 1N4R e 2N4R (**Figura 3b**). Essas isoformas podem ser diferenciadas pela presença de zero, uma ou duas inserções N-terminais (0N, 1N ou 2N, respectivamente) e pela presença de três (3R) ou quatro (4R) repetições de ligação aos microtúbulos na metade C-terminal da tau. As isoformas 4R (aquelas que incluem o éxon 10) são geralmente melhores em estabilizar microtúbulos em comparação com as isoformas 3R (GUO; NOBLE; HANGER, 2017).

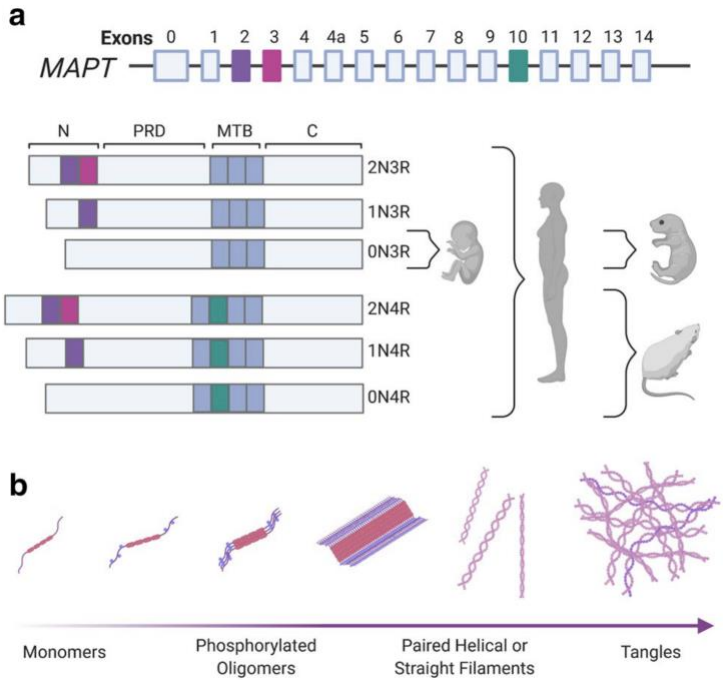

Figura 3. Formação da proteína tau. a. Tau é codificada pelo gene MAPT no cromossomo 17. Seis isoformas da proteína tau são geradas por splicing alternativo dos exons 2, 3 e 10. A inclusão do exon 10 resulta na tau com quatro domínios de ligação aos microtúbulos (4R), enquanto a exclusão do exon 10 resulta na tau com três domínios de ligação aos microtúbulos (3R). A regulação dos exons 2 e 3 pode incluir (2 N ou 1 N) ou excluir (0 N) inserções na extremidade amino-terminal. Apenas a tau 0N3R é expressa no cérebro fetal humano ou de camundongo, enquanto todas as seis isoformas de tau são expressas em humanos adultos. Em camundongos e ratos adultos, a expressão é quase exclusivamente de tau 4R. **b.** A tau fosforilada pode se agrupar para formar oligômeros, filamentos (retos e helicoidais duplos) e, eventualmente, emaranhados. N (extremidade N-terminal),

PRD (domínio rico em prolina), MTB (domínios de ligação aos microtúbulos), C (extremidade C-terminal). Retirado de KENT; SPIRES-JONES; DURRANT, 2020.

Além disso, a tau sofre diversas modificações pós-translacionais, como glicosilação, oxidação, acetilação e a mais comumente descrita, fosforilação. A tau possui 85 sítios de fosforilação (GUO; NOBLE; HANGER, 2017), e na DA, a hiperfosforilação da tau desencadeia mudanças conformacionais, promovendo formação de monômeros, oligômeros fosforilados, PHF e filamentos retos duplos, e finalmente, emaranhados neurofibrilares (HANGER; ANDERTON; NOBLE, 2009) (**Figura 3b**).

Tais alterações reduzem a afinidade da tau pelos microtúbulos, impactando no transporte intracelular (HANGER; ANDERTON; NOBLE, 2009; MEYER et al., 1995). Nesse sentido, a desestabilização dos microtúbulos prejudica o transporte axonal de vesículas, exercendo, portanto, papel fundamental na sinaptopatia na DA (**Figura 4a**). Além do axônio, estudos mais recentes localizaram a tau no núcleo onde foi associada com a manutenção da integridade do DNA, e nas espinhas dendríticas, embora sua função ainda não esteja clara (WANG; MANDELKOW, 2016). Entretanto, na DA a hiperfosforilação ocasiona perda de afinidade pelos microtúbulos (**Figura 4b**). Como consequência, a tau desassociada pode resultar em dano no DNA e em disfunção tanto no terminal pré- quanto no pós-sináptico. Ademais, estudos mostraram que agregados de tau patológica podem ser liberados no espaço extracelular e depois entrar em outros neurônios, espalhando a taupatia.

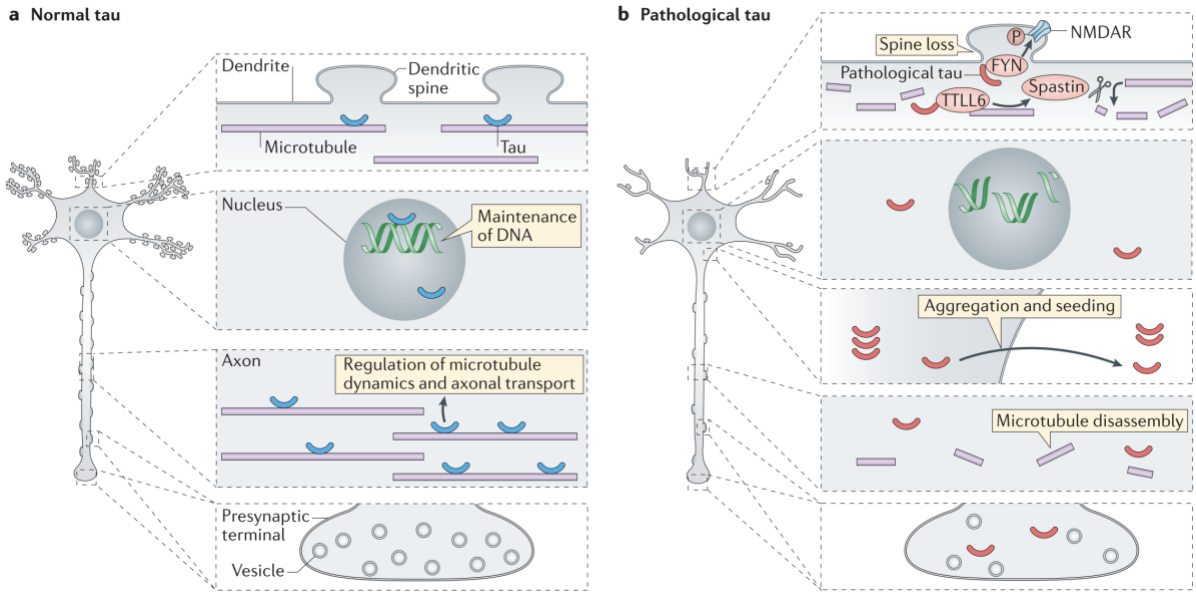

Figura 4. Funções da proteína tau. a. Em condições normais, a tau é encontrda nos axônios, estabilizando microtúbulos e influenciando o transporte axonal. Pequenas quantidades de tau também são encontradas no núcleo, onde auxilia a manter a integridade do DNA e nas espinhas dendríticas. **b.** Em condições patológicas, a tau pode se desassociar dos microtúbulos, prejudicando o transporte axonal. A tau patológica não consegue entrar no núcleo, prejudicando a manutenção do DNA. Além disso, tratamento de cultura primária de neurônios com oligômeros de Aβ ou outros estressores estimulam o deslocamento da tau para os dendritos, aumentando a translocação da enzima ligase 6 da tubulina tirosina (TTLL6), e subsequente corte dos microtúbulos pela spastina. Ademais, a tau

patológica pode servir como uma plataforma de proteínas nos dendritos, entregando proteína tirosina-quinase (FYN), a qual fosforila a subunidade 2 do receptor NMDA (NMDAR), aumentando a estabilidade da interação com a proteína de densidade pós-sinaptica 95 (PSD95), facilitando a neurotransmissão glutamatérgica e a toxicidade de Aβ. Finalmente, a tau pode formar agregados que vão para o espaço extracelular e então são encontradas em outros neurônios, espalhando a taupatia. Modificado de WANG, Y.; MANDELKOW, 2016.

De forma similar aos AβOs, estudos recentes demonstraram que acúmulo de oligômeros de tau em sinapses iniciam uma cadeia de aberrações sinápticas (COLOM-CADENA et al., 2023; TADDEI et al., 2023). Além disso, oligômeros de tau estão presentes mesmo em áreas sem emaranhados neurofibrilares. Finalmente, a presença dos emaranhados neurofibrilados isoladamente não garantem danos sinápticos, perda neuronal e desenvolvimento de demência (CRYSTAL et al., 1988).

1.2.3 Degeneração sináptica

Desde as descobertas de que placas e emaranhados neurofibrilares são compostos de Aβ e tau, respectivamente, o maior objeto de estudo na área da DA se concentrou nessas proteínas como causas diretas da morte neuronal e consequente atrofia cerebral. Contudo, é bem aceito que a degeneração sináptica é o melhor correlato neuropatológico do declínio cognitivo na DA (DEKOSKY; SCHEFF, 1990) e portanto, uma característica central a ser investigada.

Nesse sentido, uma das alterações mais centrais na degeneração sináptica na DA consiste na redução no número de sinapses. Estudos clássicos de sinaptopatia na DA focaram na densidade sináptica utilizando diferentes métodos de visualização em tecido *post-mortem* de pacientes. DeKosky et al utilizaram microscopia eletrônica (**Figura 5a**) e relataram uma redução ultraestrutural de densidade sináptica no córtex frontal do grupo com DA, de até 42% comparado com grupo controle. Contudo, eles demonstraram haver um aumento na área de contato entre um terminal pré-sináptico e terminais pós-sinápticos no grupo com DA, sugerindo um efeito compensatório (**Figura 5a**) e uma correlação negativa entre densidade sináptica e cognição (DEKOSKY; SCHEFF, 1990). Masliah et al utilizaram imunomarcação e observaram uma redução da proteína sinaptofisina em quantidade mais significativa nos córtices frontal e parietal, equivalente a 45% comparado com tecido do grupo controle (**Figura 5b**). Ademais, os autores relataram uma perda sináptica maior do que a de grandes neurônios na mesma região (MASLIAH et al., 1991). Similarmente, Terry et al reportaram redução de sinaptofisina em 40% nas regiões médio-frontal e parietal inferior e demonstraram que essa perda sináptica correlacionou melhor com a perda cognitiva comparado com número de placas amiloides (**Figura 5c**) (TERRY et al., 1991).

Tais anormalidades sinápticas já foram reportadas em diversos sistemas de neurotransmissão na DA. No sistema colinérgico, por exemplo, há uma perda significativa de neurônios colinérgicos no núcleo basal de Meynert, o que leva à diminuição da neurotransmissão colinérgica no córtex e no hipocampo, contribuindo para os déficits cognitivos observados na DA (HAMPEL et al., 2018). O sistema noradrenérgico também é afetado, com a degeneração de neurônios no locus coeruleus, levando a uma diminuição dos níveis de noradrenalina e exacerbando a disfunção cognitiva e emocional na DA (PORTELA MOREIRA et al., 2023). Além disso, atividade epileptiforme em pacientes indica um desbalanço inibitório-excitatório, sugerindo alteração dos sistemas GABAérgico e glutamatérgico. De fato, múltiplas alterações desses sistemas já foram reportadas (BELL; BENNETT; CUELLO, 2007; BIE et al., 2018; CARELLO-COLLAR et al., 2023; NUZZO et al., 2021a; ZOTT; KONNERTH, 2022).

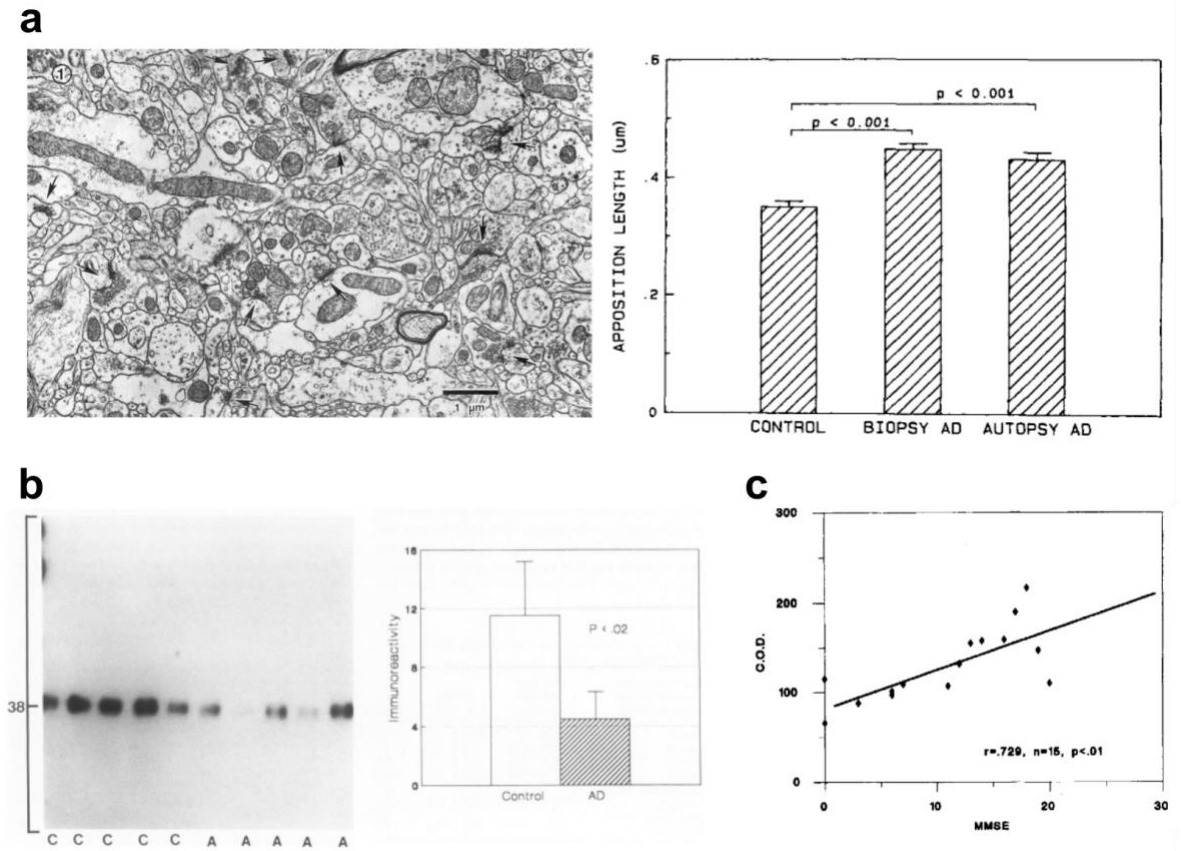

Figura 5. Estudos de densidade sináptica em tecido de pacientes com DA. a. Microscopia eletrônica mostrando sinapses típicas (indicadas com setas) da camada III do córtex frontal humano (esquerda) e a quantificação do comprimento da área de contato entre os elementos pré-sinápticos e pós-sinápticos de uma sinapse (comprimento de aposição, ⌈m) no tecido do grupo controle, de biópsia de pacientes com DA e de autópsia dos mesmos pacientes em adição a outros pacientes (direita). **b.** Detecção (esquerda) e quantificação (direita) de sinaptofisina do córtex frontal de indivíduos com DA medido por Western blot. **c.** Análise de correlação entre densidade sináptica medida por densidade óptica de marcação com sinaptofisina e severidade de perda cognitiva. Mini-Mental State Exam (MMSE). Modificado de DEKOSKY; SCHEFF, 1990; TERRY et al., 1991.

Na DA, enorme quantidade de estudos demonstra alterações no sistema glutamatérgico (**Figura 6**). A maioria das investigações foram realizadas em estágios severos da DA, quando é observada uma redução de diversos componentes do sistema. Por exemplo, níveis de glutamato e glicina estão reduzidos no cérebro post-mortem de indivíduos com DA (NUZZO et al., 2021b). Os receptores NMDAR, AMPAR e mGluRs são superestimulados ou expressos em menor quantidade (BUKKE et al., 2020) Transportadores e recaptação de glutamato por parte dos astrócitos foram investigados em modelos experimentais e em fatias de cérebro humano (BECKSTRØM et al., 1999; FAN et al., 2018; KASHANI et al., 2008; ROCHA et al., 2022; YEUNG et al., 2021a). Em um estágio mais inicial da DA, porém, são reportadas evidências de aumento da neurotransmissão glutamatérgica por meio de aumento na expressão e ativação de receptores NMDA (YEUNG et al., 2021b). Pacientes e modelos experimentais exibem hiperexcitabilidade neuronal (TARGA DIAS ANASTACIO; MATOSIN; OOI, 2022), consistente com um padrão de aumento da ativação de NMDAR, o que exacerba o influxo de cálcio e leva à excitotoxicidade. Por outro lado, a hiperexcitabilidade também já foi associada com redução de componentes do sistema GABAérgico (BUKKE et al., 2020). Contudo, os resultados são conflitantes e portanto, faz-se necessário uma integração da literatura.

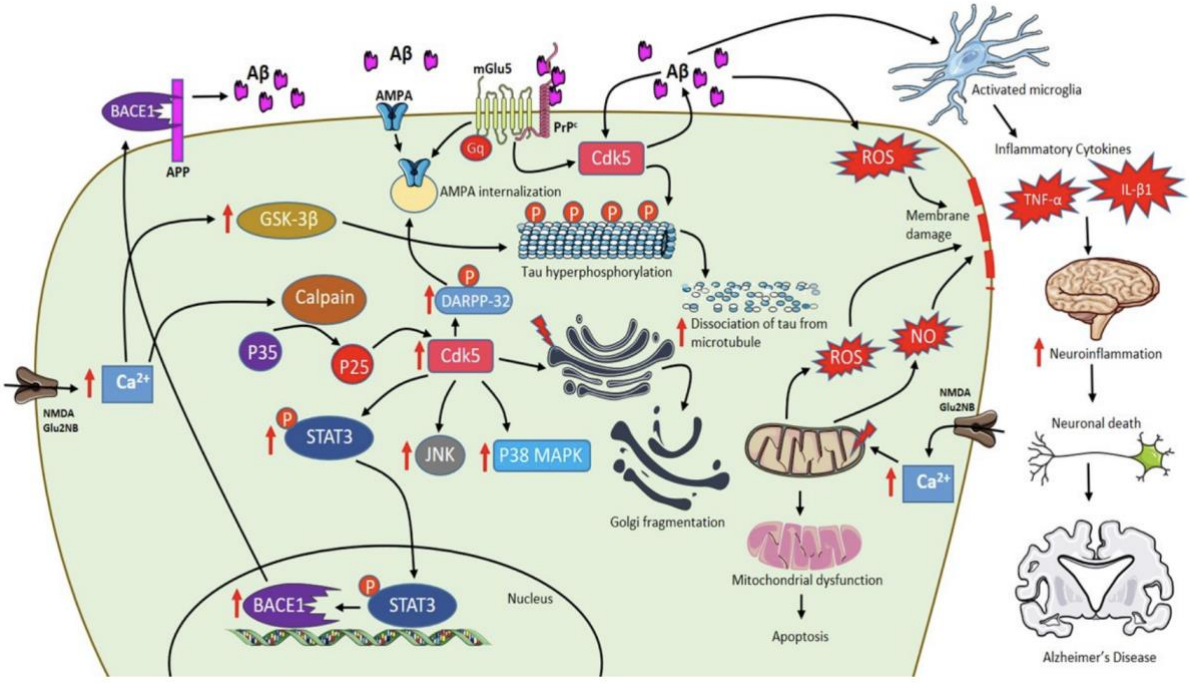

Figura 6. Alterações da sinapse glutamatérgica na doença de Alzheimer. Os peptídeos Aβ aumentam a internalização de AMPAR e a atividade dos receptores NMDA, levando a um aumento do influxo de cálcio. Isso ativa várias vias, incluindo GSK-3β, calpaína e P38 MAPK, resultando em hiperfosforilação da tau e formação de emaranhados neurofibrilares. O excesso de cálcio e espécies reativas de oxigênio (ROS) causam disfunção mitocondrial e apoptose. Aβ também ativa a microglia, levando à neuroinflamação por meio das citocinas TNF-α e IL-1β, contribuindo ainda mais para a morte neuronal. Os efeitos cumulativos dessas vias resultam em disfunção sináptica e declínio cognitivo característicos da doença de Alzheimer. Retirado de BUKKE et al., 2020.

Consistente com a importância do sistema glutamatérgico na DA, em 2003 a droga memantina foi aprovada pelo órgão americano FDA. A memantina é um antagonista não-competitivo de NMDAR e se liga na subunidade GluN2B quando o canal está aberto (JOHNSON; KOTERMANSKI, 2006; KATO, 2004; TAMPI; VAN DYCK, 2007). A memantina é recomendada para casos moderados a severos da DA e é capaz de aliviar os sintomas. Entretanto, o tratamento não interrompe a progressão da doença, indicando que ainda há grande necessidade de mais investigações acerca do papel do glutamato na patologia da DA (MOLINUEVO; LLADÓ; RAMI, 2005).

Um mecanismo que está associado com a perda sináptica é a inflamação presente na DA. Nesse contexto, foi observado um aumento da inflamação no SNC, como aumento de citocinas pró-inflamatórias, de proteínas do sistema complemento e da ativação de células gliais, como a micróglia (HEPPNER; RANSOHOFF; BECHER, 2015). A micróglia possui diversas funções, entre elas, a de vigilância do SNC, secreção de citocinas e poda de sinapses (COLONNA; BUTOVSKY, 2017). Porém, estudos em modelos da DA indicam que a micróglia possui uma assinatura típica da DA e engolfa sinapses sob sinalização do sistema complemento, consequentemente, reduzindo o número de sinapses (HONG et al., 2016; KEREN-SHAUL et al., 2017). Entretanto, o papel da micróglia e da inflamação parece ser diferente dependendo do estágio da DA (HEPPNER; RANSOHOFF; BECHER, 2015). Assim, ainda não está elucidado se a inflamação é causa ou consequência da patologia, sendo objeto de extenso debate.

As disfunções do metabolismo mitocondrial estão fortemente associadas à DA. Há décadas, pesquisas revelam que a disfunção mitocondrial e a perda sináptica são eventos presentes no início da DA (SWERDLOW, 2018). Isso se deve aos diversos papeis da mitocôndria na manutenção das sinapses incluindo assistência na geração de adenosina trifosfato e na regulação de cálcio, ambos essenciais para viabilidade neuronal (CALKINS; MANCZAK; REDDY, 2012). Ainda, na DA é observado um aumento no número e disfunção das mitocôndrias no compartimento pré-sináptico, promovendo síntese de espécies reativas de oxigênio (ROS) tóxicas. Disfunção mitocondrial e subsequente geração de ROS também são observadas pós-sinapticamente (TZIORAS et al., 2023).

Múltiplos estudos investigaram mecanismos dentro das sinapses. De fato, uma metanálise de 2016 incluiu 103 artigos com dados de proteínas sinápticas em tecido post-mortem de pacientes com DA e verificou que há perda sináptica em diversas regiões cerebrais incluindo hipocampo, córtex do cingulado, entorrinal, temporal e frontal (DE WILDE et al., 2016). A análise de marcadores sinápticos específicos concluiu que há maior alteração de marcadores pré-sinápticos do que pós-sinápticos e que isso ocorre mais robustamente no

hipocampo do que nas outras regiões analisadas (giro do cíngulo, córtex temporal e entorrinal) (**Figura 7**).

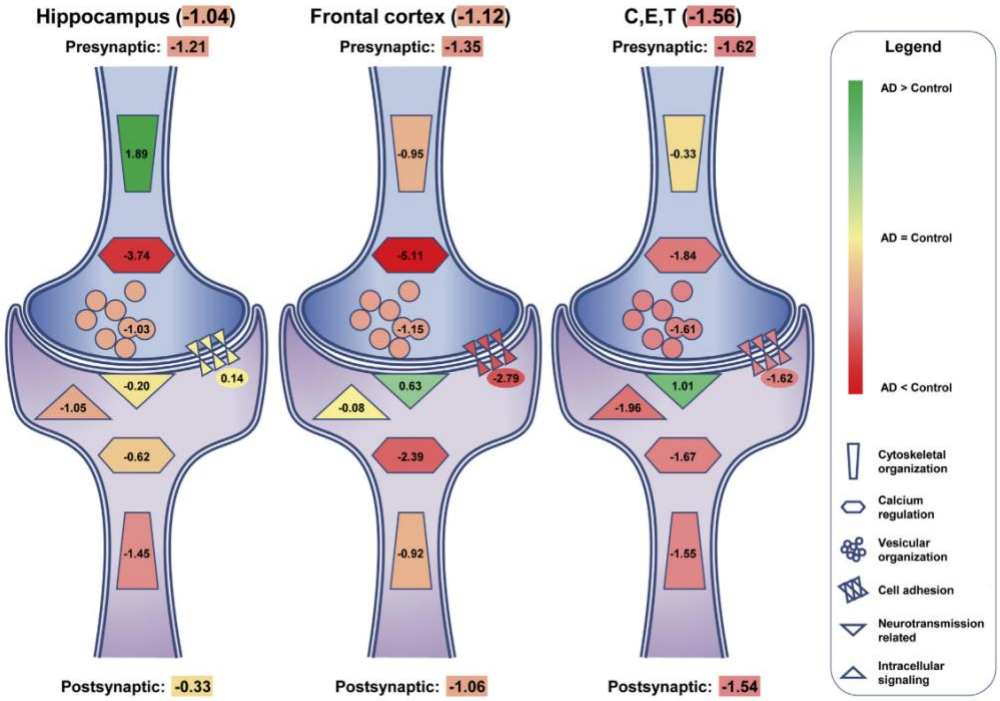

Figura 7. Alterações sinápticas são mais pronunciadas no terminal pré-sináptico do que no pós-sináptico na doença de Alzheimer. Ilustração da diferença de média padronizada (SMD) resultante da metanálise de 67 proteínas sinápticas no hipocampo, córtex frontal e regiões combinadas (giro do cíngulo, córtex temporal e entorrinal). As proteínas foram divididas em categorias funcionais listadas no quadro com a legenda e representadas por diferentes símbolos: organização do citoesqueleto (i.e actina), regulação de cálcio (i.e sinaptotagmina), organização vesicular (i.e sinaptofisina), adesão celular (i.e N-caderina), relacionada a neurotransmissão (i.e receptores), sinalização intracelular (i.e PSD95). Os valores de SMD para cada categoria estão indicados dentro dos símbolos e as cores indicam a magnitude da alteração comparada com grupo controle, sendo redução indicada por tons vermelhos e aumento indicado por tons verdes. Retirado de DE WILDE et al., 2016.

Uma grande diversidade de mecanismos de dano sináptico está diretamente ligada às formas oligoméricas de Aβ e de tau. Embora placas amiloides e emaranhados neurofibrilares sejam características clássicas da DA, diversos estudos demonstraram que AβOs e oligômeros de tau são mais neurotóxicos e produzem efeitos deletérios diretamente nas sinapses (FERREIRA; KLEIN, 2011; MILLER et al., 2014; TADDEI et al., 2023). Dessa forma, cabe explorar os efeitos dos oligômeros na sinapse no contexto da DA.

Evidências indicam que AβO pode induzir excitotoxicidade pré-sináptica por meio da ligação ao receptor σ2, aumentando o influxo de cálcio e a formação de poros na membrana, contribuindo ainda mais para o influxo de cálcio na sinapse (TZIORAS et al., 2023). A liberação

intracelular de cálcio do retículo endoplasmático liso também pode ocorrer nas espinhas dendríticas via ativação de receptores de inositol-trifosfato e receptores de rianodina.

No terminal pós-sináptico, AβO pode se ligar ao receptor de proteína priônica C e ao mGluR5 promovendo a ativação de Fyn, que ativa o receptor de inositol-trifosfato e o fator de elongação eucariótico 2, resultando na fosforilação da tau sináptica (TZIORAS et al., 2023). Além disso, AβOs se associam com a membrana mitocondrial e interagem com proteínas mitocondriais como Drp1, ABAD e ciclofilina D (CALKINS et al., 2011). Ademais, estudos demonstram que os AβOs são capazes de alterar o número de mitocôndrias em neurônios, de alterar os processos mitocondriais de fusão e fissão, bem como de transporte (CAI; TAMMINENI, 2017). Ainda, microinjeção de AβOs em camundongos induz internalização de receptores AMPA, inibindo LTP (CLEARY et al., 2005; SHANKAR et al., 2008). Além disso, foi observada perda de memória (FIGUEIREDO et al., 2013), e de espinhas dendríticas em primatas não-humanos (BECKMAN et al., 2019).

Mais recentemente, estudos com oligômeros de tau indicaram efeitos similares aos causados pelos AβOs. Isto é, a aplicação de oligômeros de tau extraídos de pacientes com DA em camundongos resulta em redução de LTP e em dano de memória (FÁ et al., 2016). Ademais, adição de oligômeros de tau em cultura primária de neurônios parece causar perda de espinhas dendríticas (KANIYAPPAN et al., 2017).

1.2.4 Biomarcadores e estágios da DA

Historicamente, o diagnóstico da DA consistia em critérios clínicos baseados na presença de sintomas cognitivos e comportamentais por entrevista médica e na exclusão de outras doenças. A confirmação era possível somente com exame post-mortem indicando a presença dos marcos histopatológicos da DA. Contudo, o exame histopatológico sugeria presença de outras patologias em 20% dos casos (MCKHANN et al., 1984). A necessidade de diagnósticos mais precisos e em vida, redirecionou a concentração de investigações para o desenvolvimento de biomarcadores multimodais *in vivo* da DA capazes de identificar a neuropatologia característica da doença. De fato, estudos com biomarcadores demonstraram que a patologia da DA se inicia 20 a 30 anos antes do aparecimento dos sintomas (JANSEN et al., 2015).

Nesse sentido, a amiloidose pode ser detectada por espécies de Aβ no LCR ($A\beta_{1-40}$, $A\beta_{1-42}$, razão $A\beta_{40/42}$) ou por tomografia de emissão de pósitron (PET) e indivíduos com níveis

anormais desses biomarcadores são classificados como amiloide-positivos (A+) (**Figura 8 - superior**). (HAMPEL et al., 2021b).

Taupatia pode ser indicada por aumento de produção, fosforilação (p-tau) em diferentes sítios (i.e fosforilação na treonina 181 ‘p-tau181’) e secreção de tau no cérebro, detectada por quantificação no LCR (p-tauX), por PET e mais recentemente, no sangue. Indivíduos com aumento anormal de biomarcadores de tau são classificados como tau-positivos (T+) (**Figura 8 – centro)** (OSSENKOPPELE; VAN DER KANT; HANSSON, 2022).

Finalmente, neurodegeneração pode ser identificada por atrofia de regiões tipicamente afetadas na DA, como córtex cerebral e hipocampo por meio de imagem de ressonância magnética (MRI) ou por redução nos níveis de captação de glicose cerebral com PET. Além disso, são considerados biomarcadores de neurodegeneração no LCR as proteínas associadas com injúria neuronal, como a proteína filamentar de cadeia leve (NfL) presente no axônio e “tau-total” (t-tau), um marcador que engloba todas as isoformas de tau, independentemente do estado fosforilação. Indivíduos com aumento anormal desses biomarcadores são classificados como neurodegeneração-positivos (N+) (**Figura 8 – inferior**) (HAMPEL et al., 2021b; HESSE et al., 2000, 2001; VISSER et al., 2022).

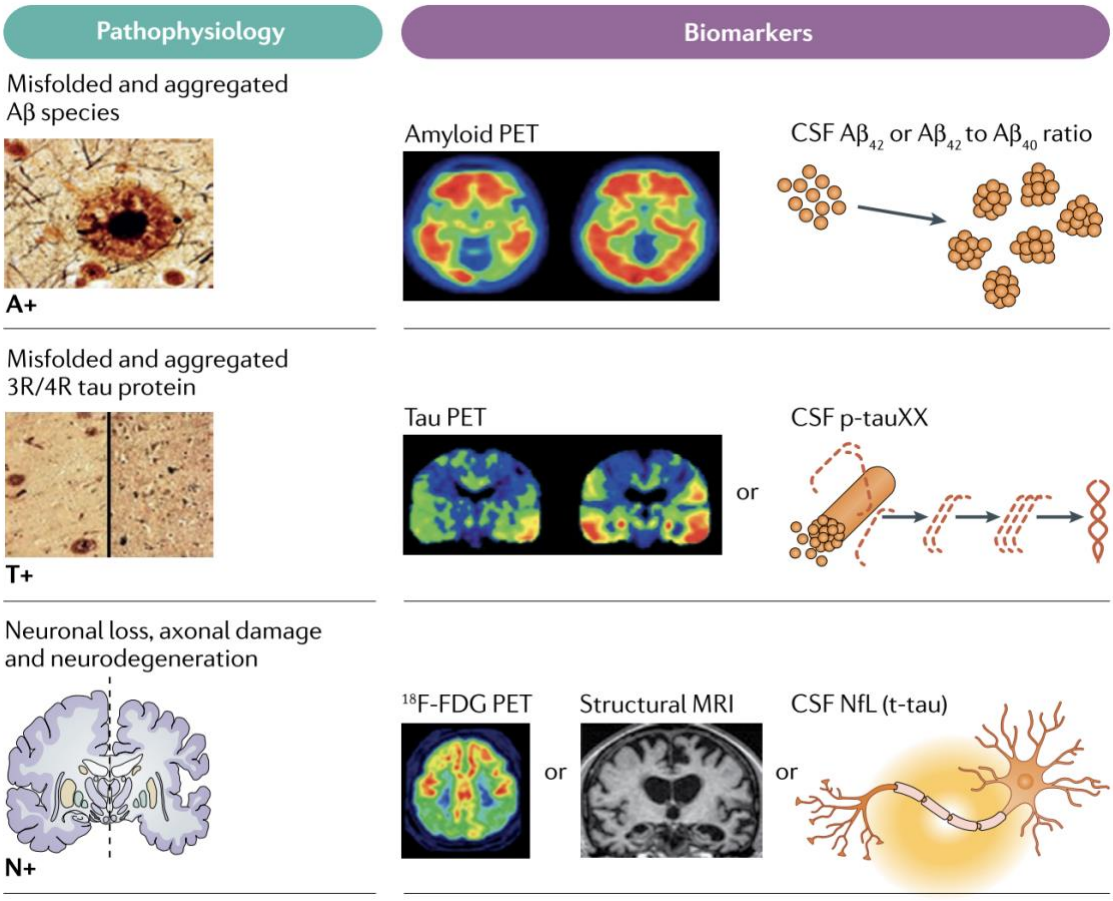

Figura 8. Biomarcadores da patologia clássica da DA. Marcos clássicos da patologia da DA (placas amiloides, emaranhados neurofibrilares, atrofia, morte neuronal e dano axonal) e os biomarcadores associados aos marcos e ao estado patológico. Tomografia por emissão de pósitrons (PET). Imagem por ressonância magnética (MRI). Líquido encefalorraquidiano (CSF). Proteína neurofilamentar de cadeia leve (NfL). Tau-total (t-tau). Retirado de HAMPEL et al., 2021b.

Incorporando esses biomarcadores, Jack et al desenvolveram um modelo postulando a ordem temporal de anormalidade dos biomarcadores em função da progressão da doença (**Figura 9**) (JACK et al., 2013). Assim, a ordem estabelecida das alterações seria: níveis de 1) Aβ no LCR; 2) Aβ por imagem PET; 3) tau no LCR; 4) de glicose por PET ou atrofia por MRI estrutural; e 5) dano cognitivo.

Similarmente aos biomarcadores da patologia clássica da DA, outras características proeminentes podem ser medidas nos fluidos e por imagem PET. Nesse sentido, reatividade astrocitária é medida por meio de deprenyl-PET ou quantificada nos fluidos utilizando marcadores como proteína ácida fibrilar glial (GFAP) e chitinase-3-like protein 1 (YKL40). Ademais, marcadores de neuroinflamação são considerados o PET-TSPO e o marcador triggering receptor expressed on myeloid cells 2 (TREM2) cujos biomarcadores refletem ativação microglial, além de citocinas como CXCL10 no LCR (HAMPEL et al., 2021b) Alterações vasculares são visualizadas por MRI ou refletidas por aumento dos níveis de marcadores associados com quebra da barreira hematoencefálica, como o fator solúvel de crescimento derivado de plaquetas (sPDGF) no LCR (HAMPEL et al., 2021b).

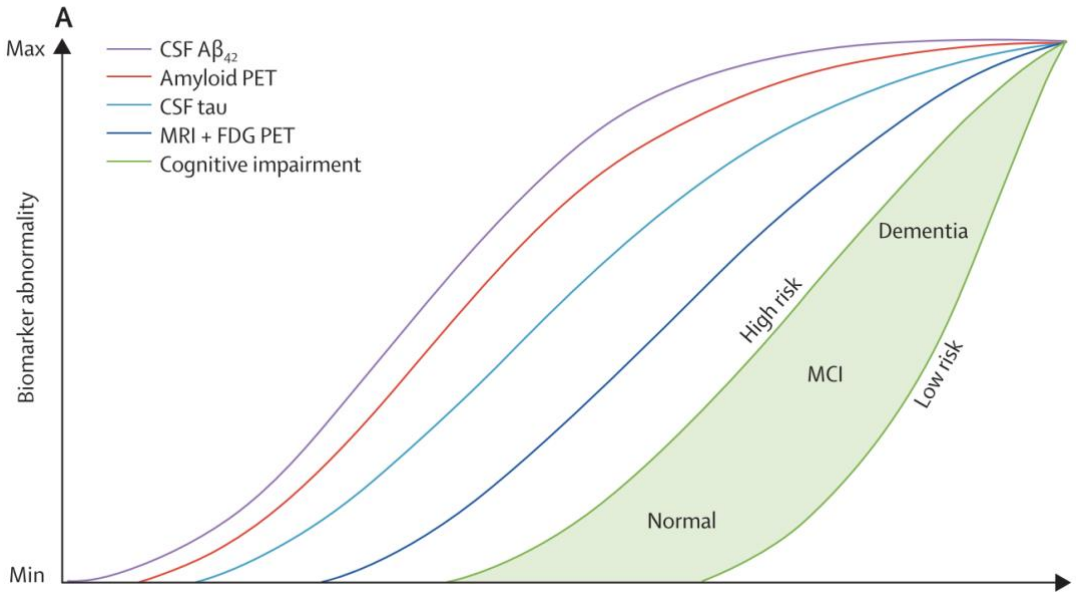

Figura 9. Modelo da evolução temporal de biomarcadores na cascata patológica da doença de Alzheimer. As curvas demonstram os níveis de biomarcadores em função da progressão da DA. A área preenchida em verde representa estágios cognitivos (normal, de declínio leve (MCI), demência). Modificado de JACK et al., 2013.

Ademais, diversos biomarcadores sinápticos são utilizados na DA. Dentre eles, a proteína associada ao sinaptossomo de 25kDa (SNAP25), neurogranina (Ng), proteína associada a crescimento-43 (GAP43), sinaptotagmina, sinaptofisina, pentraxinas e AMPAR encontram-se elevados no LCR (**Figura 9**) (COLOM-CADENA et al., 2020; TZIORAS et al., 2023). Além disso, a ordem temporal de anormalidade de alguns desses biomarcadores foi proposta de modo similar ao modelo de Jack (**Figura 10**).

Notavelmente, estudos clínicos sugerem que anormalidades dos biomarcadores sinápticos ocorrem antes da neurodegeneração axonal (LAN et al., 2022;MILÀ-ALOMÀ et al., 2021), refletida pela perda de volume hipocampal e pelos níveis aumentados de NfL no LCR. Apesar de t-tau ser considerado um biomarcador de neurodegeneração em função do aumento dos níveis após lesão axonal (JACK et al., 2018), pesquisas atuais relataram uma forte associação entre t-tau no LCR e os biomarcadores sinápticos em indivíduos nos estágios iniciais do espectro da DA (MORAR et al., 2022;PEREIRA; WESTMAN; HANSSON, 2017;QIANG et al., 2022;TIBLE et al., 2020). Isto ocorre tanto em indivíduos com comprometimento cognitivo leve quanto na DA pré-clínica, quando há aumento de biomarcadores da DA, porém não há comprometimento cognitivo. De fato, em modelo mais recente, foi proposto que níveis de t-tau se tornam anormais antes de alterações no volume hipocampal e nos níveis de NfL (**Figura 10**) (ZETTERBERG; BENDLIN, 2021). Nesse sentido, níveis de t-tau foram relatados como triplicados nos estágios iniciais da DA (VOS et al., 2013), antes de uma perda neuronal substancial, sugerindo que t-tau pode ser um biomarcador mais específico para mudanças sinápticas precoces do que para a neurodegeneração em estágios posteriores. Essa relação ressalta o potencial do t-tau no LCR como um indicador precoce de degeneração sináptica na DA, em vez de degeneração neuronal evidente.

Embora existam muitos estudos demonstrando alterações sinápticas no LCR, a metodologia invasiva não reflete diferenças regionais no cérebro. Assim, o uso de PET é considerado mais apropriado. Nesse sentido, os marcadores que permitem imageamento de densidade sináptica por PET são os radiofármacos [^{11}C]UCB-J e [^{18}F]SDM8, os quais se ligam na glicoproteína de vesícula sináptica 2A (SV2A, do inglês, *synaptic vesicle glycoprotein 2A*) integrante da membrana da vesícula pré-sináptica (FINNEMA et al., 2016; NAGANAWA et al., 2021). A glicoproteína SV2A está distribuída de forma ubíqua e homogênea pelas sinapses cerebrais, assim como a sinaptofisina (HEURLING et al., 2019). A redução de sua expressão está correlacionada com performance inferior em avaliações neuropsicológicas e com o diagnóstico de DA (ROBINSON et al., 2014). Dessa forma, o PET-SV2A oferece uma oportunidade inovadora e valiosa para diagnóstico de sinaptopatias e para estudos clínicos

experimentais longitudinais e multimodais, ou seja, a avaliação contínua do paciente em combinação com biomarcadores de fluído e avaliações neuropsicológicas.

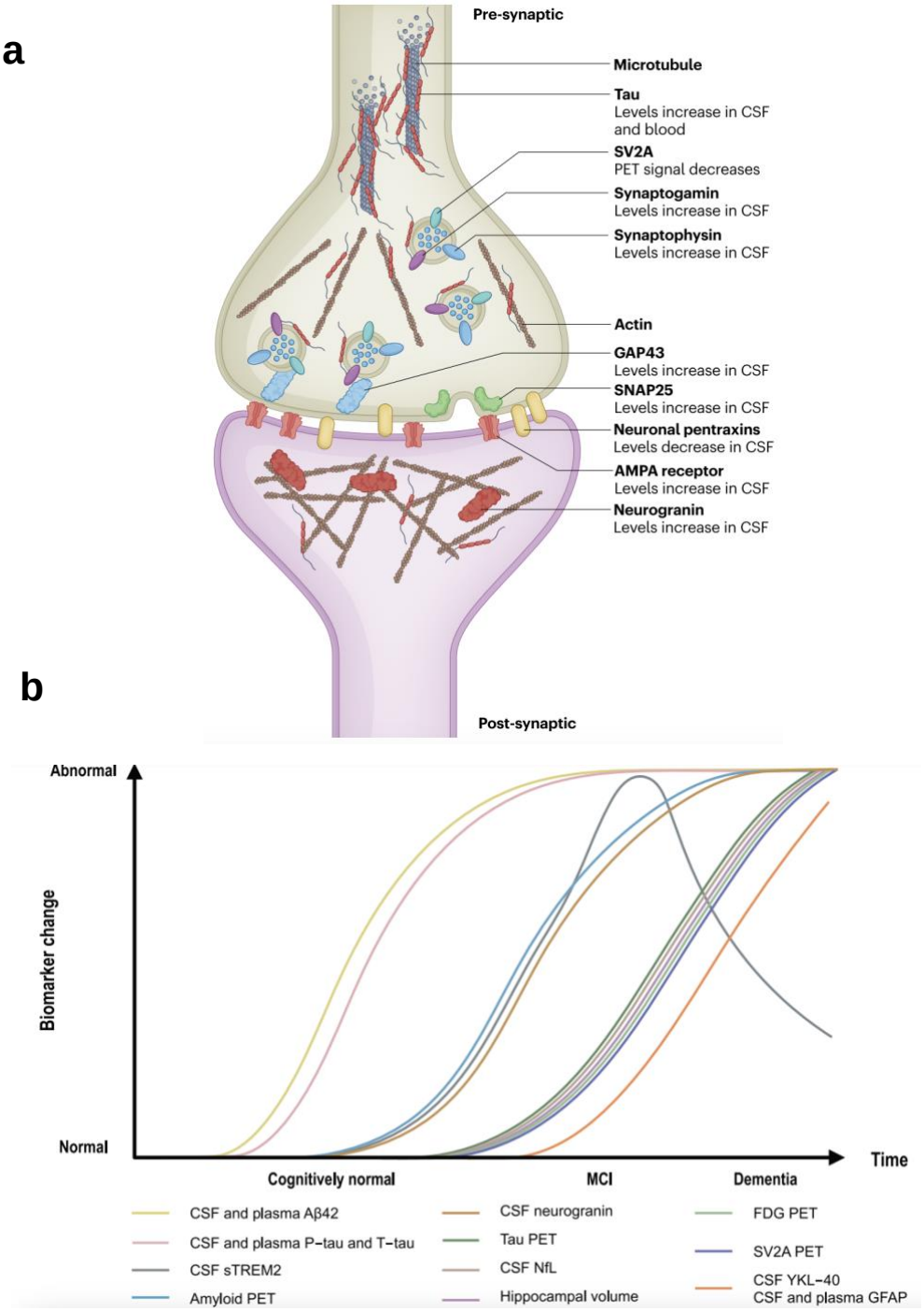

Figura 10. Biomarcadores sinápticos e ordem temporal de anormalidades na doença de Alzheimer. a. Biomarcadores sinápticos. **b.** As curvas demonstram os níveis de biomarcadores em função da progressão da DA. Modificado de TZIORAS et al., 2023; ZETTERBERG; BENDLIN, 2021.

1.3 MODELO TgF344-AD

Animais geneticamente modificados são frequentemente utilizados como ferramenta experimental para investigação da DA. O zebrafish, *Caenorhabditis elegans* e *Drosophila melanogaster* são modelos usados a fim de estudar mecanismos envolvidos na patologia da DA. Entretanto, por se tratar de animais com sistema nervoso muito mais simples e por serem animais mais distantes filogeneticamente dos humanos, esses modelos são considerados bastante limitados (JUCKER, 2010).

Frequentemente os modelos transgênicos da DA expressam mutações humanas/humanizadas da APP, PS1 ou PS2. Contudo, não contemplam de modo robusto as características centrais da DA, como a perda neuronal e a (**Tabela 1**). Além disso, esse modelo apresenta alteração comportamental antes mesmo da deposição de Aβ no cérebro (JUCKER, 2010). Por essa razão, foi desenvolvido um modelo de ratos transgênicos da DA, TgF344-AD, com background de ratos Fischer 344 que superexpressam genes humanos da APP e da PS1 e cujo diferencial é a manifestação progressiva e completa das características da DA: amiloidose cerebral que precede a taupatia, crescente morte neuronal e declínio cognitivo tardio (COHEN et al., 2013).

A fim de investigar a DA, existem versões adaptadas das ferramentas utilizadas em estudos clínicos para animais. De fato, o PET consiste em uma adaptação miniaturizada do PET para pequenos animais, mantendo os princípios metodológicos do PET e radiofármacos utilizados na clínica (ZIMMER et al., 2014b). Assim, o uso dessa ferramenta confere um valor translacional sem precedentes. Nesse sentido, estudos recentes com PET em animais transgênicos demonstram alterações espaciais e temporais similares à DA (ZIMMER et al., 2014a).

1.4 JUSTIFICATIVA

Com o aumento da expectativa de vida, espera-se que a incidência de doenças neurodegenerativas aumente dos atuais 57 milhões de pessoas com demência mundialmente (NICHOLS et al., 2022) para o triplo de casos até 2050 (ALZHEIMER; ASSOCIATION, 2024). Visto que a DA é responsável por cerca de 60 a 80% dos casos de demência, (ALZHEIMER; ASSOCIATION, 2024) seu estudo é de alta relevância. Assim, a prevenção, o tratamento e respostas de saúde pública são urgentemente necessários para enfrentar essa crescente crise de saúde (CUMMINGS et al., 2024).

Tabela 1. Características da DA em modelos animais

Modelo animal	A	T	N	G	S	L	C
rTgTauEC	Não	Sim	Sim	Sim	Sim	Sim	Sim
TauΔK280	Não	Sim	Não	ND	Sim	Sim	Sim
TauRDΔK280	Não	Sim	Sim	Sim	Sim	Sim	Sim
ARTE10	Não	Não	Não	Sim	Sim	ND	Sim
APP E693Δ-Tg (Osaka)	Não	Não	Sim	Sim	Sim	Sim	Sim
hTau.P301S	Não	Sim	Sim	Sim	ND	ND	Sim
Tau P301L	Não	Sim	ND	Sim	ND	ND	ND
Tau P301S (Line PS19)	Não	Sim	Sim	Sim	Sim	Sim	Sim
TgCRND8	Sim	Não	Sim	Sim	Sim	Sim	Sim
TASTPM (TAS10 x TPM)	Sim	Não	Não	Sim	ND	ND	Sim
TAS10 (thy1-APPswe)	Sim	Não	Não	Sim	Sim	Não	Sim
APP NL-F Knock-in	Sim	Não	Não	ND	Sim	ND	Sim
APP NL-G-F Knock-in	Sim	Não	Não	Sim	Sim	ND	ND
PS/APP	Sim	Não	Sim	ND	ND	ND	Sim
APP23	Sim	Não	Sim	ND	Não	Não	ND
PDAPP (line 109)	Sim	Não	Sim	Sim	Sim	Sim	Sim
APPswe/PSEN1dE9 (line 85)	Sim	Não	Sim	Sim	Sim	Sim	Sim
5xFAD (B6SJL)	Sim	Não	Sim	Sim	Sim	Sim	Sim
APP (V717I) x PS1 (A246E)	Sim	Não	Não	Sim	ND	Sim	Sim
APP (V717I)	Sim	Não	Não	Sim	ND	Sim	ND
J20 (PDGF-APPSw,Ind)	Sim	Não	Sim	Sim	Sim	ND	Sim
Tg2576	Sim	Não	Não	ND	Sim	Sim	Sim
APPSwDI x NOS2 KO	Sim	Sim	Sim	ND	ND	ND	Sim
APP (Swedish) (R1.40)	Sim	ND	ND	ND	ND	Não	ND
JNPL3 (P301L)	ND	Sim	Sim	Sim	ND	ND	ND
3xTg	Sim	Sim	ND	Sim	ND	Sim	Sim

Presença (sim), ausência (não) ou dados não disponíveis (ND) acerca de placas amiloides (A), emaranhados neurofibrilares (T), morte neuronal (N), gliose (G), perda sináptica (S), alterações em LTP/LTD (L), dano cognitivo (C). Modificado de alzforum.org (https://www.alzforum.org/research-models/alzheimers-disease (acessado em julho de 2024)

Nesse sentido, estudar a degeneração sináptica na DA é de grande pertinência devido ao papel crítico que a perda sináptica desempenha na progressão da doença. A perda de sinapses é um efeito desencadeado por processos patológicos como amiloidose, taupatia, inflamação e outros mecanismos que ocorrem na DA (SELKOE; HARDY, 2016). Dentre esses processos, a perda sináptica é a que mais fortemente se correlaciona com o declínio cognitivo, uma vez que a função sináptica é fundamental para o desempenho cognitivo (DEKOSKY; SCHEFF, 1990;TERRY et al., 1991). Ainda, dada a presença significativa de sinapses glutamatérgicas no SNC e a vulnerabilidade delas na DA (CONWAY, 2020), focar nesse sistema pode oferecer melhor entendimento da sinaptopatia. Portanto, compreender e abordar a degeneração sináptica

pode fornecer insights vitais para a fisiopatologia da DA e criar oportunidades para intervenções terapêuticas mais eficazes.

Dessa forma, o desenvolvimento de compostos que interrompam ou reduzam o dano ou a perda de sinapses em combinação com o uso de biomarcadores facilitarão o desenvolvimento clínico de tais medicamentos. Nesse contexto, a capacidade de medir sensivelmente a densidade sináptica no cérebro de um paciente vivo, através de tecnologias como a imagem por PET-SV2A e a concentração de proteínas sinápticas no LCR (i.e neurogranina, SNAP25, AMPAR), oferece uma base sólida para usar esses tipos de medições como biomarcadores (COLOM-CADENA et al., 2020) que possam efetivamente melhorar a qualidade de vida dos pacientes. Nesse sentido, a utilização de modelos como o TgF344-AD, que mimetiza a patologia humana da DA (COHEN et al., 2013), é uma ferramenta importante para estudar os mecanismos de degeneração sináptica e testar novos tratamentos voltados para a proteção das sinapses.

Portanto, esta tese aborda alterações sinápticas na DA, sobre as quais hipotetizamos que 1) ocorra uma depleção generalizada do sistema glutamatérgico em indivíduos com DA, 2) t-tau mais correlacionado com biomarcadores sinápticos do que com biomarcadores de neurodegeneração em pacientes no espectro da DA e 3) o modelo TgF344-AD reflete a redução de densidade sináptica encontrada em indivíduos com DA.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Investigar alterações sinápticas que ocorrem na DA.

2.2 OBJETIVOS ESPECÍFICOS

I. Realizar uma revisão sistemática com metanálise abrangente sobre o sistema glutamatérgico no cérebro de indivíduos com DA.

II. Comparar a contribuição da disfunção sináptica e da degeneração axonal para os níveis do biomarcador total-tau no líquido cefalorraquidiano de indivíduos no espectro da DA.

III. Quantificar a densidade sináptica cerebral de SV2A de ratos machos e fêmeas TgF344-AD via PET com [^{18}F]SDM8.

PARTE II

CAPÍTULO 1

REVISÃO SISTEMÁTICA COM METANÁLISE DO SISTEMA GLUTAMATÉRGICO NA DOENÇA DE ALZHEIMER

(Publicado no periódico Molecular Psychiatry em fevereiro de 2024)

Neste capítulo apresentamos uma revisão sistemática com metanálise para examinar se há anormalidades glutamatérgicas consistentes no cérebro humano com DA. Analisamos 16 componentes do sistema glutamatérgico, incluindo transportadores, receptores, componentes do ciclo glutamato-glutamina e recaptação de glutamato no cérebro humano com DA. A busca recuperou 6.936 artigos, dos quais 63 atenderam aos critérios de inclusão. Mostramos que o cérebro de indivíduos com DA apresenta níveis reduzidos de glutamato, aspartato, além de recaptação reduzida. Também encontramos níveis reduzidos de AMPAR-GluA2/3, hipofunção do receptor NMDA e redução seletiva dos níveis da subunidade NMDAR-GluN2B no cérebro como um todo. Ademais, verificamos diferenças regionais de glutamato, aspartato, recaptação de glutamato, AMPAR-GluA2/3, hipofunção de NMDAR e de níveis da subunidade NMDAR-GluN2B no córtex entorrinal e hipocampo. Outros parâmetros estudados não foram alterados.

Nossos achados mostram a depleção do sistema glutamatérgico e enfatizam a importância de entender a neurotoxicidade mediada pelo glutamato na DA. Este estudo tem implicações para o desenvolvimento de terapias e biomarcadores na DA.

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SYSTEMATIC REVIEW

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The glutamatergic system in Alzheimer's disease: a systematic review with meta-analysis

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Glutamatergic neurotransmission system dysregulation may play an important role in the pathophysiology of Alzheimer's disease (AD). However, reported results on glutamatergic components across brain regions are contradictory. Here, we conducted a systematic review with meta-analysis to examine whether there are consistent glutamatergic abnormalities in the human AD brain. We searched PubMed and Web of Science (database origin-October 2023) reports evaluating glutamate, glutamine, glutaminase, glutamine synthetase, glutamate reuptake, aspartate, excitatory amino acid transporters, vesicular glutamate transporters, glycine, D-serine, metabotropic and ionotropic glutamate receptors in the AD human brain (PROSPERO #CDRD42022299518). The studies were synthesized by outcome and brain region. We included cortical regions, the whole brain (cortical and subcortical regions combined), the entorhinal cortex and the hippocampus. Pooled effect sizes were determined with standardized mean differences (SMD), random effects adjusted by false discovery rate, and heterogeneity was examined by I^2 statistics. The search retrieved 6 936 articles, 63 meeting the inclusion criteria (N = 709CN/786AD; mean age 75/79). We showed that the brain of AD individuals presents decreased glutamate (SMD −0.82; I^2 74.54%; $P < 0.001$) and aspartate levels (SMD −0.64; I^2 89.71%; P 0.006), and reuptake (SMD −0.75; I^2 83.04%; $P < 0.001$. We also found reduced α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR)-GluA2/3 levels (SMD −0.63; I^2 95.55%; P 0.046), hypofunctional N-methyl-D-aspartate receptor (NMDAR) (SMD −0.60; I^2 91.47%; $P < 0.001$) and selective reduction of NMDAR-GluN2B subunit levels (SMD −1.07; I^2 41.81%; $P < 0.001$). Regional differences include lower glutamate levels in cortical areas and aspartate levels in cortical areas and in the hippocampus, reduced glutamate reuptake, reduced AMPAR-GluA2/3 in the entorhinal cortex, hypofunction of NMDAR in cortical areas, and a decrease in NMDAR-GluN2B subunit levels in the entorhinal cortex and hippocampus. Other parameters studied were not altered. Our findings show depletion of the glutamatergic system and emphasize the importance of understanding glutamate-mediated neurotoxicity in AD. This study has implications for the development of therapies and biomarkers in AD.

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INTRODUCTION

Glutamate is the most abundant excitatory neurotransmitter in the human brain. It is synthesized through multiple metabolic pathways, stored in vesicles by vesicular glutamate transporters (VGluTs), and released during synaptic transmission. After interacting with metabotropic (mGlu), and ionotropic receptors such as N-methyl-D-aspartate (NMDAR), kainate (GluK), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR), glutamate is removed from the synaptic cleft by excitatory amino acid transporters (EAATs), which also take up aspartate [1]. Glycine and D-serine are essential neuromodulators of the glutamatergic receptor NMDA [2, 3]. It is well established that glutamate neurotransmission is central in orchestrating crucial processes, such as learning and memory [4], which are affected in Alzheimer's disease (AD) dementia.

AD is classically characterized by the accumulation of insoluble amyloid β (Aβ) and tau proteins, followed by neurodegeneration and cognitive decline. In addition, it has been demonstrated that synapse loss is a prominent feature in the AD brain and is highly associated with dementia symptoms [5–8]. Consequently, growing efforts have been made to elucidate underlying mechanisms associated with synaptic failure in AD progression.

In the last decades, numerous publications have reported diverse alterations in the glutamatergic neurotransmission in AD [9]. Studies have found changes in glutamate levels and receptors, dysfunction in the glutamate reuptake system, and loss of

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glutamatergic neurons. However, inconsistent results concerning glutamatergic components across brain regions have been reported. These discrepancies challenge the understanding of the glutamatergic system changes in AD.

To our knowledge, no comprehensive meta-analysis has assessed the glutamatergic system in the AD brain tissue. Therefore, the accumulated evidence is long overdue for a robust statistical analysis. Thus, we aimed to perform a systematic review and meta-analysis to determine whether the sum of the literature points to glutamatergic system changes in the AD human brain.

MATERIAL AND METHODS

Search strategy and selection criteria

The systematic review and meta-analysis was conducted in conformity with the PRISMA guidelines [10]. Two unfiltered databases were searched: PubMed and Web of Science, from inception year to October 31, 2023 (last search) by C.S. Complete search terms can be found in Supplementary Method S1). Additionally, two authors (C.S and G.L.) scrutinized the reference lists from included reports in this meta-analysis and related reviews from the last five years.

Included reports evaluated selected glutamatergic outcomes in clinically or neuropathologically defined AD human brain compared to cognitively normal (CN) individuals. Briefly, reports were excluded if they were/had: a review and meta-analysis; not peer-reviewed original data; no AD compared to CN data from human brain tissue; no selected glutamatergic outcomes; not in English; no clinical or neuropathological diagnostic criteria for AD; an AD group presenting another concomitant neurodegenerative disease; a CN group presenting inflammatory, neuropsychiatric, neurological disorder; any group receiving drugs affecting the glutamatergic system; or insufficient information to calculate the standard mean difference (SMD). A detailed list of the exclusion criteria can be found in Supplementary Method S2.

Records retrieved were uploaded into the program, Rayyan QCRI [11], to aid the screening process. Two authors (C.S. and L.U.dR., L.M., A.R. or G.L.) independently removed duplicates, manually, screened abstracts and eligible full texts, and extracted data. Conflicts were resolved by a third author (B.B.).

Statistical analysis

Study characteristics and sample size, mean and standard deviation (SD)/standard error of the mean (SEM) were collected directly from text or were estimated manually from the graphs with a digital ruler as described previously [12]. Outcomes sought for collection were quantified levels of AMPAR, AMPAR GluA subunits, NMDAR, NMDAR-GluN subunits, GluK, mGlu, EAAT, VGluT, glutamate, glutamine, glutamine synthetase (GS), glutaminase, aspartate, glutamate uptake, aspartate uptake or binding, D-serine, and glycine. Both quantitative and semi-quantitative methods were included, prioritizing the more precise method when necessary. Investigators were contacted twice by email to clarify information or provide missing SD/SEM.

Two authors (C.S. and G.L.) independently assessed methodological quality using an adapted bias tool (Supplementary Method S3). If checklist items were not provided or unclear, the section was labeled as high risk. Bias results are described as a ratio of the number of studies in each synthesis (high risk: low risk: some concerns). Publication bias was estimated by visual inspection of funnel plots and by Egger's regression asymmetry test.

A minimum of three studies were required for each synthesis[10]. Studies were grouped by outcome and brain region. The regions synthesized were a) a composite of cortical (CX) and subcortical regions pooled as "whole brain" (additional information on Supplementary Table S1); b) CX; c) entorhinal cortex (EC); and d) hippocampus (HP). Sample size, mean, and SD from each group were used as input in RStudio 4.2.1 [13] to generate forest plots with SMD with 95% confidence interval (CI) by Hedge's g method providing a more precise estimate of the effect size. To adjust for statistical artifacts produced by studies with small sample sizes, unbiased correction was applied. To mitigate between-study variability, we employed a random-effects model using a DerSimonian and Laird estimate based on the effect size. *Z*-test estimated the significance and was corrected by false discovery rate with 5% *q* value, and the adjusted *P*-value was considered significant when < 0.05. If a study measured the same outcome in multiple subregions (i.e. frontal and temporal cortices), we proceeded as previously described [14]. Briefly, SMD was calculated for each subregion using a fixed-effect model, and pooled effect size was estimated as described above.

Heterogeneity was assessed by I^2 statistic, demonstrating the percentage of the overall variance in pooled estimates attributed to between-study variability [15]. Sensitivity analysis was performed to account for the effect of studies with high risk of bias, and the Jackknife method [16] was used to assess whether any article was skewing the results in each synthesis.

RESULTS

Study selection and characteristics

We found 5267 records after duplicate removal. Following pre-established criteria, 171 reports were selected for full-text evaluation, from which only one article could not be retrieved. Eighty-eight reports were included for data collection. Fourteen corresponding authors were contacted for missing values or clarification; however, nine did not reply or could not provide the data. An additional 47 records were included from the reference list or recent reviews. Altogether, 146 studies were excluded after eligibility assessment and data extraction (Supplementary Table S2). Ultimately, 63 studies assessing 709 CN and 786 AD individuals met the inclusion criteria for our meta-analysis (Fig. 1). Forty-eight studies reported AD clinical diagnosis confirmed by neuropathological examination, while 15 studies had neuropathological diagnosis alone. Overall, CN and AD (CN/AD) individuals had mean age of 75/79 years, 58/50% males, and post-mortem interval of 14.5/12.4 hours. Additionally, the risk of bias assessment revealed a ratio of studies we considered high risk: low risk: some concerns as 29:9:25, representing 46, 14, and 40%, respectively. Study characteristics and statistics are described in Table 1 and Supplementary Table S3. Unless specified, all results correspond to the whole brain (see Methods; and are reported as SMD [lower; upper limit of CI].

Meta-analysis

Glutamate – reuptake, transporters, storage, recycling, and aspartate. We meta-analyzed glutamate reuptake studies in the post-mortem brain of AD individuals compared to CN. The analysis showed a decline in all regions meta-analyzed, whole brain (Fig. 2A, 11 studies; N = 67 CN/79 AD; SMD = −0.75[−1.10; −0.39]; I^2 = 83.07%; P < 0.001), CX, and HP (Supplementary Fig. S1A–B). On the transporter level, we included EAAT2, VGluT1 and VGluT2 (Fig. 2B–D), but none of them were altered (EAAT2: nine studies; N = 159CN/189AD; SMD = −0.25[−0.67; 0.17]; I^2 = 91.64%; P = 0.378), (VGluT1: seven studies; N = 165CN/154AD; SMD = −0.62|−1.36; 0.12|; I^2 = 88.53%; P = 0.198), (VGluT2: three studies; N = 96CN/110AD; SMD = −0.11[−0.25; 0.03]; I^2 = 2.97%; P = 0.259). Similarly, EAAT2 and VGluT1 levels in the CX remained unaltered (Supplementary Fig. S1C-D). Overall, Jackknife's test did not affect any of these results (Supplementary Table S4), and Egger's test indicated no significant publication bias (Supplementary Table S5). Moreover, risk of bias assessment revealed that the ratio of studies with high risk, low risk, and some concerns was 6:0:5 for glutamate reuptake; 5:1:3 for EAAT2; 5:0:2 for VGluT1, and 2:0:1 for VGluT2. Removing studies with a high risk of bias did not change the results (Supplementary Table S6), however, VGlut1 and

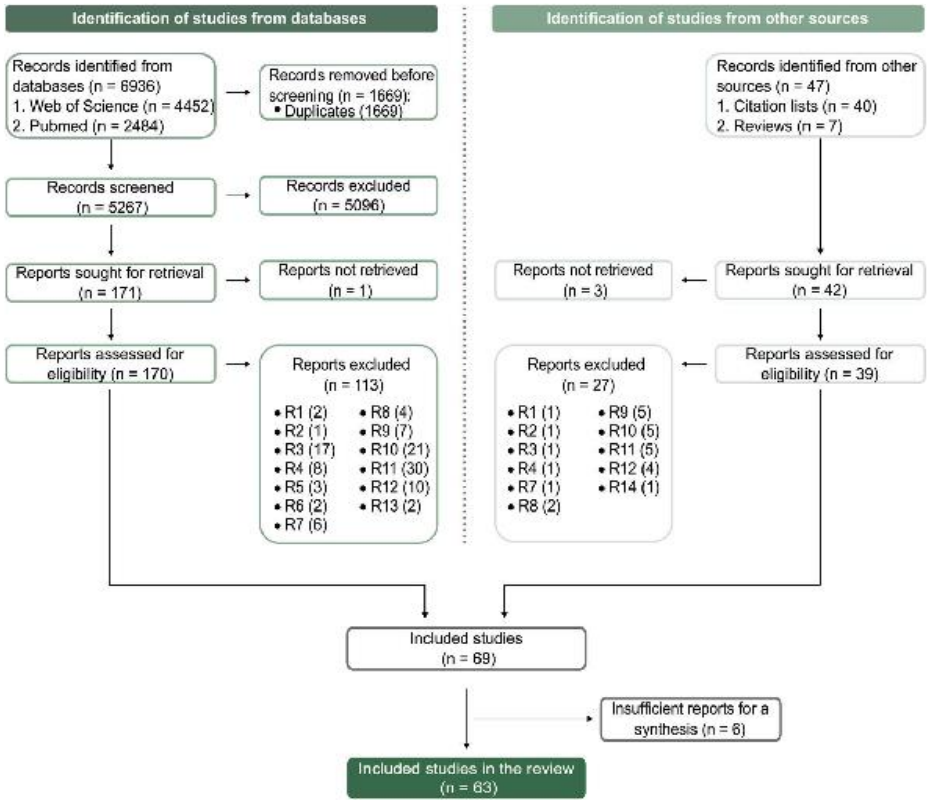


Fig. 1 Study selection. Flowchart of included studies. Exclusion reasons (R) are detailed in Supplementary Method S2.

VGlut2 had insufficient studies for sensitivity analysis. Additionally, we meta-analyzed levels of glutamate, GS, glutamine, and aspartate in the brain (Fig. 2E-H). Accordingly, we found a significant decrease of glutamate levels in both the whole brain (six studies; N = 57CN/58AD; SMD = 0.82[1.17; 0.47]; I^2 – 74.54%; $P < 0.001$) and CX (Supplementary Fig. S1E). Similarly, we found decreased levels of aspartate in all regions analyzed, whole brain (five studies; N 48CN/47AD; SMD −0.61|−1.01; −0.23]; I^2 – 89.71%; P – 0.006), CX and HP (Supplementary Fig. S1G-H). However, we found no significant difference of whole brain GS expression (three studies; N = 27CN/46AD; SMD = 0.33[1.18; 1.78]; $I^2 = 93.01\%$; $P = 0.782$) or glutamine levels (four studies; N – 43CN/43 AD; SMD — 0.12[0.37; 0.13]; I^2 – 64.65%; P – 0.686) nor in the CX (Supplementary Fig. S1F). Furthermore, most of the included studies had low risk of bias or some concerns in glutamate, glutamine and aspartate synthesis, with a bias ratio of 1:3:2, 0:2:2, and 0:2:3, respectively. Conversely, all studies from GS were labeled as high risk. Nevertheless, removal of the study with high risk of bias from the glutamate analysis did not change overall statistical significance. Additionally, Jackknife's test confirmed significant decrease in glutamate levels regardless of any study removed. On the other hand, glutamine levels achieved statistical significance following removal of Gueli and Taibi 2013 [17] ($P < 0.001$), and GS expression resulted in significant decrease ($P = 0.020$) upon removal of Hensley et al. [18]. Moreover, publication bias was significant only for GS ($P < 0.01$). The limited number of studies on EAAT1, EAAT3, VGluT3 and glutaminase prevented their meta-analysis.

Glutamate receptors

Glutamatergic receptors and their subunits were assessed (Fig. 3A–I) in the post-mortem AD brain. Importantly, specific binding to open-NMDAR was quantified in the included studies, thus reported here as such. Meta-analysis of AMPAR density revealed no differences in the AD whole brain (four studies; N – 29CN/30AD; SMD – −0.41[−0.97; 0.15]; I^2 – 93.34%; P 0.253) or HP (Supplementary Fig. S2A) compared to CN. Similarly, the subunit AMPAR-GluA1 was not altered (six studies; N – 60CN/59AD; SMD – 0.59[1.76; 0.57]; I^2 – 92.75%; $P = 0.317$), though we observed a decrease in the HP and EC (Supplementary Fig. S2B-C). Alternatively, AMPAR-GluA2/3 levels were lower in the whole brain (seven studies; N – 68CN/75AD; SMD – −0.63|−1.18; −0.09|; I^2 – 95.55%; P – 0.046) and EC, but not in the HP or CX (Supplementary Fig. S2D-F). Risk of bias analysis indicated a ratio of 1:1:2 for AMPAR, 5:0:1 for AMPAR-GluA1, and 4:1:2 for AMPAR-GluA2/3. Sensitivity analysis indicated that discarding high risk studies from AMPAR-GluA2/3 synthesis resulted in a non-significant change instead (P – 0.221) (Supplementary Table S6). Conversely, AMPAR and AMPAR-GluA1 findings were unaltered. Jackknife's test showed that overall, no single study removal changed the results, except for AMPAR-GluA1, in which case Keihan Falsafi et al. [19] removal resulted in significant AMPAR-GluA1 reduction ($P < 0.001$) (Supplementary Table S4). Moreover, Egger's test was negative for AMPAR and its subunits (Supplementary Table S5). Of note, only one GluA4 study was eligible for data collection [20], thus not meta-analyzed.

Table 1. Characteristics of included studies.

Characteristics												
	Subjects, n		Mean age (SD), y		% Male		Mean PMI (SD), h					
Study	CN	AD	CN	AD	CN	AD	CN	AD	Diagnosis	Outcomes synthesized	Detection method	Bias score
Maxfield et al. [55]	7	16	77 (10.58)	79 (6)	ND	ND	5 (5.29)	6 (6)	Clin. & Neuropath.	Glu reuptake	Binding	9
Cross et al. [56]	4	4	75 (14)	77 (16)	75.0	75.0	41 (58)	18 (46)	Clin. & Neuropath.	Glu reuptake	Binding	8
Rothstein et al. [57]	17	15	67 (16)	76 (8)	70.6	60.0	10.4 (4.4)	9.2 (2.8)	Clin. & Neuropath.	Glu reuptake	Uptake	8
Cowburn et al. [58]	6	6	78 (12.24)	78 (24.49)	33.3	33.3	8.2 (9.80)	10 (17.15)	Clin. & Neuropath.	Glu reuptake	Binding	8
Cowburn et al. [59]	6	6	78 (5)	78 (10)	33.3	33.3	8 (4)	10 (7)	Clin. & Neuropath.	Glu reuptake	Binding	6
Hardy et al. [60]	7	8	65 (15.87)	83 (25.45)	57.1	50.0	11 (10.58)	9 (14.14)	Clin. & Neuropath.	Glu reuptake	Uptake	6
Xuereb et al. [61]	5	5	78 (9)	80 (4)	40.0	20.0	ND	ND	Clin. & Neuropath.	Glu reuptake	Binding	5
Li et al. [62]	4	12	76.5 (12)	79.6 (8.66)	ND	ND	5 (4)	8 (13.86)	Clin. & Neuropath.	Glu reuptake EAAT2	mRNA Binding	7
Beckstrom et al. [63]	10	10	80.5 (21.50)	83 (1.91)	60.0	40.0	5.2 (6.61)	5 (1.2)	Neuropath.	Glu reuptake EAAT2	WB Uptake	6
Chalmers et al. [64]	6	6	84 (4.89)	89 (4.89)	33.3	33.3	11-23	3-15	Clin. & Neuropath.	Glu reuptake AMPAR | GluK	Binding	7
Simpson et al. [64]	9	8	73 (15)	77 (8.48)	44.4	33.3	37 (15)	35 (25.46)	Clin. & Neuropath.	Glu reuptake open-NMDAR	Autorad.	8
Young et al. [65]	9	8	73.88 (6.97)	83.75 (6.89)	66.7	62.5	21.22 (12.69)	12.9 (8.3)	Clin. & Neuropath.	LAAT2	IHC	11
Woltjer et al. [66]	13	22	81 (20.55)	84.3 (10.31)	61.5	54.5	9.8 (11.3)	11.4 (11.4)	Clin. & Neuropath.	LAAT2	LUSA	10
Kobayash et al. [67]	19	18	81.9 (7.8)	91 (8.8)	68.4	38.9	447.7 (219)	421.5 (212.4)	Clin. & Neuropath.	EAAT2	mRNA IHC	10
Tian et al. [68]	6	11	70.2 (8.2)	73.5 (6.6)	83.3	63.6	11.2 (4)	8 (1.6)	Neuropath.	EAAT2	WB	6
Hoshi et al. [69]	5	8	78.2 (2.68)	80.25 (10.9)	80.0	12.5	7 (8.4)	3.6 (0.5)	Clin. & Neuropath.	EAAT2	IHC	5
Garcia-Esparcia et al. [33]	39	20	61.8 (87.42)	80.5 (30.85)	56.4	45.0	3-9	2.5-17.5	Neuropath.	EAAT2 | VGluT1	qRT-PCR	8
Poirel et al. [30]	63	64	78.1 (11.11)	86.7 (9.6)	ND	ND	10.92 (7.54)	6.65 (5.47)	Clin. & Neuropath.	EAAT2 VGluT1 | VGluT2	WB	7
Kashani et al. [39]	5	5	68.6 (12.39)	70.8 (3.27)	20.0	60.0	14.2 (23.43)	8.4 (6.88)	Clin. & Neuropath.	VGluT1	Autorad.	7
Mitew et al. [32]	5	6	ND	ND	ND	ND	ND	ND	Neuropath.	VGluT1	IF	6
Rodrigues-Perdigon et al. [28]	16	15	74.7 (10.68)	81.06 (6.14)	43.8	33.3	ND	ND	Clin. & Neuropath.	VGluT1	WB	6
Sokolow et al. [34]	7	8	89.4 (8.73)	86 (9.33)	42.9	14.3	7.4 (1.4)	6.9 (0.8)	Clin. & Neuropath.	VGlut1 | VGlut2	IMCS	8
Kirvell et al. [31]	34	43	78.2 (13.23)	82.6 (6.58)	66.7	55.7	41.29 (24.67)	45.18 (25.60)	Clin. & Neuropath.	VGluT1 | VGluT2	WB	6

Table 1. continued

Characteristics												
	Subjects, n		Mean age (SD), y		% Male		Mean PMI (SD), h					
Study	CN	AD	CN	AD	CN	AD	CN	AD	Diagnosis	Outcomes synthesized	Detection method	Bias score
Sasaki et al. [70]	10	9	70.5 (11.80)	69 (15.80)	80.0	66.7	8.5 (6.1)	7.2 (4.6)	Clin. & Neuropath.	Glu | Asp	ELISA	12
Hyman et al. [71]	4	6	62.5 (8.80)	79.8 (7.80)	ND	ND	12 (18.28)	8 (18.62)	Clin. & Neuropath.	Glu	HPLC	5
Tommaso et al. [71]	10	10	84.5 (4.43)	85 (2.85)	100.0	100.0	6 (0.63)	5.7 (1.76)	Clin. & Neuropath.	Glu | Gln	HPLC	13
Gudi et al. [17]	13	13	72.7 (34.97)	71.1 (27.4)	ND	ND	31.24 (2.09)	30.09 (2.41)	Clin. & Neuropath.	Glu | Gln Asp	HPLC	12
Gramsbergen et al. [72]	12	12	82.6 (6.58)	79.1 (7.62)	25.0	25.0	55.6 (22.52)	40.6 (22.17)	Clin. & Neuropath.	Glu | Gln Asp	HPLC	8
Burbaeva et al. [73]	9	11	ND	ND	22.2	18.2	ND	ND	Clin. & Neuropath.	GS	WB	7
Hensley et al. [74]	7	22	79.7 (9.4)	78.1 (10.5)	57.1	40.9	4.4 (4)	4.6 (3.1)	Clin. & Neuropath.	GS	Protein	6
Jørgensen et al. [75]	11	13	71 (6.63)	74 (7.21)	ND	ND	27 (26.53)	23 (25.24)	Neuropath.	GS	Antigen	7
Jansen et al. [76]	9	8	70 (15)	77 (22.62)	62.5	66.7	13 (5)	17 (7)	Clin. & Neuropath.	AMPAR | open NMDAR GluK	Binding	8
Geddes et al. [42]	8	10	78.87 (8.11)	81.6 (8.3)	75.0	80.0	11.25 (6.23)	8.4 (6.28)	Clin. & Neuropath.	AMPAR | GluK	Binding	12
Dewar et al. [38]	6	6	75 (7.34)	82 (7.34)	50.0	33.3	10 (2)	11 (2)	Clin. & Neuropath.	AMPAR | GluK mGlu	Binding	10
Pellegrini-Giampietro et al. [77]	10	9	67 (9.48)	73 (9)	60.0	55.6	18 (1)	15 (2)	Neuropath.	GluA1	In situ hyb.	10
González et al. [78]	21	13	53.5 (9.86)	78.76 (3.53)	62.0	69.0	7.23 (4.93)	8 (5.81)	Neuropath.	GluA1	WB	5
Yasuda et al. [79]	6	8	81.3	77.4	ND		ND	ND	Clin. & Neuropath.	GluA1 GluA2/3	WB	3
Falsafi et al. [19]	24	24	82.9 (5.43)	80.41 (4.93)	50.0	50.0	ND	ND	Neuropath.	GluA1 GluA2/3 GluN1	WB	7
Wakabayashi et al. [80]	7	7	68.8 (12.6)	78.5 (10.3)	57.1	57.1	4.8 (3.6)	4.5 (2.9)	Clin. & Neuropath.	GluA1 GluN1	WB	6
Thorns et al. [81]	12	18	ND	ND	ND	ND	ND	ND	Clin. & Neuropath.	GluA2/3	IHC	3
Young et al. [82]	9	8	73.9 (6.97)	83.75 (6.69)	66.7	62.5	21.22 (12.69)	12.9 (8.3)	Clin. & Neuropath.	GluA2/3 | GluN1 GluN2A	IHC	11
Gong et al. [83]	10	12	74.8 (9.89)	78.41 (7.53)	70.0	66.7	7.3 (10.43)	6.9 (8.31)	Clin. & Neuropath.	GluA2/3 | GluN1 mGlu	WB	10
Tsamis et al. [84]	6	6	78.2 (7.02)	77.83 (2.31)	66.7	66.7	13.66 (6.47)	13 (5.1)	Neuropath.	GluA2/3 | GluN1 mGlu	IHC	9
Wang et al. [20]	5	6	66 (2.73)	69 (2.44)	100.0	50.0	11 (1)	8 (1)	Clin. & Neuropath.	GluA1 GluA2/3 GluN1 | GluN2A | GluN2B	WB	6
Tsang et al. [85]	12	22	73.2 (13.85)	78.8 (9.38)	50.0	54.5	14.4 (10.39)	14.2 (31.11)	Clin. & Neuropath.	open-NMDAR	Autorad.	12
Ulas et al. [86]	8	10	78.9 (8.11)	81.6 (8.3)	75.0	60.0	11.25 (6.23)	8.4 (6.28)	Clin. & Neuropath.	open NMDAR	Autorad.	10

Table 1. continued

Characteristics												
	Subjects, n		Mean age (SD), y		% Male		Mean PMI (SD), h					
Study	CN	AD	CN	AD	CN	AD	CN	AD	Diagnosis	Outcomes synthesized	Detection method	Bias score
Ninomiya et al. [87]	10	8	79 (6.3)	81 (8.9)	60.0	25.0	9 (5.05)	9 (2.83)	Clin. & Neuropath.	open NMDAR	Autorad.	8
Scheuer et al. [88]	20	21	75.6 (9.8)	81.2 (7.5)	50.0	33.3	38.1 (11.1)	30.8 (15.2)	Neuropath.	open NMDAR	Autorad.	8
Palmer et al. [89]	8	8	73 (8)	74 (7)	50.0	60.0	11 (7)	10 (6)	Clin. & Neuropath.	open NMDAR	Autorad.	8
Shimohama et al. [90]	5	5	79 (4.47)	80 (6.7)	ND	ND	ND	ND	Clin. & Neuropath.	open NMDAR	Autorad.	5
Mouradian et al. [91]	21	22	73 (9.16)	75 (9.58)	66.7	68.2	16 (8.70)	15 (7.04)	Neuropath.	open NMDAR	Autorad.	5
Ulas et al. [92]	/	/	77.7 (10.31)	78.4 (6.61)	57.1	57.1	6.2 (0.7)	3.7 (0.6)	Clin. & Neuropath.	GluN1	In situ hyb.	14
Berchtold et al. [93]	33	27	84.4 (9)	85.7 (6.3)	42.4	40.7	3.83 (1.91)	4.41 (2.79)	Clin. & Neuropath.	GluN1	qPCR	11
Elnagar et al. [94]	8	/	77.6 (14.97)	61.14 (3.18)	37.5	57.1	7.69 (1.21)	4.76 (0.93)	Clin. & Neuropath.	GluN1	WB	6
Bi et al. [95]	9	9	69.1 (8.35)	83.77 (10.04)	55.6	22.2	10 (6.67)	5.61 (2.03)	Clin. & Neuropath.	GluN1 | GluN2A | GluN2B	qRT-PCR	8
Sze et al. [96]	6	6	85 (6.81)	82.5 (6.41)	100.0	50.0	10.5 (5.61)	12.6 (7.64)	Clin. & Neuropath.	GluN1 | GluN2A | GluN2B	WB	6
Marshal et al. [97]	9	26	72 (12)	74 (10)	44.4	57.7	12 (4)	12 (9)	Neuropath.	GluN2B	IHC	9
Albasanz et al. [37]	5	11	76 (7.58)	79.1 (7.6)	60.0	54.5	9.2 (2.5)	11.18 (5.93)	Neuropath.	mGlu	Binding	10
Lee et al. [98]	13	18	48-87	67-93	ND	ND	6-23	3-14	Clin. & Neuropath.	mGlu	WB	3
Cowburn et al. [43]	6	6	78 (12.24)	78 (24.49)	33.3	33.3	8 (9.80)	10 (17.15)	Clin. & Neuropath.	GluK	Binding	8
Geddes et al. [45]	5	5	ND	ND	60.0	60.0	ND	ND	Neuropath.	GluK	Binding	3
Seidl et al. [22]	8	8	55 (12.8)	61.7 (5.4)	75.0	75.0	26.9 (10.3)	19.9 (12.7)	Neuropath.	Glu | Gln | Asp	HPLC	10
D'Aniello et al. [99]	5	5	75.8 (9.62)	78.2 (13.47)	ND	ND	13.2 (3.1)	14.4 (3.9)	Clin. & Neuropath.	Asp	HPLC	12

SD standard deviation, *CN* cognitively normal, *AD* Alzheimer's disease, *AMPAR* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *AMPAR-GluA* AMPAR-subunit, *Asp* aspartate, *EAAT2* excitatory amino acid transporter 2, *GS* glutamine synthetase, *Glu* glutamate, *Gln* glutamine, *GluK* kainate receptor, *mGlu* metabotropic glutamate receptor, *open-NMDAR* N-methyl-D-aspartate receptor, *ND* not determined, *NMDAR-GluN* NMDAR-subunit, *VGlut* vesicular glutamate transporter, *Autorad.* autoradiography, *In situ hyb.* in situ hybridization, *WB* western blotting, *IHC* immunohistochemistry, *Clin* clinical, *Neuropathol.* neuropathological.

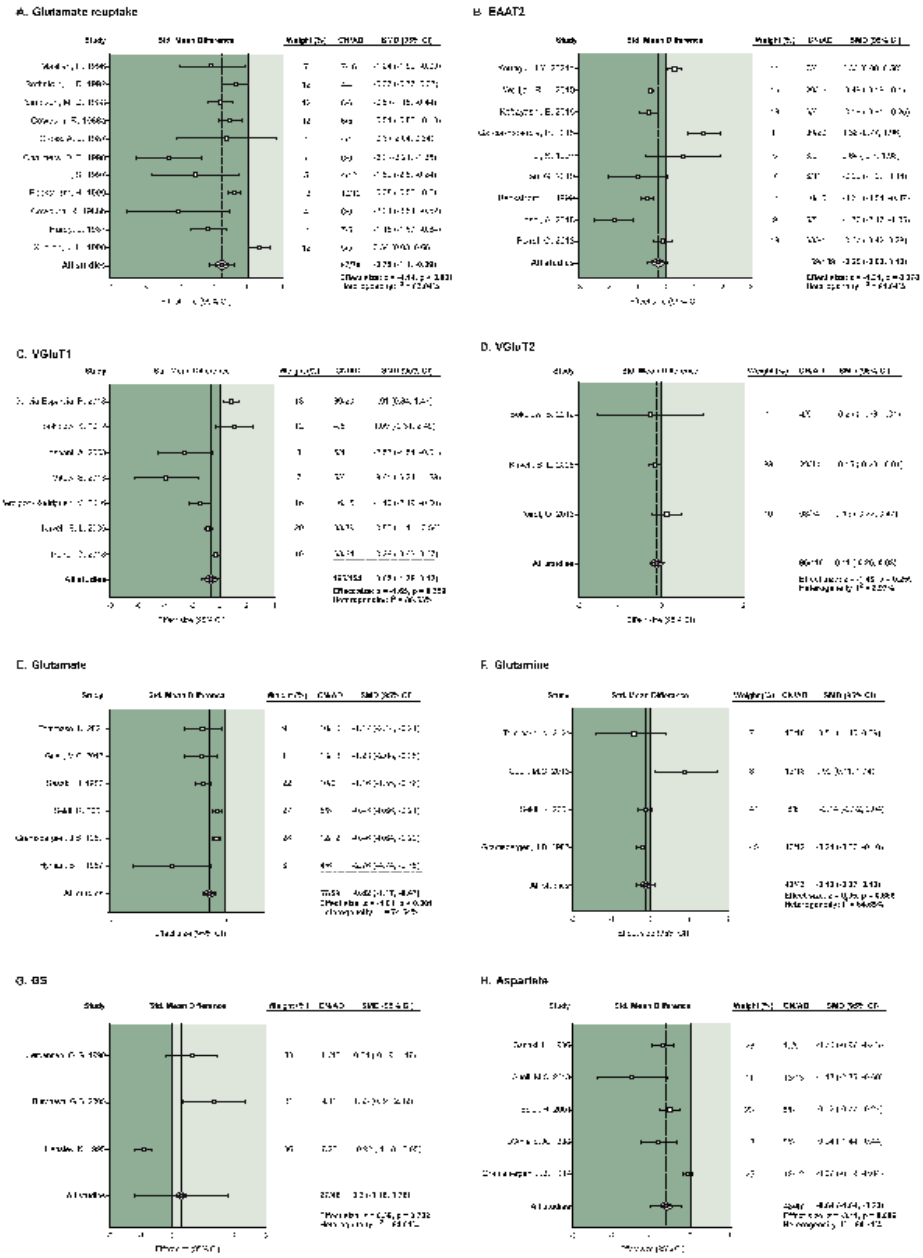


Fig. 2 Forest plots of included studies evaluating glutamate reuptake, transporters, storage, recycling, and aspartate. Overall standardized mean difference (SMD) of glutamate reuptake (**A**), EAAT2 (**B**), VGluT1 (**C**), VGluT2 (**D**), glutamate (**E**), glutamine (**F**), GS (**G**) and aspartate (**H**). Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. EAAT2 excitatory amino acid transporter 2, VGluT vesicular glutamate transporter, GS glutamine synthetase, CN cognitively normal, AD Alzheimer's disease, CI confidence interval.

Additionally, we observed a marked reduction of specific glutamate binding to open-NMDAR in the AD whole brain (nine studies; N = 93CN/97AD; SMD = −0.6[−0.92; −0.28]; $I^2 = 91.47\%$; $P < 0.001$) and CX but not HP (Supplementary Fig. 53A-B). Interestingly, of all the NMDAR subunits analyzed (GluN1, GluN2A, and GluN2B), only the NMDAR-GluN2B was significantly reduced in the whole brain (GluN2B: four studies; N = 23CN/28AD; SMD = −1.07[−1.45; −0.68]; $I^2 = 41.81\%$; $P < 0.001$). (GluN2A: four

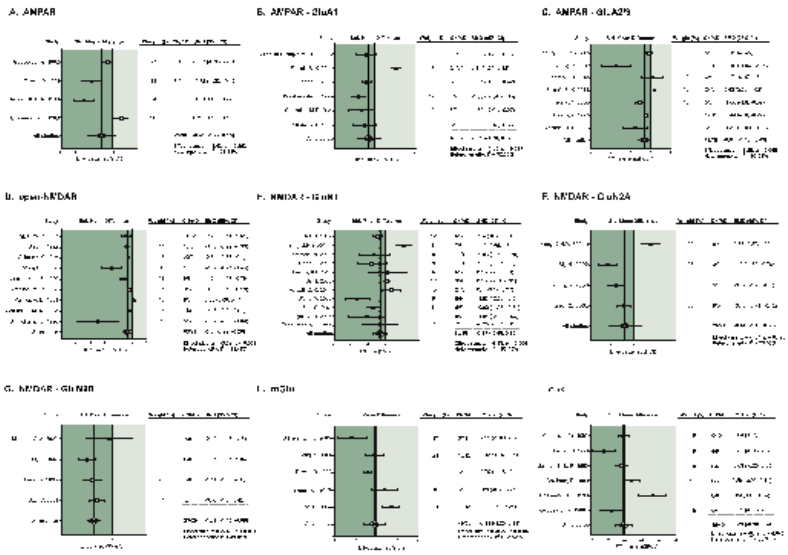


Fig. 3 Forest plots of included studies evaluating glutamate receptors. Overall standardized mean difference (SMD) of AMPAR (**A**), AMPAR-GluA1 (**B**), AMPAR-GluA2/3 (**C**), open-NMDAR (**D**), NMDAR-GluN1 (**E**), NMDAR-GluN2A (**F**), NMDAR-GluN2B (**G**), mGlu (**H**) and GluK (**I**). Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. AMPAR α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, AMPAR-GluA AMPAR-subunit, GluK kainate receptor, mGlu metabotropic glutamate receptor, open-NMDAR N-methyl-D-aspartate receptor, NMDAR-GluN NMDAR-subunit, CN cognitively normal, AD Alzheimer's disease, CI confidence interval.

studies; N — 26CN/24AD; SMD — -0.41 -1.18; 0.36|; I^2 — 93.30%; *P* 0.123), (GluN1: eleven studies; N 98CN/98AD; SMD -0.27|-0.68; 0.13|: I^2 — 86.40%; *P* — 0.504). The same happens in HP and EC (Supplementary Figs. S3–4). Risk of bias assessment indicated a ratio of 2:1:6 for open-NMDAR, 5:3:3 for NMDAR-GluN1, 2:1:1 for NMDAR-GluN2A and 2:0:2 for NMDAR-GluN2B. Subsequent exclusion of studies with high risk of bias did not affect the result of open-NMDAR or NMDAR-GluN1, however, insufficient remaining studies with NMDAR-GluN2A and NMDAR-GluN2B prevented same analysis (Supplementary Table S6). Jackknife's test from the whole brain analysis did not change the overall result of open-NMDAR (Supplementary Table S4), GluN1 or GluN2B, although GluN2B publication bias was detected by Egger's test and visual inspection (*P* — 0.020) (Supplementary Table S5). Alternatively, Jackknife's test indicated that the removal of Yeung et al. 2021b significantly modified the result of the GluN2A synthesis (*P* — 0.002) (Supplementary Table S4). Other NMDAR subunits (GluN2C-D, GluN3A-B) and neuromodulators, glycine [21, 22] and D-serine [22], were not reported due to an insufficient number of eligible studies.

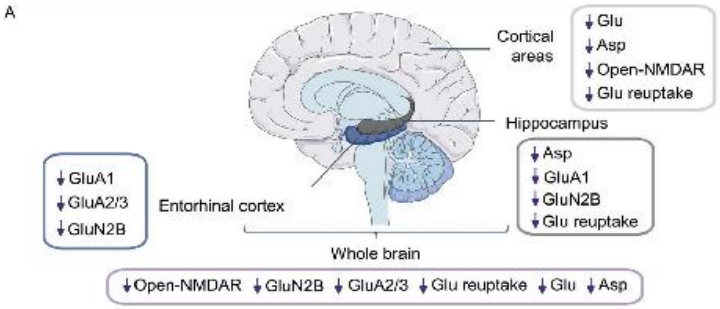
Density of mGlu and GluK (Fig. 3H, I) were not significantly altered in the whole brain (mGlu: five studies; N = 40/53; SMD = 0.15[1.23; 0.93]; I^2 = 88.52%; *P* = 0.845) (GluK: six studies; N = 40/40; SMD = -0.03[-0.30; 0.24]; I^2 = 86.31%; *P* = 0.846) or in the HP (Supplementary Fig. S5). Additionally, the risk of bias ratio was 1:0:4 and 2:1:3 for mGlu and GluK, respectively, and further exclusion of studies with high risk did not change the overall results (Supplementary Table S6). Neither Jackknife's method nor Egger's test showed differences for these outcomes (Supplementary Tables S4–5).

DISCUSSION

Here, we present the most comprehensive meta analysis evaluating the scientific literature about the glutamatergic system in AD. We examined the expression/levels/function of glutamate, aspartate, glutamine, glutamatergic receptors and transporters, and glutamate recycling proteins. Our results indicate a global dysfunction in the glutamatergic neurotransmission system in the human AD brain (**Fig. 4**) and support future studies focusing on currently available and novel drugs that modulate the glutamatergic system in AD.

More specifically, our meta-analysis revealed reduced glutamate reuptake in all analyzed brain regions (whole brain, CX, and HP), suggesting elevated glutamate/aspartate levels in the synaptic cleft that can lead to neuronal death through excitotoxicity [23]. In contrast to our reuptake findings, the observed decrease in aspartate (whole brain, CX, and HP) and glutamate levels (whole brain and CX) in the AD brain tissue may not reflect their concentration in the synaptic cleft but overall loss. Interestingly, glutamate levels were not altered in the CSF of AD patients, as reported in another meta-analysis [24], indicating that regional variability cannot be dismissed. To our knowledge, this is the first study to perform a meta-analysis on aspartate levels in the AD brain. Importantly, although EAAT2 levels appeared normal, studies have shown that AD individuals have a higher proportion of splice variants linked to impaired reuptake [25]. In addition, EAAT2 is mostly located in astrocytes and cellular density differs across brain regions, which may have an important influence on interpreting these results [26]. Thus, further investigation of EAAT2 expression in AD is warranted. Furthermore, we found no alteration in GS expression or glutamine levels, and in contrast to glutamate, these results are mirrored in the CSF [24]. Unfortunately, glutaminase studies were insufficient to provide a complete glutamate glutamine cycle analysis in the AD brain. Nonetheless, collectively, our results indicate that AD individuals present impaired aspartate/glutamate reuptake and aspartate levels but partial conservation of the astrocytic glutamate glutamine cycle.

A

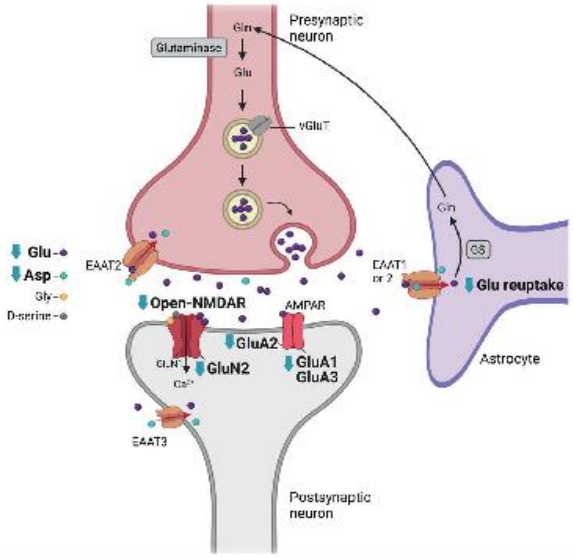


B

Outcome	Region	SMD	P-value	CN (N)	AD (N)	Bias score
Glutamate reuptake	WB	-0.75	< 0.001	67	79	6 5 0
Glutamate reuptake	CX	-0.99	< 0.001	66	76	5 5 0
Glutamate reuptake	HP	-0.76	0.015	26	22	1 3 0
Glutamate	WB	-0.82	< 0.001	57	58	1 2 3
Glutamate	CX	-0.76	0.018	53	52	0 2 3
Aspartate	WB	-0.64	0.006	48	47	0 3 2
Aspartate	CX	-0.58	0.016	48	47	0 3 2
Aspartate	HP	-0.86	0.006	27	25	0 2 1
AMPAR - GluA1	EC	-1.08	0.003	18	21	3 0 0
AMPAR - GluA1	HP	-1.20	< 0.001	30	27	3 1 0
AMPAR - GluA2/3	WB	-0.63	0.046	68	75	4 2 1
AMPAR - GluA2/3	EC	-0.84	0.008	30	33	3 0 1
Open-NMDAR	WB	-0.60	< 0.001	93	97	2 6 1
Open-NMDAR	CX	-0.58	0.006	73	79	2 4 1
NMDAR - GluN2B	WB	-1.07	< 0.001	23	28	2 2 0
NMDAR - GluN2B	EC	-1.49	0.005	20	20	2 1 0
NMDAR - GluN2B	HP	-0.94	0.005	23	28	2 2 0

High risk
Some concerns
Low risk

C



VGluT1 and VGluT2 are presynaptic markers and despite consistent evidence of synapse loss in AD [27], we observed normal levels of both markers. Importantly, we included conflicting reports of VGluT1 expression showing a decrease throughout the brain [28–32], normal levels in the frontal [33] and temporal cortices [31], and an increase in the parietal cortex [34]. Conversely, VGluT2 levels were consistently normal. Interestingly, a meta-analysis found a decline in "vesicular organization" proteins in the post-mortem AD brain [35], although this category mostly included synaptophysin, another

Fig. 4 Summary of changes in the glutamatergic system in the AD brain. A Brain illustration of results shown by analyzed region: WB, CX, HP and EC. Arrows represent decrease of glutamatergic component in the human post-mortem AD brain. **B** Table showing alterations in the glutamatergic synapse, divided by glutamatergic component and region analyzed. Risk of bias assessment and the number of reports considered as high risk, with some concerns or with low risk is indicated next to the respective label. **C** Illustration of a glutamatergic synapse and the alterations of components in the AD brain indicated by the arrows. Glutaminase, D-serine and glycine were not meta-analyzed due to insufficient number of studies. AD Alzheimer's disease, CN cognitively normal, SMD standardized mean difference, Regions: WB whole brain, CX cortical areas, HP hippocampus, EC entorhinal cortex. Components: Asp aspartate, AMPAR α amino 3 hydroxy 5 methyl 4 isoxazolepropionic acid receptor, AMPAR-GluA AMPAR-subunit, D-serine; EAAT excitatory amino acid transporter, Gln glutamine, Glu glutamate, Gly glycine, GS glutamine synthetase, open-NMDAR N-methyl-D-aspartate receptor, NMDAR-GluN NMDAR-subunit, VGluT vesicular glutamate transporter.

presynaptic marker. Taken together, VGluTs may not be driving the vesicular dysregulation in AD.

Furthermore, we showed unchanged glutamate binding levels to AMPAR in the whole brain composite and HP. However, when we analyzed specific AMPAR subunits, we observed a significant reduction in AMPAR-GluA1, but unchanged AMPAR-GluA2/3. Both subunits were decreased in the EC, a region preceding tau accumulation in the HP [36]. In contrast, we found no alteration of mGlu and GluK glutamate binding in the AD brain. Importantly, we pooled different mGlu, despite known differences, because studies reported their results as such [37, 38], possibly hindering selective differences. Two positron emission tomography (PET) studies [39, 40] were not included because they measured mGlu in living patients, and we considered them inappropriate to combine with post-mortem tissue. Regarding GluK, studies exhibited conflicting results, showing no differences [41–43], increased [44, 45] and decreased [38] binding in AD, despite homogeneous methods.

We further found a decrease in open-NMDAR in the whole brain and CX. This is a relevant finding, considering that NMDAR channel opening is necessary for NMDAR signaling, suggesting widespread NMDAR hypoactivation in AD. This is likely a progression from excitotoxicity, an effect of excessive NMDAR activation observed in the early stages of AD and reported by multiple studies [46]. This is consistent with AD post-mortem tissue, which usually represents more advanced AD pathology, thus, more likely exhibiting the effects of long-term excitotoxicity. Importantly, memantine, an open-NMDAR channel blocker used to treat moderate to severe AD cases [47], is believed to reduce excitotoxicity [48]. Alternatively, although not meta-analyzed due to the limited number of studies, one cannot rule out a reduced density or internalization of NMDAR. We meta-analyzed expression of NMDAR subunits and observed a selective NMDAR-GluN2B decrease in the whole brain, HP, and EC, whereas GluN1 and GluN2A remained consistently normal. Typically, NMDAR has two glycine-binding GluN1 subunits and two glutamate-binding GluN2A-D or GluN3 subunits, and the most common assembly is the GluN1 GluN2A GluN2B in hippocampal synapses [49]. In this context, the fact that we found a reduction of GluN2B, but not GluN2A could indicate a compensatory assembly of GluN2A-containing NMDAR. Preclinically, NMDAR-GluN2B antagonists have demonstrated potential in rescuing the harmful effects of Aβ dysmetabolism, including synapse loss, reduced long-term potentiation (LTP), and increased long-term depression (LTD) [50–52], thus showing a significant role of GluN2B-containing NMDARs in synaptic plasticity disruptions. The decrease in GluN2B containing NMDAR levels we observed in the AD brain further supports this understanding. Moreover, conflicting results regarding the prominent role of GluN2A and GluN2B on LTP/LTD and their location on synaptic/extrasynaptic sites [53, 54] increment the challenge to understand the contribution of these subunits to AD and how memantine alleviates AD symptoms. Our results reinforce the important role of NMDAR in AD and support further efforts to understand the excitotoxicity dynamics across the AD continuum.

Strengths and limitations

To enhance the robustness of the meta-analysis, this study used stringent criteria, requiring the inclusion of a minimum of three studies for synthesis and adopting a conservative threshold for considering studies with a higher risk of bias. A composite of cortical and subcortical regions was pooled due to insufficient data to integrate brain regions individually. Whenever possible, we performed meta-analysis of the individual region reported. Thus, whole brain results may mask subtle differences. Nonetheless, this analysis is relevant to examine alterations of glutamatergic components as a meaningful process throughout the brain. Furthermore, the low number of studies and high heterogeneity in some synthesis affect the statistical power. Finally, controls are not necessarily "disease free individuals", but have been controlled for absence of dementia or AD pathophysiology, and other brain or psychiatric diseases. One should keep in mind that a systematic review with meta-analysis is a summary of the literature, which cannot completely eliminate the potential for bias or error since negative data have a higher probability of not being published.

CONCLUSION

This sum of the literature indicated a global glutamatergic dysfunction in AD, as well as a regional vulnerability and a decrease of specific components, likely reflecting a response to excitotoxicity. More studies are required to elucidate the contribution of glutamate system abnormalities in AD pathophysiology. Finally, we recommend further investigation of the glutamatergic system in earlier stages of the disease.

DATA AVAILABILITY

Data will be provided upon reasonable request.

CODE AVAILABILITY

Scripts used for statistical analysis will be provided upon reasonable request.

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AUTHOR CONTRIBUTIONS

Study conception and design: C.S., G.C.C., B.B., and E.R.Z. Article screening and data extraction: C.S., L.U.dR., L.M., A.R. and G.L. Data preparation: C.S. and B.B. Meta analysis: C.S. and M.A.B. Data interpretation: C.S., B.B. and E.R.Z. Manuscript preparation and figure elaboration: C.S. Intelectual content: all authors. All authors revised and approved the final version of the manuscript.

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COMPETING INTERESTS

E.R.Z. served in the SAB of Novo Nordisk, serves on SAB of Next Innovative Therapeutics (Nintx) and serves on the SAB and is a Co founder of MASIMA. P.R.N. served in the SAB of Novo Nordisk, Eisai and Ely Lily and as a Consultant in Eisai and Cerveau radiopharmaceuticals. Other authors declare no conflict of interest.

ADDITIONAL INFORMATION

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Supplementary information

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Supplementary Method S1. Search terms

Search terms appropriately used for each database.

Base Terms

Pubmed	11 May 2022: ("alzheimer disease"[MeSH Terms] OR "alzheimer"[All fields] AND "disease"[All fields]) OR ("alzheimer disease"[All fields] OR "alzheimer's"[All fields]) AND (("vGlut"[All fields]) OR ("AMPAR"[All fields] OR "AMPA"[All fields] AND "Receptor"[All fields]) OR ("NMDAR"[All fields] OR "NMDA"[All fields] AND "Receptor"[All fields]) OR ("mGluR1"[All fields]) OR ("mGluR5"[All fields]) OR ("EAAT1"[All fields]) OR ("GLAST"[All Fields]) OR ("Glutamine synthetase"[All fields]) OR ("Glutamate uptake"[All fields]) OR (“Glutamatergic system”[All fields]) OR (“GLT1”[All fields]) OR (“Glutamate transporter 1” [All fields]) OR (“Glutamate transporter-1” [All fields]) OR (“GLT-1”[All fields]) OR (“EAAT2”[All fields]) OR (“Glutamate aspartate transporter”[All fields])) 31 October 2023: ("alzheimer disease"[MeSH Terms] OR ("alzheimer"[All Fields] AND "disease"[All Fields]) OR "alzheimer disease"[All Fields] OR "alzheimer's"[All Fields]) AND ("postmortem"[All Fields] OR "post-mortem"[All Fields] OR "post-mortem"[All Fields]) AND (("d-serine"[All Fields] OR "serine"[All Fields]) OR "glycine"[All Fields])
Web of Science	11 May 2022: (ALL = (Alzheimer*) AND ALL= ("vGlut" OR “AMPA” OR “NMDA” OR "mGluR1" OR "mGluR5" OR "EAAT1" OR "GLAST" OR "Glutamine synthetase" OR "Glutamate uptake" OR "GLT1" OR "Glutamate transporter 1" OR "Glutamate transporter-1" OR "GLT-1" OR "EAAT2" OR "Glutamate aspartate transporter")) 31 October 2023: (ALL = (Alzheimer*) AND ALL= ("postmortem" OR "post-mortem") AND ALL= ("glycine" OR "d-serine" OR "serine"))

Supplementary Method S2. Detailed exclusion criteria

Reports were excluded if it was or had: 1) a review and meta-analysis;
2) not peer-reviewed original data;
3) no AD and/or control group;
4) not glutamate-related;
5) retracted;
6) not in English;
7) no clinical or neuropathological diagnostic criteria for AD;
8) AD group presenting other concomitant neurodegenerative disease or receiving drugs affecting the glutamatergic system (i.e.. memantine, carbamazepine);
9) control group presenting inflammatory, neuropsychiatric, neurological and/or receiving drugs affecting the glutamatergic system or indicating any neurological/psychiatric disease;
10) no selected glutamatergic outcomes;
11) not quantitative or data were insufficient to calculate the standard mean difference (SMD); 12) data not from brain tissue;
13) less than three samples in each group;
14) same data were previously published and included in this study;
15) not available;
16) to be synthesized with less than three eligible studies;
17) data from animal, in vitro, or in silico studies.

Supplementary Method S3. Risk of bias questionnaire

To assess methodological quality, included reports were appraised and rated employing an adapted questionnaire from the JBI Critical Appraisal Checklist for Case Control Studies by two authors independently (C.S. and G.L.). The adapted questionnaire was composed of seven sections or items (i.e., how comparable the groups were, if investigators were blind from diagnosis and method description). A few items were subdivided (i.e. if the groups were age-, sex-, and PMI- matched). To each sub-item, the possible answers were ‘yes’, ‘no’ or ‘unclear’. If any of the sub-items was labeled ‘no’ or ‘unclear’, the item received score of zero. The studies were rated in overall as ‘high risk’ (score 0-7), with ‘some concerns’ (score 8-10), or ‘low risk’ (score 11-15) and described as a ratio of the number of studies in each category (high risk: low risk: some concerns).

CHECKLIST FOR CASE CONTROL STUDIES

Critical Appraisal tools for use in JBI Systematic Reviews

INTRODUCTION

JBI is an international research organisation based in the Faculty of Health and Medical Sciences at the University of Adelaide, South Australia. JBI develops and delivers unique evidence-based information, software, education and training designed to improve healthcare practice and health outcomes. With over70 Collaborating Entities, servicing over 90 countries, JBI is a recognised global leader in evidence-based healthcare.

JBI Systematic Reviews

The core of evidence synthesis is the systematic review of literature of a particular intervention, condition or issue. The systematic review is essentially an analysis of the available literature (that is, evidence) and a judgment of the effectiveness or otherwise of a practice, involving a series of complex steps. JBI takes a particular view on what counts as evidence and the methods utilised to synthesise those different types ofevidence. In line with this broader view of evidence, JBI has developed theories, methodologies and rigorous processes for the critical appraisal and synthesis of these diverse forms of evidence in order to aid in clinical decision-making in healthcare. There now exists JBI guidance for conducting reviews of effectiveness research, qualitative research, prevalence/incidence, etiology/risk, economic evaluations, text/opinion, diagnostic test accuracy, mixed-methods, umbrella reviews and scoping reviews. Further information regarding JBI systematic reviews can be found in the JBI Evidence Synthesis Manual.

JBI Critical Appraisal Tools

All systematic reviews incorporate a process of critique or appraisal of the research evidence. The purpose of this appraisal is to assess the methodological quality of a study and to determine the extent to which a study has addressed the possibility of bias in its design, conduct and analysis. All papers selected for inclusion in the systematic review (that is – those that meet the inclusion criteria described in the protocol) need to be subjected to rigorous appraisal by two critical appraisers. The results of this appraisal can then be used to inform synthesis and interpretation of the results of the study. JBI Critical appraisal tools have been developed by the JBI and collaborators and approved by the JBI Scientific Committee following extensive peer review. Although designed for use in systematic reviews, JBI critical appraisal tools can also be used when creating Critically Appraised Topics (CAT), in journal clubs and as an educational tool.

JBI CRITICAL APPRAISAL CHECKLIST FOR CASE CONTROL STUDIES

Reviewer_____ Date_____

Author_____ Year_____ Record Number_____

	Yes (score:maximum)	No (score: 0)	Unclear (score: 0)
1. Were cases and controls comparable and matched appropriately? (Score: 3)	☐	☐	☐
2. Were the same criteria used for identification of cases and controls? (Score: 2)	☐	☐	☐
3. Was the outcome measured in a standard, valid and reliable way? (Score: 2)	☐	☐	☐
4. Was the outcome measured in the same way for cases and controls? (Score: 3)	☐	☐	☐
5. Were confounding factors identified and were the strategies to deal with them stated? (Score:2)	☐	☐	☐
6. Was the conflict of interests disclosed? (Score:1)	☐	☐	☐
7. Was the investigator blind for group condition when the outcome was evaluated? (Score: 2)	☐	☐	☐

Maximum score: 15

Low risk of bias: score 11 or +Some concerns: score 8 to 10High risk of bias: score 7 or –

Overall risk of bias: HIGH RISK ☐ SOME CONCERNS ☐ LOW-RISK ☐

Comments/observations (e.g., there is a relevant information in the paper that was not contemplated in the questions or if there is a need to contact the authors):

EXPLANATION OF CASE CONTROL STUDIES CRITICAL APPRAISAL

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Case Control Studies Critical Appraisal Tool

Answers: Yes, No, Unclear.

Explain using direct quotes from the study or state any assumptions.

1. **Were cases and controls comparable and matched appropriately?**

   See if the subjects are* :

   A. age-matched (yes/no/unclear),

   B. PMI-matched (yes/no/unclear),

   C. sex-matched (yes/no/unclear).

   *if they do not mention these information, you can do the statistics to confirm it.

   D. See if they mention the age of the subjects (yes/no/unclear).Full

   score: 3/4 or 4/4 yes answers.

   Explanation:

   The control group should be representative of the source population that produced the cases. This is usually done by individual matching; wherein controls are selected for each case on the basis of similarity with respect to certain characteristics other than the exposure of interest. Frequency or group matching is an alternative method. Selection bias may result if the groups are not comparable. The study should include clear definitions of the source population. Sources from which cases and controls were recruited should be carefully looked at. For example, cancer registries may be used to recruit participants in a study examining risk factors for lung cancer, which typify population-based case control studies. Study participants may be selected from the target population, the source population, or from a pool of eligible participants (such as in hospital- based case control studies).

2. **Were the same criteria used for identification of cases and controls?**

   a. See if there was a control group definition (yes/no/unclear).

   b. See if there was neuropathological confirmation (AD group), when applicable (only in post-mortem studies) (yes/no/unclear).

   c. See if the source where controls and AD were recruited is the same (yes/no/unclear).

   d. See if the population of the study is representative of the real disease condition(yes/no/unclear).

   Full score: 3/4 or 4/4 yes answers.

   Explanation:

   It is useful to determine if patients were included in the study based on either a specified diagnosis or definition. This is more likely to decrease the risk of bias. Characteristics are another useful approach to matching groups, and studies that did not use specified diagnostic methods or definitions should provide evidence on matching by key characteristics. A case should be defined

clearly. It is also important that controls must fulfil all the eligibility criteria defined for the cases except for those relating to diagnosis of the disease.

3. Was the outcome measured in a standard, valid and reliable way?

See if the analysis is quantitative (yes answer) or semi-quantitative (no answer).

Explanation:

The study should clearly describe the method of measurement of the outcome. Assessing validity requires that a 'gold standard' is available to which the measure can be compared. The validity of outcome measurement usually relates to whether a current measure is appropriate. Case control studies may investigate many different 'outcomes' that may or may not be associated with the condition. In these cases, reviewers should use the main outcome of interest for their review to answer this question when using this tool at the study level. Read the methods section of the paper. If for e.g. lung cancer is assessed based on existing definitions or diagnostic criteria, then the answer to this question is likely to be yes. If lung cancer is assessed using observer reported, or self-reported scales, the risk of over- or under-reporting is increased, and objectivity is compromised. Importantly, determine if the measurement tools used were validated instruments as this has a significant impact on outcome assessment validity. Having established the objectivity of the outcome measurement (e.g. lung cancer) instrument, it's important to establish how the measurement was conducted. Were those involved in collecting data trained or educated in the useof the instrument/s? (e.g. radiographers). If there was more than one data collector, were they similar in terms of level of education, clinical or research experience, or level of responsibility in the piece of research being appraised?

4. Was the outcome measured in the same way for cases and controls?

Explanation:

As in item 3, the study should clearly describe the method of measurement of outcome. The outcome measures should be clearly defined and described in detail. Assessment of outcome or risk factors should have been carried out according to same procedures or protocols for both cases and controls.

5. Were confounding factors identified and were the strategies to deal with them stated?

A. See if the cause of death for control and AD group was described (yes/no/unclear).

B. See if it is mentioned that the participants did **NOT** have inflammatory/psychiatric conditions, or were **NOT** using drugs (yes/no/unclear) and/or if participants that had inflammatory, psychiatric conditions, or were using drugs were excluded (yes/no/unclear).

Full score: 1/2 yes answers.

Explanation:

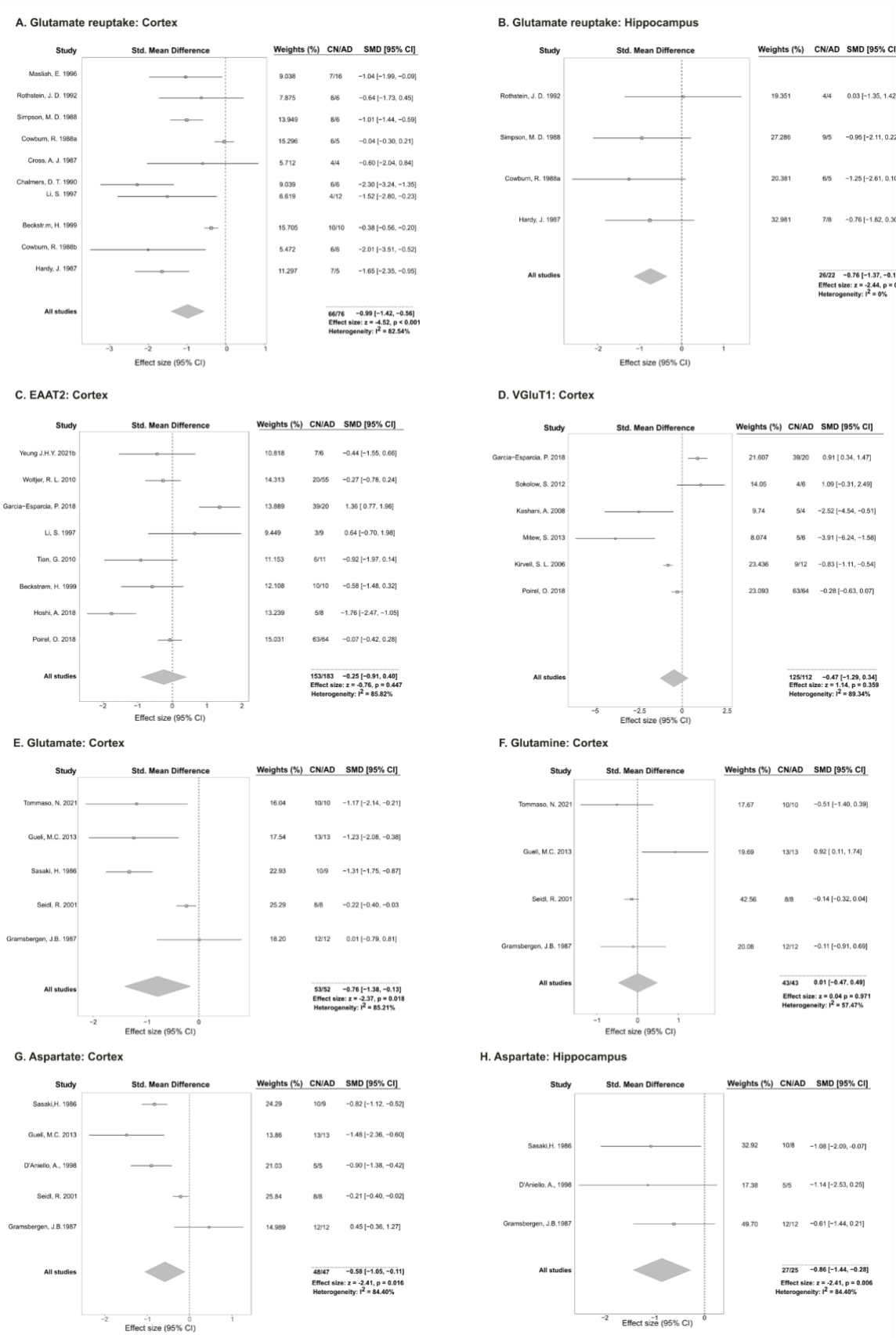
Confounding has occurred where the estimated outcome effect is biased by the presence of some difference between the comparison groups (apart from the outcome investigated/of interest). Typical confounders include baseline characteristics, prognostic factors, or concomitant exposures (e.g. smoking). A confounder is a difference between the comparison groups and it influences the direction of the study results. A high quality study at the level of case control design will identify thepotential confounders and measure them (where possible). This is difficult for studies where behavioural, attitudinal or lifestyle factors may impact on the results. Strategies to deal with effects of confounding factors may be dealt within the study design or in data analysis. By matching or stratifying sampling of participants, effects of confounding factors can be adjusted for. Whendealing with adjustment in data analysis, assess the statistics used in the study. Most will be some form of multivariate regression analysis to account for the confounding factors measured. Look out

for a description of statistical methods as regression methods such as logistic regression are usually employed to deal with confounding factors/variables of interest.

6. **Was the conflict of interest disclosed?**

7. **Was investigator blinded for group condition when the outcome was evaluated?**
   See if the analysis of the results was done blinded.

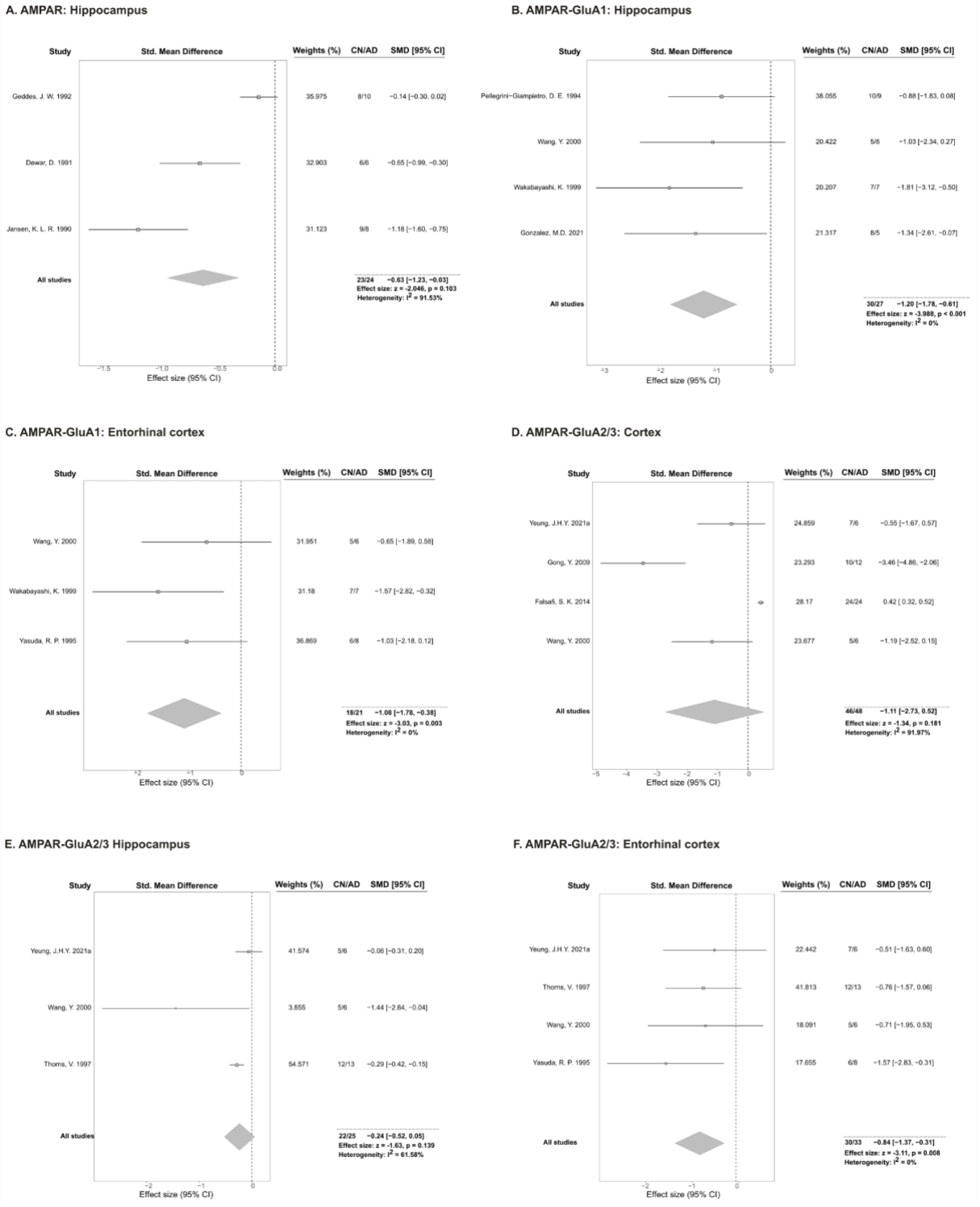
Supplementary Figure S1. Forest plots of included studies evaluating glutamate reuptake, transporters, storage, recycling, and aspartate in cortical areas, hippocampus, and entorhinal cortex



Forest plots of included studies evaluating glutamate reuptake, transporters, storage, and recycling, and aspartate in

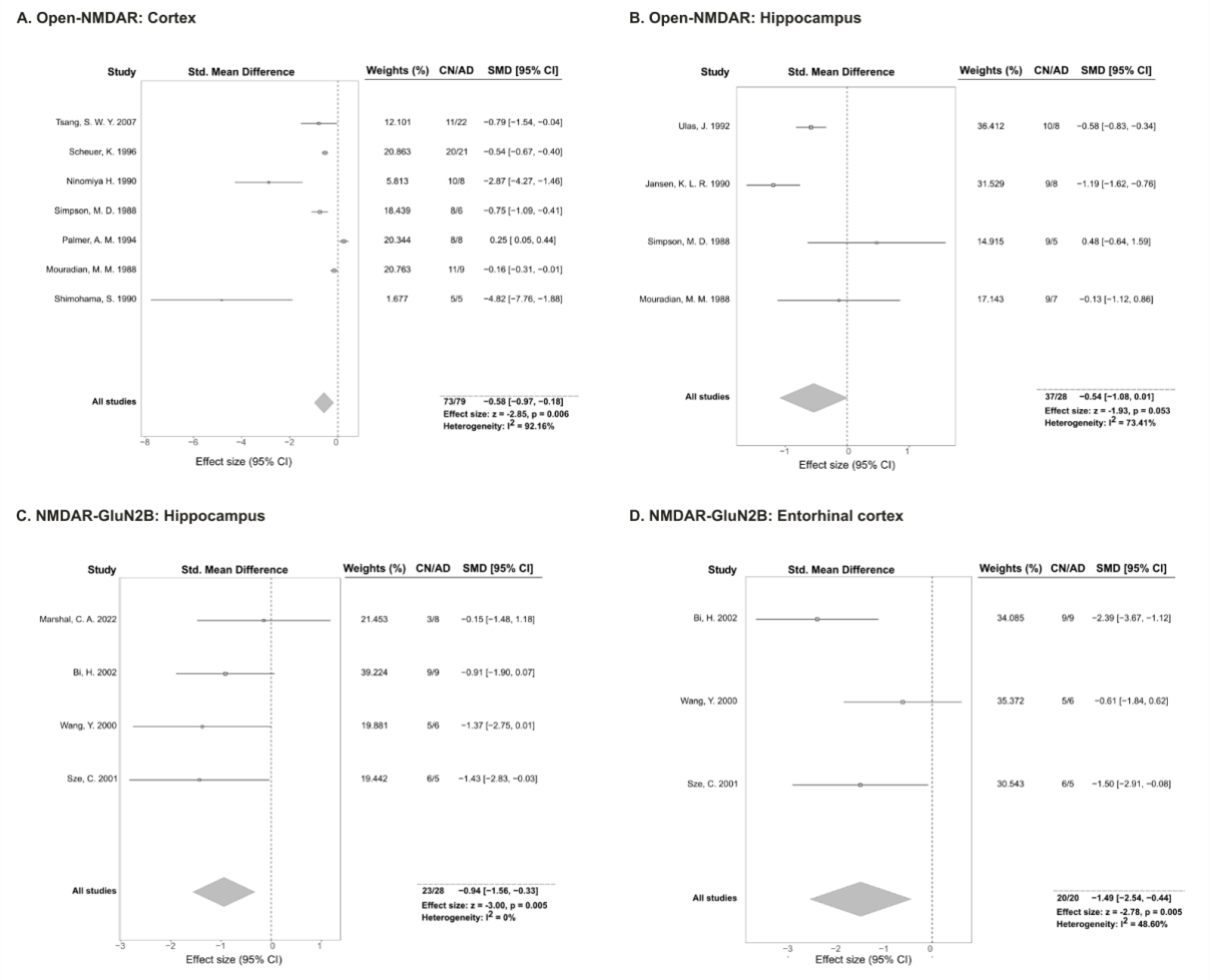
cortical areas, hippocampus, and entorhinal cortex. Overall standardized mean difference (SMD) of Glutamate reuptake (A-B), EAAT2 (C), VGluT1 (D), glutamate (E), glutamine (F), aspartate (G-H). Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. EAAT (excitatory amino acid transporter 2). VGluT (vesicular glutamate transporter). CN (cognitively normal). AD (Alzheimer's disease). CI (confidence interval).

Supplementary Figure S2. Forest plots of included studies evaluating glutamate AMPAR in cortical areas, hippocampus, and entorhinal córtex



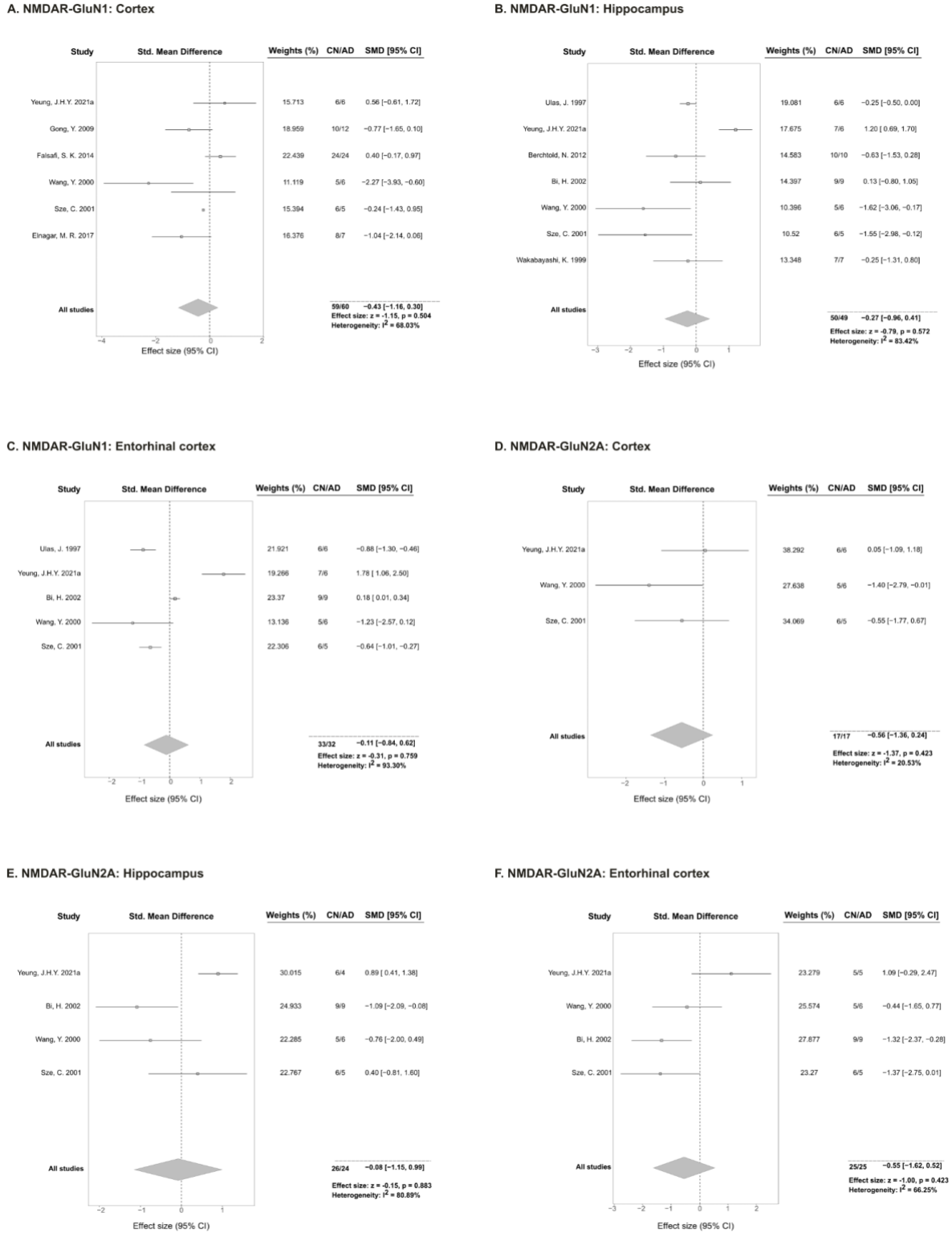
Forest plots of included studies evaluating glutamate AMPAR in cortical areas, hippocampus and entorhinal cortex. Overall standardized mean difference (SMD) of AMPAR (A), AMPAR-GluA1 (B-C), AMPAR-GluA2/3 (D-F). Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor). AMPAR-GluA (AMPAR-subunit). CN (cognitively normal). AD (Alzheimer's disease). CI (confidence interval).

Supplementary Figure S3. Forest plots of included studies evaluating glutamate NMDAR in cortical areas, hippocampus, and entorhinal córtex



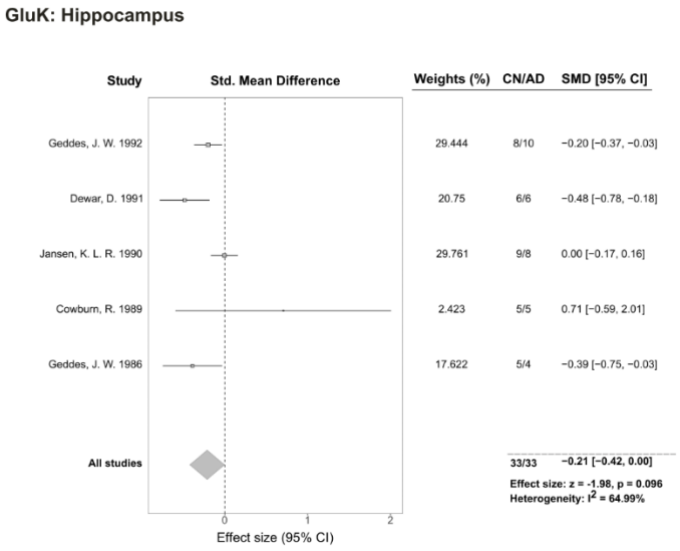
Forest plots of included studies evaluating glutamate NMDAR in cortical areas, hippocampus, and entorhinal cortex. Overall standardized mean difference (SMD) of open-NMDAR (A-B), NMDAR-GluN2B (C-D). Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. open-NMDAR (N-methyl-D-aspartate receptor). NMDAR-GluN (NMDAR-subunit). CN (cognitively normal). AD (Alzheimer's disease). CI (confidence interval).

Supplementary Figure S4. Forest plots of included studies evaluating glutamate NMDAR-GluN1 and -GluN2A in cortical areas, hippocampus, and entorhinal cortex



Forest plots of included studies evaluating glutamate NMDAR-GluN1 and -GluN2A in cortical areas, hippocampus, and entorhinal cortex. Overall standardized mean difference (SMD) of open-NMDAR-GluN1 (A-C), NMDAR-GluN2A (D-E). Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. NMDAR-GluN (NMDAR-subunit). CN (cognitively normal). AD (Alzheimer's disease). CI (confidence interval).

Supplementary Figure S5. Forest plots of included studies evaluating glutamate GluK in the hippocampus



Forest plots of included studies evaluating glutamate GluK in the hippocampus. Overall standardized mean difference (SMD) of GluK in the hippocampus. Effect sizes are represented by horizontal line ± 95% CI. Summary effect size is represented by a diamond. Studies are listed from lower to higher risk of bias. GluK (kainate receptor). CN (cognitively normal). AD (Alzheimer's disease). CI (confidence interval).

Supplementary Table S1. Description of original regions from included studies

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Sasaki et al. (72)	Whole brain	Superior frontal cortex,Orbital cortex,Cingulate cortex,Inferior temporal cortex,Insular cortex,Angular cortex,Occipital cortex,Hippocampus,Caudate nucleus,Putamen,Globus pallidus interna,Globus pallidus externa,Amygdaloid nucleus,Dorsomedial thalamus,Ventrolateral thalamus,Substantia nigra,Accumbens nucleus,Hypothalamus,Raphe nucleus,Mamillary body	Aspartate
D'Aniello et al. (101)	Whole brain	Superior frontal cortex,Orbital cortex,Cingulate cortex,Inferior temporal cortex,Insular cortex,Angular cortex,Occipital cortex	Aspartate
Gramsbergen et al. (74)	Whole brain	Hippocampus, frontal cortex	Aspartate
Gueli et al. (17)	Whole brain	Temporal cortex	Aspartate
Seidl et al. (23)	Whole brain	Temporal cortex, occipital cortex, caudate nucleus, thalamus	Aspartate
Sasaki et al. (72)	Cortex	Superior frontal cortex,Orbital cortex,Cingulate cortex,Inferior temporal cortex,Insular cortex,Angular cortex,Occipital cortex	Aspartate
Seidl et al. (23)	Cortex	Temporal cortex, occipital cortex	Aspartate
D'Aniello et al. (101)	Cortex	Frontal cortex, parietal cortex, temporal cortex	Aspartate
Gramsbergen et al. (74)	Cortex	Frontal cortex	Aspartate
Gueli et al. (17)	Cortex	Temporal cortex	Aspartate
Sasaki et al. (72)	Hippocampus	Hippocampus	Aspartate
D'Aniello et al. (101)	Hippocampus	Hippocampus	Aspartate
Gramsbergen et al. (74)	Hippocampus	Hippocampus	Aspartate

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Chalmers et al.(45)	Whole brain	Frontal cortex layers I-II, Frontal cortex layers III,Frontal cortex layers IV,Frontal cortex layers V-VI,Frontal cortex white matter	AMPAR
Dewar et al.(83)	Whole brain	Subiculum, Hippocampal CA1, CA3,CA4,DG	AMPAR
Geddes et al.(43)	Whole brain	Hippocampal CA1 or, CA1 pyr,CA1 rad,CA3 or,CA3 pyr,CA3 rad,gd out,gd in,infragranular,hilus,lm,sub mol,sub pyr,sub pm,phg in,phg out	AMPAR
Jansen et al.(80)	Whole brain	Hippocampal CA1, CA3, DG, Entorhinal cortex	AMPAR
Dewar et al.(83)	Hippocampus	Hippocampal CA1, CA3,CA4, DG	AMPAR
Geddes et al.(43)	Hippocampus	Hippocampal CA1 or, CA1 pyr,CA1 rad,CA3 or,CA3 pyr,CA3 rad,gd out,gd in,infragranular,hilus,lm	AMPAR
Jansen et al.(80)	Hippocampus	Hippocampal CA1, CA3, DG	AMPAR
Falsafi et al.(21)	Whole brain	Cortex (unspecified)	AMPAR - GluA1
González et al.(87)	Whole brain	Hippocampus	AMPAR - GluA1
Pellegrini-Giampietro et al.(83)	Whole brain	Dentate gyrus	AMPAR - GluA1
Wakabayashi et al.(85)	Whole brain	Dentate gyrus, Entorhinal cortex	AMPAR - GluA1
Wang et al.(20)	Whole brain	Entorhinal cortex, Frontal cortex, Hippocampus	AMPAR - GluA1
Yasuda et al.(84)	Whole brain	Entorhinal cortex	AMPAR - GluA1
González et al.(87)	Hippocampus	Hippocampus	AMPAR - GluA1

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Pellegrini-Giampietro et al.(83)	Hippocampus	Dentate gyrus	AMPAR - GluA1
Wakabayashi et al.(85)	Hippocampus	Dentate gyrus	AMPAR - GluA1
Wang et al.(20)	Hippocampus	Hippocampus	AMPAR - GluA1
Wakabayashi et al.(85)	Entorhinal cortex	Entorhinal cortex	AMPAR - GluA1
Wang et al.(20)	Entorhinal cortex	Entorhinal cortex	AMPAR - GluA1
Yasuda et al.(84)	Entorhinal cortex	Entorhinal cortex	AMPAR - GluA1
Falsafi et al.(21)	Whole brain	Cortex (unspecified)	AMPAR - GluA2/3
Gong et al.(88)	Whole brain	Middle frontal gyrus	AMPAR - GluA2/3
Thorns et al.(87)	Whole brain	Hippocampal CA1, CA3, Entorhinal cortex	AMPAR - GluA2/3
Tsamis et al.(88)	Whole brain	Striatum	AMPAR - GluA2/3
Wang et al.(20)	Whole brain	Entorhinal cortex, Frontal cortex, Hippocampus	AMPAR - GluA2/3
Yasuda et al.(84)	Whole brain	Entorhinal cortex	AMPAR - GluA2/3
Yeung et al.(21)	Whole brain	Hippocampal Str.ori_CA1, Str.pyr_CA1, Str.rad_CA1, Str.ori_CA2, Str.pyr_CA2, Str.rad_CA2, Str.ori_CA3, Str.pyr_CA3, Str.rad_CA3,	AMPAR - GluA2/3

Study (Reference number from main text)	Pooled as	Original regions	Outcome
		Str.mol_DG, Str_gran_DG, Hilus_DG, Subiculum, Entorhinal cortex, Superior temporal gyrus	
Thorns et al.(87)	Hippocampus	Hippocampal CA1, CA3	AMPAR - GluA2/3
Wang et al.(20)	Hippocampus	Hippocampus	AMPAR - GluA2/3
Yeung et al.(21)	Hippocampus	Hippocampus Str.ori_CA1, Str.pyr_CA1, Str.rad_CA1, Str.ori_CA2, Str.pyr_CA2, Str.rad_CA2, Str.ori_CA3, Str.pyr_CA3, Str.rad_CA3, Str.mol_DG, Str_gran_DG, Hilus_DG	AMPAR - GluA2/3
Thorns et al.(87)	Entorhinal cortex	Entorhinal Cortex	AMPAR - GluA2/3
Wang et al.(20)	Entorhinal cortex	Entorhinal cortex	AMPAR - GluA2/3
Yasuda et al.(84)	Entorhinal cortex	Entorhinal cortex	AMPAR - GluA2/3
Yeung et al.(21)	Entorhinal cortex	Entorhinal cortex	AMPAR - GluA2/3
Falsafi et al.(21)	Cortex	Cortex (unspecified)	AMPAR - GluA2/3
Gong et al.(88)	Cortex	Middle frontal gyrus	AMPAR - GluA2/3
Wang et al.(20)	Cortex	Frontal cortex	AMPAR - GluA2/3
Yeung et al.(21)	Cortex	Superior temporal gyrus	AMPAR - GluA2/3
Beckstrøm et al.(66)	Whole brain	Cingulate gyrus, Inferior temporal gyrus	EAAT2
Garcia-Esparcia et al.(73)	Whole brain	Frontal cortex	EAAT2

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Hoshi et al.(72)	Whole brain	Inferior temporal cortex, Medial temporal cortex, Superior temporal cortex	EAAT2
Kobayash et al.(68)	Whole brain	Entorhinal cortex layer I_II, Entorhinal cortex layer III_IV	EAAT2
Li et al.(65)	Whole brain	Frontal cortex	EAAT2
Poirel et al.(34)	Whole brain	Frontal cortex	EAAT2
Tian et al.(69)	Whole brain	Frontal cortex	EAAT2
Woltjer et al.(67)	Whole brain	Frontal cortex, Hippocampus	EAAT2
Yeung et al.(66)	Whole brain	Str.ori_CA1, Str.pyr_CA1, Str.rad_CA1, Str.ori_CA2, Str.pyr_CA2, Str.rad_CA2, Str.ori_CA3, Str.pyr_CA3, Str.rad_CA3, Str.mol_DG, Str_gran_DG, Hilus_DG, Subiculum, Entorhinal cortex, Superior temporal gyrus	EAAT2
Beckstrøm et al.(66)	Cortex	Inferior temporal gyrus	EAAT2
Garcia-Esparcia et al.(73)	Cortex	Frontal cortex	EAAT2
Hoshi et al.(72)	Cortex	Inferior temporal cortex, Medial temporal cortex, Superior temporal cortex	EAAT2
Li et al.(65)	Cortex	Frontal cortex	EAAT2
Poirel et al.(34)	Cortex	Frontal cortex	EAAT2
Tian et al.(69)	Cortex	Frontal cortex	EAAT2
Woltjer et al.(67)	Cortex	Frontal cortex	EAAT2
Yeung et al.(66)	Cortex	Superior temporal gyrus	EAAT2
Gramsbergen et al.(76)	Whole brain	Hippocampus, Frontal cortex	Glutamate

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Gueli et al.(17)	Whole brain	Temporal cortex	Glutamate
Hyman et al.(75)	Whole brain	terminal zone of the perforant pathway	Glutamate
Sasaki et al.(74)	Whole brain	Orbital cortex, Cingulate cortex, Inferior temporal cortex, Insular cortex,Angular cortex,Occipital cortex,Hippocampus,Caudate nucleus,Putamen,Globus pallidus interna,Globus pallidus externa, Amygdaloid nucleus, Dorsomedial thalamus, Ventrolateral thalamus, Substantia nigra, Accumbens nucleus, Hypothalamus, Mamillary body	Glutamate
Tommaso et al.(22)	Whole brain	Superior frontal gyrus	Glutamate
Seidl et al.(23)	Whole brain	Temporal cortex, occipital cortex, caudate nucleus, thalamus	Glutamate
Gramsbergen et al.(76)	Cortex	Frontal cortex	Glutamate
Gueli et al.(17)	Cortex	Temporal cortex	Glutamate
Sasaki et al.(74)	Cortex	Orbital cortex, cingulate cortex, Inferior temporal cortex, Insular cortex,Angular cortex,Occipital cortex	Glutamate
Tommaso et al.(22)	Cortex	superior frontal gyrus	Glutamate
Seidl et al.(23)	Cortex	Temporal cortex, occipital cortex	Glutamate
Gramsbergen et al.(76)	Whole brain	Hippocampus, frontal cortex	Glutamine
Gueli et al.(17)	Whole brain	Temporal cortex	Glutamine
Tommaso et al.(22)	Whole brain	superior frontal gyrus	Glutamine
Seidl et al.(23)	Whole brain	Temporal cortex, occipital cortex, caudate nucleus, thalamus	Glutamine

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Gramsbergen et al.(76)	Cortex	Frontal cortex	Glutamine
Gueli et al.(17)	Cortex	Temporal cortex	Glutamine
Tommaso et al.(22)	Cortex	superior frontal gyrus	Glutamine
Seidl et al.(23)	Cortex	Temporal cortex, occipital cortex	Glutamine
Burbaeva et al.(78)	Whole brain	Prefrontal cortex (BA10)	GS
Hensley et al.(78)	Whole brain	Hippocampus, Inferior parietal lobe	GS
Jørgensen et al.(83)	Whole brain	Frontal cortex	GS
Chalmers et al.(45)	Whole brain	Frontal cortex layers III, Frontal cortex layers I-II, Frontal cortex layers IV, Frontal cortex layers V-VI,Frontal cortex white matter	GluK
Cowburn et al.(44)	Whole brain	Temporal cortex, Frontal cortex, Parietal cortex, Hippocampus, Caudate	GluK
Dewar et al.(83)	Whole brain	Subiculum, Hippocampal CA1, CA3,CA4,DG	GluK
Geddes et al.(43)	Whole brain	CA1 or, CA1 pyr, CA1 rad, CA3 or, CA3 pyr, CA3 rad, gd out, gd in, infragranular, hilus, lm, sub mol, sub pyr, sub pm, phg in, phg out	GluK
Geddes et al.(46)	Whole brain	Hippocampus Inner mol, Outer mol, Hilus, H3, H2, H1, S. Or, S.L.M, S.Luc	GluK
Jansen et al.(80)	Whole brain	Hippocampus CA1, CA3, Entorhinal cortex, DGM	GluK
Cowburn et al.(44)	Hippocampus	hippocampus	GluK
Dewar et al.(83)	Hippocampus	Hippocampal CA1, CA3, CA4, DG	GluK
Geddes et al.(43)	Hippocampus	Hippocampal CA1 or, CA1 pyr, CA1 rad, CA3 or, CA3 pyr, CA3 rad, gd out, gd in, infragranular, hilus, lm	GluK

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Geddes et al.(46)	Hippocampus	Hippocampal Inner mol, Outer mol, Hilus, H3, H2, H1, S. Or, S.L.M, S.Luc	GluK
Jansen et al.(80)	Hippocampus	Hippocampal CA1, CA3, DGM	GluK
Albasanz et al.(38)	Whole brain	Left frontal lobe	mGlu
Dewar et al.(83)	Whole brain	Subiculum, CA1, CA3, CA4, DG	mGlu
Gong et al.(88)	Whole brain	Middle frontal gyrus	mGlu
Lee et al.(102)	Whole brain	Hippocampus	mGlu
Tsamis et al.(88)	Whole brain	Striatum	mGlu
Berchtold et al.(98)	Whole brain	Hippocampus	NMDAR - GluN1
Bi et al.(100)	Whole brain	Entorhinal cortex, Hippocampus	NMDAR - GluN1
Elnagar et al.(99)	Whole brain	Middle frontal gyrus	NMDAR - GluN1
Falsafi et al.(21)	Whole brain	Cortex (unspecified)	NMDAR - GluN1
Gong et al.(88)	Whole brain	Middle frontal gyrus	NMDAR - GluN1
Sze et al.(100)	Whole brain	Caudate, Entorhinal cortex, Hippocampus, Occipital cortex	NMDAR - GluN1
Tsamis et al.(88)	Whole brain	Striatum	NMDAR - GluN1
Ulas et al.(96)	Whole brain	Hippocampal CA1, CA2, CA3, Granule cells, Hilus, Subiculum, Entorhinal cortical layers, Perirhinal layers	NMDAR - GluN1

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Wakabayashi et al.(85)	Whole brain	Dentate gyrus	NMDAR - GluN1
Wang et al.(20)	Whole brain	Entorhinal cortex, Frontal cortex, Hippocampus	NMDAR - GluN1
Yeung et al.(21)	Whole brain	Str.ori_CA1, Str.pyr_CA1,Str.rad_CA1,Str.ori_CA2,Str.pyr_CA2,Str.rad_CA2,Str.ori_CA3,Str.pyr_CA3,Str.rad_CA3,Str.mol_DG,Str_gran_DG,Hilus_DG, Subiculum, Entorhinal cortex, Superior temporal gyrus	NMDAR - GluN1
Berchtold et al.(98)	Hippocampus	Hippocampus	NMDAR - GluN1
Bi et al.(100)	Hippocampus	Hippocampus	NMDAR - GluN1
Sze et al.(100)	Hippocampus	Hippocampus	NMDAR - GluN1
Ulas et al.(96)	Hippocampus	Hippocampal CA1, CA2, CA3, granule cells, Hilus	NMDAR - GluN1
Wakabayashi et al.(85)	Hippocampus	Dentate gyrus	NMDAR - GluN1
Wang et al.(20)	Hippocampus	Hippocampus	NMDAR - GluN1
Yeung et al.(21)	Hippocampus	Str.ori_CA1, Str.pyr_CA1,Str.rad_CA1,Str.ori_CA2,Str.pyr_CA2,Str.rad_CA2,Str.ori_CA3,Str.pyr_CA3,Str.rad_CA3,Str.mol_DG,Str_gran_DG,Hilus_DG, Subiculum, Entorhinal cortex, Superior temporal gyrus	NMDAR - GluN1
Bi et al.(100)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN1
Sze et al.(100)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN1

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Ulas et al.(96)	Entorhinal cortex	Entorhinal cortex layers	NMDAR - GluN1
Wang et al.(20)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN1
Yeung et al.(21)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN1
Yeung et al.(21)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN1
Elnagar et al.(99)	Cortex	Middle frontal gyrus	NMDAR - GluN1
Falsafi et al.(21)	Cortex	Cortex (unspecified)	NMDAR - GluN1
Gong et al.(88)	Cortex	Middle frontal gyrus	NMDAR - GluN1
Sze et al.(100)	Cortex	Occipital cortex	NMDAR - GluN1
Wang et al.(20)	Cortex	Frontal cortex	NMDAR - GluN1
Yeung et al.(21)	Cortex	Superior temporal gyrus	NMDAR - GluN1
Bi et al.(100)	Whole brain	Entorhinal cortex, Hippocampus	NMDAR - GluN2A
Sze et al.(100)	Whole brain	Entorhinal cortex, Hippocampus, Occipital cortex	NMDAR - GluN2A
Wang et al.(20)	Whole brain	Entorhinal cortex	NMDAR - GluN2A
Wang et al.(20)	Whole brain	Frontal cortex	NMDAR - GluN2A
Wang et al.(20)	Whole brain	Hippocampus	NMDAR - GluN2A

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Yeung et al.(21)	Whole brain	Str.ori_CA1, Str.pyr_CA1,Str.rad_CA1,Str.ori_CA2,Str.pyr_CA2,Str.rad_CA2,Str.ori_CA3,Str.pyr_CA3,Str.rad_CA3,Str.mol_DG,Str_gran_DG,Hilus_DG, Subiculum, Entorhinal cortex, Superior temporal gyrus	NMDAR - GluN2A
Bi et al.(100)	Hippocampus	Hippocampus	NMDAR - GluN2A
Sze et al.(100)	Hippocampus	Hippocampus	NMDAR - GluN2A
Wang et al.(20)	Hippocampus	Hippocampus	NMDAR - GluN2A
Yeung et al.(21)	Hippocampus	Str.ori_CA1, Str.pyr_CA1,Str.rad_CA1,Str.ori_CA2,Str.pyr_CA2,Str.rad_CA2,Str.ori_CA3,Str.pyr_CA3,Str.rad_CA3,Str.mol_DG,Str_gran_DG,Hilus_DG	NMDAR - GluN2A
Bi et al.(100)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2A
Sze et al.(100)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2A
Wang et al.(20)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2A
Yeung et al.(21)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2A
Sze et al.(100)	Cortex	Occipital cortex	NMDAR - GluN2A
Wang et al.(20)	Cortex	Frontal cortex	NMDAR - GluN2A
Yeung et al.(21)	Cortex	Superior temporal gyrus	NMDAR - GluN2A

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Bi et al.(100)	Whole brain	Entorhinal cortex, Hippocampus	NMDAR - GluN2B
Marshal et al.(101)	Whole brain	Hippocampus	NMDAR - GluN2B
Sze et al.(100)	Whole brain	Caudate, Entorhinal cortex, Hippocampus, Occipital cortex	NMDAR - GluN2B
Wang et al.(20)	Whole brain	Entorhinal cortex, Frontal cortex, Hippocampus	NMDAR - GluN2B
Bi et al.(100)	Hippocampus	Hippocampus	NMDAR - GluN2B
Marshal et al.(101)	Hippocampus	Hippocampus	NMDAR - GluN2B
Sze et al.(100)	Hippocampus	Hippocampus	NMDAR - GluN2B
Wang et al.(20)	Hippocampus	Hippocampus	NMDAR - GluN2B
Bi et al.(100)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2B
Sze et al.(100)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2B
Wang et al.(20)	Entorhinal cortex	Entorhinal cortex	NMDAR - GluN2B
Jansen et al.(80)	Whole brain	Hippocampal CA1, CA3, DGM, Entorhinal cortex	open-NMDAR
Mouradian et al.(95)	Whole brain	Frontal (A4), Frontal (A9), Hippocampus, Occipital (A17), Parietal (A39), Temporal (A28, A22)	open-NMDAR

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Ninomiya et al.(92)	Whole brain	Frontal cortex	open-NMDAR
Palmer et al.(94)	Whole brain	Frontal cortex, Temporal cortex	open-NMDAR
Scheuer et al.(92)	Whole brain	Frontal Cortex, Parietal cortex	open-NMDAR
Shimohama et al.(94)	Whole brain	Frontal Cortex	open-NMDAR
Simpson et al.(67)	Whole brain	Caudate, Frontal cortex, Hippocampus, Putamen, Temporal cortex	open-NMDAR
Tsang et al.(89)	Whole brain	Right hemisphere Orbitofrontal Gyrus	open-NMDAR
Ulas et al.(90)	Whole brain	Hippocampal CA1 pyr, CA1 or, CA1 rad, CA3 or, CA3 pyr, CA3 rad, GD in, GD out, Hilus, Infragranular, Lm, Phg in, Phg out, Sub mol, Sub pm, Sub pyr	open-NMDAR
Jansen et al.(80)	Hippocampus	Hippocampal CA1, CA3, DGM	open-NMDAR
Mouradian et al.(95)	Hippocampus	Hippocampus	open-NMDAR
Simpson et al.(67)	Hippocampus	Hippocampus	open-NMDAR
Ulas et al.(90)	Hippocampus	Hippocampal CA1 pyr, CA1 or, CA1 rad, CA3 or, CA3 pyr, CA3 rad, GD in, GD out, Hilus, Infragranular, Lm	open-NMDAR
Mouradian et al.(95)	Cortex	Frontal (A4), Frontal (A9), Occipital (A17), Parietal (A39), Temporal (A28, A22)	open-NMDAR
Ninomiya et al.(92)	Cortex	Frontal cortex	open-NMDAR
Palmer et al.(94)	Cortex	Frontal cortex	open-NMDAR
Palmer et al.(94)	Cortex	Temporal cortex	open-NMDAR
Scheuer et al.(92)	Cortex	Frontal Cortex, Parietal cortex	open-NMDAR

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Shimohama et al.(94)	Cortex	Frontal Cortex	open-NMDAR
Simpson et al.(67)	Cortex	Frontal cortex, Temporal cortex	open-NMDAR
Tsang et al.(89)	Cortex	Right hemisphere Orbitofrontal Gyrus	open-NMDAR
Beckstrøm et al.(66)	Whole brain	Cingulate gyrus, Inferior temporal gyrus	Glutamate reuptake
Chalmers et al.(45)	Whole brain	Frontal cortex layers I-II, Frontal cortex layers III,Frontal cortex layers IV,Frontal cortex layers V-VI,Frontal cortex white matter	Glutamate reuptake
Cowburn et al.(100)	Whole brain	Temporal cortex	Glutamate reuptake
Cowburn et al.(65)	Whole brain	Hippocampus, Frontal cortex, Parietal cortex, Caudate	Glutamate reuptake
Cross et al.(67)	Whole brain	Frontal-parietal cortex	Glutamate reuptake
Hardy et al.(67)	Whole brain	Frontal cortex, Hippocampus, Occipital cortex, Parietal cortex, Temporal cortex, Caudate, Globus pallidus	Glutamate reuptake
Li et al.(65)	Whole brain	Midfrontal cortex	Glutamate reuptake
Masliah et al.(56)	Whole brain	Frontal cortex	Glutamate reuptake
Rothstein et al.(64)	Whole brain	Hippocampus, Striatum, Motor cortex	Glutamate reuptake
Simpson et al.(67)	Whole brain	Frontal cortex, Temporal cortex, Caudate, Putamen, Hippocampus	Glutamate reuptake
Xuereb et al.(64)	Whole brain	Principal anterior, Lateral dorsal, Anterior ventral lateral external part, Anterior ventral lateral internal part, Posterior ventral lateral dorsal part, Posterior ventral lateral ventral part, Posterior ventral lateral caudal part, Ventral posterolateral, Ventral posteromedial, Basal ventral medial, Mediodorsal, Anterior paraventricular, Paracentral, Central medial, Centromedian, Para	Glutamate reuptake

Study (Reference number from main text)	Pooled as	Original regions	Outcome
		fascicular, Pulvinar lateral, Pulvinar medial, Reticular, Zona incerta, Subthalamus	
Cowburn et al.(65)	Hippocampus	Hippocampus	Glutamate reuptake
Hardy et al.(67)	Hippocampus	Hippocampus	Glutamate reuptake
Rothstein et al.(64)	Hippocampus	Hippocampus	Glutamate reuptake
Simpson et al.(67)	Hippocampus	Hippocampus	Glutamate reuptake
Beckstrøm et al.(66)	Cortex	Cingulate gyrus, Inferior temporal gyrus	Glutamate reuptake
Chalmers et al.(45)	Cortex	Frontal cortex layers I-II, Frontal cortex layers III,Frontal cortex layers IV,Frontal cortex layers V-VI,Frontal cortex white matter	Glutamate reuptake
Cowburn et al.(100)	Cortex	Temporal cortex	Glutamate reuptake
Cowburn et al.(65)	Cortex	Frontal Cortex, Parietal cortex	Glutamate reuptake
Cross et al.(67)	Cortex	Frontal-parietal cortex	Glutamate reuptake
Hardy et al.(67)	Cortex	Frontal cortex, Occipital cortex, Parietal cortex, Temporal cortex	Glutamate reuptake
Li et al.(65)	Cortex	Midfrontal cortex	Glutamate reuptake
Masliah et al.(56)	Cortex	Frontal cortex	Glutamate reuptake
Rothstein et al.(64)	Cortex	Motor Cortex	Glutamate reuptake
Simpson et al.(67)	Cortex	Frontal cortex, Temporal cortex	Glutamate reuptake
Garcia-Esparcia et al.(73)	Whole brain	Frontal cortex	VGLUT1
Kashani et al.(72)	Whole brain	Prefrontal cortex	VGLUT1
Kirvell et al.(32)	Whole brain	Temporal cortex, Parietal cortex, Occipital cortex	VGLUT1
Mitew et al.(33)	Whole brain	Inferior temporal cortex	VGLUT1

Study (Reference number from main text)	Pooled as	Original regions	Outcome
Poirel et al.(34)	Whole brain	Frontal cortex	VGLUT1
Rodriguez-Perdigon et al.(73)	Whole brain	Hippocampus	VGLUT1
Sokolow et al.(35)	Whole brain	Parietal cortex	VGLUT1
Garcia-Esparcia et al.(73)	Cortex	Frontal cortex	VGLUT1
Kashani et al.(72)	Cortex	Prefrontal cortex	VGLUT1
Kirvell et al.(32)	Cortex	Temporal cortex, Parietal cortex, Occipital cortex	VGLUT1
Mitew et al.(33)	Cortex	Inferior temporal cortex	VGLUT1
Poirel et al.(34)	Cortex	Frontal cortex	VGLUT1

Original regions were pooled as “whole brain”, cortical areas, hippocampus or entorhinal cortex. Regions not included: cerebellum, brain stem, and spinal cord. Abbreviations: AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor); AMPAR-GluA (AMPAR-subunit); EAAT (excitatory amino acid transporter); GS (glutamine synthetase); GluK (kainate receptor); mGlu (metabotropic glutamate receptor); open-NMDAR (N-methyl-D-aspartate receptor). NMDAR-GluN (NMDAR-subunit). VGluT (vesicular glutamate transporter).

Supplementary Table S2. List of excluded studies after full-text eligibility

Study PMID	Study	Reason
28083916	(Tracy and Gan, 2017)	1
32475008	(Schnöder et al., 2020)	1
2900537	(Greenamyre et al., 1988)	1
3713780	(Procter et al., 1986)	2
18586353	(Simpson et al., 2010)	3
14751773	(Mishizen-Eberz et al., 2004)	3
20805102	(Epis et al., 2010)	3
20535486	(Thal et al., 2010)	3
30606734	(Lleó et al., 2019)	3
1842701	(Chessell et al., 1991)	3
19774677	(Sultana, Banks and Butterfield, 2010)	3
15144856	(Carter et al., 2004)	3
28176002	(Busse et al., 2018)	3
7477679	(Le Prince et al., 1995)	3
25012223	(Robinson et al., 2014)	3
34143915	(Ishibashi et al., 2021)	3
34603012	(Salcedo et al., 2021)	3
34048087	(Lyons et al., 2021)	3
31954399	(Mecca et al., 2020)	3
15287897	(Matthew R Hynd, Scott and Dodd, 2004)	3
19295912	(Williams et al., 2009)	3
2557558	(Jansen, Faull and Dragunow, 1989)	3
17292512	(Abbott et al., 2008)	4
32483284	(Cho et al., 2020)	4
25622143	(Orr et al., 2015)	4
25031178	(Neuman et al., 2015)	4
20416976	(Scott et al., 2011)	4
1387617	(Porter et al., 1992)	4
11148253	(Masliah et al., 2001)	4
33492290	(Nakano et al., 2021)	4
35707770	(Kenanoglu et al., 2022)	4
21743130	(Chen et al., 2011)	5
25213836	(Li et al., 2014)	5
19726654	(Wang et al., 2009)	5
10416226	(Gonzalo-Ruiz, 1999)	6
22677667	(Burbaeva et al., 2012)	6
2878980	(Greenamyre et al., 1987)	7
29450841	(Chan et al., 2018)	7
10412022	(Chan, Griffin and Mattson, 1999)	7
8164524	(García-Ladona et al., 1994)	7
30291374	(Müller Herde et al., 2019)	7

Study PMID	Study	Reason
10436347	(Procter, Qurne and Francis, 1999)	7
4071042	(Geddes et al., 1985)	7
15605986	(Dalfó et al., 2004)	8
25261450	(Mohamed et al., 2015)	8
2160518	(Penney et al., 1990)	8
35109872	(Muñoz-Castro et al., 2022)	8
3029638	(Monaghan et al., 1987)	8
2878639	(Ellison et al., 1986)	8
32896673	(Nuzzo et al., 2020)	9
32925049	(Enache et al., 2020)	9
22232349	(Ringman et al., 2012)	9
11793164	(Panegyres, Zafiris-Toufexis and Kakulas, 2002)	9
34145880	(Matthews et al., 2021)	9
15030408	(Matthew R. Hynd, Scott and Dodd, 2004)	9
26455863	(Fluteau et al., 2015)	9
3031556	(Maragos et al., 1987)	9
2570384	(Procter et al., 1989)	9
2898011	(Procter, Lowe, et al., 1988)	9
3339353	(Procter, Palmer, et al., 1988)	9
1980143	(Lowe et al., 1990)	9
27041503	(Tracy et al., 2016)	10
25942042	(Madeira et al., 2015)	10
1683703	(Smith et al., 1991)	10
11071482	(Honig et al., 2000)	10
11432984	(Hynd, Scott and Dodd, 2001)	10
24312364	(Pirttimaki et al., 2013)	10
7690114	(Carlson, Penney and Young, 1993)	10
15931666	(Fang et al., 2005)	10
27986924	(Prieto et al., 2017)	10
14751437	(Hashimoto et al., 2004)	10
19339596	(Deshpande et al., 2009)	10
2879613	(Geddes et al., 1986)	10
1966604	(Harrison et al., 1990)	10
9697936	(D'Aniello et al., 1998)	10
2858129	(Greenamyre et al., 1985)	10
11274307	(Antuono et al., 2001)	10
7970158	(Masliah et al., 1994)	10
6133580	(Smith et al., 1983)	10
7824065	(Balazs and Leon, 1994)	10
8397299	(Chouinard, Gaitan and Wood, 1993)	10
29859871	(Tiernan et al., 2018)	10
11461977	(Lauderback et al., 2001)	10
34039651	(Domínguez-Álvaro et al., 2021)	10
34965419	(Simoes et al., 2021)	10
34065927	(Ahmad et al., 2021)	10

Study PMID	Study	Reason
29186695	(Savas et al., 2017)	13
12938024	(Westphalen, Scott and Dodd, 2003)	13
11826152	(Scott et al., 2002)	11
17361039	(Jacob et al., 2007)	11
8773259	(Ikonomovic, Sheffield and Armstrong, 1995)	11
8205474	(Armstrong et al., 1994)	11
8871942	(Armstrong and Ikonomovic, 1996)	11
23616440	(Lim et al., 2013)	11
11844890	(Tsai et al., 2002)	11
20882066	(Chen et al., 2010)	11
18983893	(Jiang and Jia, 2009)	11
19832840	(Mäkitie et al., 2010)	11
11746426	(Robinson, 2001)	11
1361232	(Gunnersen and Haley, 1992)	11
10630204	(Ikonomovic et al., 1999)	11
8616634	(Akbarian, Smith and Jones, 1995)	11
12408226	(Thai, 2002)	11
24950944	(Burbaeva et al., 2014)	11
11004532	(Oyama, Yamamoto and Titani, 2000)	11
24312282	(Kravitz, Gaisler-Salomon and Biegon, 2013)	11
8285589	(Hyman et al., 1994)	11
19319679	(Pow and Cook, 2009)	11
11532724	(Dracheva et al., 2001)	11
9466422	(Aronica et al., 1998)	11
17913914	(Bell, Bennett and Cuello, 2007)	11
24156266	(Leuba et al., 2014)	11
9447566	(Brown et al., 1997)	11
9291943	(Ikonomovic et al., 1997)	11
33380492	(Halbgebauer et al., 2021)	11
20980075	(Marcello et al., 2012)	11
7722505	(Scott, Tannenberg and Dodd, 1995)	11
2761669	(Akiyama et al., 1989)	11
4047524	(Arai et al., 1985)	11
3014387	(Palmer et al., 1986)	11
2905920	(Represa et al., 1988)	11
29025866	(Goetzl et al., 2018)	12
31422081	(Lin, Yang and Lane, 2019)	12
14749132	(Zoia et al., 2004)	12
10805335	(Ferrarese et al., 2000)	12
10520940	(Tumani et al., 1999)	12
10847559	(Kuiper et al., 2000)	12
24973618	(Busse et al., 2014)	12
8102356	(Miulli, Norwell and Schwartz, 1993)	12
21597934	(Vermeiren et al., 2011)	12
25577411	(Timmer et al., 2015)	12

Study PMID	Study	Reason
2926384	(Hoyer and Nitsch, 1989)	12
1734749	(Pomara et al., 1992)	12
1508400	(Tohgi et al., 1992)	12
7702702	(Fisher et al., 1994)	12
9871505	(Fisher et al., 1998)	14
18624794	(Duerson et al., 2009)	16
32304284	(Treyer et al., 2020)	16
1980999	(Dewar et al., 1990)	16
30909027	(Ishibashi et al., 2019)	16
19501936	(Rupsingh et al., 2011)	16
8819138	(Haug et al., 1996)	16

Excluded studies with their respective PMID and exclusion reason number. Exclusion reasons are described in the Supplementary Method S2.

Supplementary Table S3. Report of effect estimates for each study

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
Aspartate	Whole brain	Sasaki et al.(74)	10	9	12	-0.70	-0.97	-0.43
Aspartate	Whole brain	Gueli et al.(17)	13	13	12	-1.48	-2.36	-0.60
Aspartate	Whole brain	D`Aniello et al.(101)	5	5	10	-0.94	-1.44	-0.44
Aspartate	Whole brain	Seidl et al.(23)	8	8	10	-0.52	-0.77	-0.27
Aspartate	Whole brain	Gramsbergen et al.(76)	12	12	8	-0.07	-0.19	0.04
Glutamate	Whole brain	Tommaso et al.(22)	10	10	13	-1.17	-2.14	-0.21
Glutamate	Whole brain	Gueli et al.(17)	13	13	12	-1.23	-2.08	-0.38
Glutamate	Whole brain	Sasaki et al.(74)	10	9	12	-1.16	-1.55	-0.76
Glutamate	Whole brain	Seidl et al.(23)	8	8	10	-0.43	-0.66	-0.21
Glutamate	Whole brain	Gramsbergen et al.(76)	12	12	8	-0.46	-0.64	-0.29
Glutamate	Whole brain	Hyman et al.(75)	4	6	5	-2.76	-4.74	-0.78
Glutamine	Whole brain	Tommaso et al.(22)	10	10	13	-0.51	-1.40	0.39
Glutamine	Whole brain	Gueli et al.(17)	13	13	12	0.92	0.11	1.74
Glutamine	Whole brain	Seidl et al.(23)	8	8	10	-0.14	-0.32	0.04
Glutamine	Whole brain	Gramsbergen et al.(76)	12	12	8	-0.24	-0.37	-0.10
AMPAR - GluA1	Whole brain	Pellegrini-Giampietro et al.(82)	10	9	10	-0.88	-1.83	0.08
AMPAR - GluA1	Whole brain	Falsafi et al.(19)	24	24	7	2.11	1.39	2.82

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
AMPAR - GluA1	Whole brain	Wang et al.(20)	5	6	6	-0.86	-1.31	-0.42
AMPAR - GluA1	Whole brain	Wakabayashi et al.(85)	7	7	6	-1.68	-2.34	-1.03
AMPAR - GluA1	Whole brain	González et al.(83)	8	5	5	-1.34	-2.61	-0.07
AMPAR - GluA1	Whole brain	Yasuda et al.(84)	6	8	3	-1.03	-2.18	0.12
AMPAR - GluA2/3	Whole brain	Yeung et al. (84)	5	6	11	-0.19	-0.45	0.08
AMPAR - GluA2/3	Whole brain	Gong et al.(87)	10	12	10	-3.46	-4.86	-2.06
AMPAR - GluA2/3	Whole brain	Tsamis et al.(88)	6	6	9	0.17	-0.97	1.30
AMPAR - GluA2/3	Whole brain	Falsafi et al.(19)	24	24	7	0.42	0.32	0.52
AMPAR - GluA2/3	Whole brain	Wang et al.(20)	5	6	6	-1.08	-1.60	-0.56
AMPAR - GluA2/3	Whole brain	Thorns et al.(86)	12	13	3	-0.44	-0.60	-0.27
AMPAR - GluA2/3	Whole brain	Yasuda et al.(84)	6	8	3	-1.57	-2.83	-0.31
EAAT2	Whole brain	Yeung et al.(21)	7	6	11	0.33	0.08	0.58
EAAT2	Whole brain	Woltjer et al.(67)	20	55	10	-0.49	-0.58	-0.40
EAAT2	Whole brain	Kobayashi et al.(66)	6	6	10	-0.58	-0.91	-0.25
EAAT2	Whole brain	Garcia-Esparcia et al.(71)	39	20	8	1.36	0.77	1.96
EAAT2	Whole brain	Li et al.(63)	3	9	7	0.64	-0.70	1.98
EAAT2	Whole brain	Tian et al.(69)	6	11	6	-0.92	-1.97	0.14
EAAT2	Whole brain	Beckstrøm et al.(64)	10	10	6	-0.61	-0.84	-0.37

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
EAAT2	Whole brain	Hoshi et al.(70)	5	8	5	-1.76	-2.47	-1.05
EAAT2	Whole brain	Poirel et al.(31)	63	64	4	-0.07	-0.42	0.28
AMPAR	Whole brain	Geddes et al.(46)	8	10	12	-0.17	-0.33	-0.01
AMPAR	Whole brain	Dewar et al.(81)	6	6	10	-0.82	-1.22	-0.42
AMPAR	Whole brain	Jansen et al.(80)	9	8	8	-1.10	-1.51	-0.70
AMPAR	Whole brain	Chalmers et al.(45)	6	6	7	0.37	0.10	0.65
Glutamate reuptake	Whole brain	Masliah et al.(56)	7	16	9	-1.04	-1.99	-0.09
Glutamate reuptake	Whole brain	Rothstein et al.(58)	4	4	8	-0.36	-0.74	0.03
Glutamate reuptake	Whole brain	Simpson et al.(65)	8	6	8	-0.80	-1.15	-0.44
Glutamate reuptake	Whole brain	Cowburn et al.(60)	6	5	8	-0.51	-0.84	-0.18
Glutamate reuptake	Whole brain	Cross et al.(57)	4	4	8	-0.60	-2.04	0.84
Glutamate reuptake	Whole brain	Chalmers et al.(45)	6	6	7	-2.30	-3.24	-1.35
Glutamate reuptake	Whole brain	Li et al.(63)	4	12	7	-1.52	-2.80	-0.23
Glutamate reuptake	Whole brain	Beckstrøm et al.(64)	10	10	6	-0.38	-0.56	-0.20

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
Glutamate reuptake	Whole brain	Cowburn et al.(59)	6	6	6	-2.01	-3.51	-0.52
Glutamate reuptake	Whole brain	Hardy et al.(61)	7	5	6	-1.16	-1.67	-0.64
Glutamate reuptake	Whole brain	Xuereb et al.(62)	5	5	5	0.34	0.03	0.66
GS	Whole brain	Jørgensen et al.(79)	11	13	7	0.64	-0.19	1.47
GS	Whole brain	Burbaeva et al.(77)	9	11	7	1.33	0.34	2.32
GS	Whole brain	Hensley et al.(78)	7	22	6	-0.91	-1.18	-0.65
GluK	Whole brain	Geddes et al.(46)	8	10	12	-0.04	-0.20	0.11
GluK	Whole brain	Dewar et al.(81)	6	6	10	-0.61	-0.95	-0.28
GluK	Whole brain	Jansen et al.(80)	9	8	8	-0.09	-0.26	0.08
GluK	Whole brain	Cowburn et al.(44)	6	6	8	0.20	-0.05	0.44
GluK	Whole brain	Chalmers et al.(45)	6	6	7	0.83	0.43	1.24
GluK	Whole brain	Geddes et al.(43)	5	4	3	-0.39	-0.75	-0.03
mGlu	Whole brain	Albasanz et al.(38)	5	11	10	-2.29	-3.69	-0.90
mGlu	Whole brain	Gong et al.(87)	10	12	10	-0.13	-0.97	0.71
mGlu	Whole brain	Dewar et al.(81)	6	6	10	-0.79	-1.18	-0.40
mGlu	Whole brain	Tsamis et al.(88)	6	6	9	0.82	-0.37	2.02
mGlu	Whole brain	Lee et al.(102)	13	18	3	1.43	0.62	2.24

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
NMDAR - GluN1	Whole brain	Ulas et al.(96)	6	6	14	-0.40	-0.68	-0.12
NMDAR - GluN1	Whole brain	Yeung et al.(66)	7	6	11	1.15	0.66	1.64
NMDAR - GluN1	Whole brain	Berchtold et al.(97)	10	10	11	-0.63	-1.53	0.28
NMDAR - GluN1	Whole brain	Gong et al.(87)	10	12	10	-0.77	-1.65	0.10
NMDAR - GluN1	Whole brain	Tsamis et al.(88)	6	6	9	0.16	-0.97	1.30
NMDAR - GluN1	Whole brain	Bi et al.(99)	9	9	8	0.15	-0.01	0.31
NMDAR - GluN1	Whole brain	Falsafi et al.(19)	24	24	7	0.40	-0.17	0.97
NMDAR - GluN1	Whole brain	Wang et al.(20)	5	6	6	-1.63	-2.36	-0.90
NMDAR - GluN1	Whole brain	Sze et al.(100)	6	5	6	-0.69	-1.08	-0.31
NMDAR - GluN1	Whole brain	Elnagar et al.(98)	8	7	6	-1.04	-2.14	0.06
NMDAR - GluN1	Whole brain	Wakabayashi et al.(85)	7	7	6	-0.25	-1.31	0.80
NMDAR - GluN2A	Whole brain	Yeung et al.(66)	6	4	11	0.83	0.37	1.29
NMDAR - GluN2A	Whole brain	Bi et al.(99)	9	9	8	-1.20	-1.62	-0.78
NMDAR - GluN2A	Whole brain	Wang et al.(20)	5	6	6	-0.82	-1.25	-0.39
NMDAR - GluN2A	Whole brain	Sze et al.(100)	6	5	6	-0.43	-0.74	-0.12
NMDAR - GluN2B	Whole brain	Marshall et al.(101)	3	8	9	-0.15	-1.48	1.18
NMDAR - GluN2B	Whole brain	Bi et al.(99)	9	9	8	-1.47	-1.97	-0.96
NMDAR - GluN2B	Whole brain	Wang et al.(20)	5	6	6	-1.14	-1.68	-0.60

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
NMDAR - GluN2B	Whole brain	Sze et al.(100)	6	5	6	-0.86	-1.30	-0.42
Open-NMDAR	Whole brain	Tsang et al.(89)	11	22	12	-0.79	-1.54	-0.04
Open-NMDAR	Whole brain	Ulas et al.(90)	10	8	10	-0.65	-0.92	-0.39
Open-NMDAR	Whole brain	Scheuer et al.(92)	20	21	8	-0.54	-0.67	-0.40
Open-NMDAR	Whole brain	Ninomiya et al.(91)	10	8	8	-2.87	-4.27	-1.46
Open-NMDAR	Whole brain	Jansen et al.(80)	9	8	8	-1.24	-1.68	-0.79
Open-NMDAR	Whole brain	Simpson et al.(65)	9	8	8	-0.39	-0.59	-0.18
Open-NMDAR	Whole brain	Palmer et al.(93)	8	8	8	0.25	0.05	0.44
Open-NMDAR	Whole brain	Mouradian et al.(95)	11	9	5	-0.15	-0.30	-0.01
Open-NMDAR	Whole brain	Shimohama et al.(94)	5	5	5	-4.82	-7.76	-1.88
VGluT1	Whole brain	Garcia-Esparcia et al.(71)	39	20	8	0.91	0.34	1.47
VGluT1	Whole brain	Sokolow et al.(35)	4	6	8	1.09	-0.31	2.49
VGluT1	Whole brain	Kashani et al.(72)	5	4	7	-2.52	-4.54	-0.51
VGluT1	Whole brain	Mitew et al.(33)	5	6	6	-3.91	-6.24	-1.58
VGluT1	Whole brain	Rodriguez-Perdigon et al.(73)	16	15	6	-1.40	-2.19	-0.60
VGluT1	Whole brain	Kirvell et al.(32)	9	12	6	-0.83	-1.11	-0.54
VGluT1	Whole brain	Poirel et al.(31)	63	64	4	-0.28	-0.63	0.07

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
EAAT2	Cortex	Yeung et al.(21)	7	6	11	-0.44	-1.55	0.66
EAAT2	Cortex	Woltjer et al.(67)	20	55	10	-0.27	-0.78	0.24
EAAT2	Cortex	Garcia-Esparcia et al.(71)	39	20	8	1.36	0.77	1.96
EAAT2	Cortex	Li et al.(63)	3	9	7	0.64	-0.70	1.98
EAAT2	Cortex	Tian et al.(69)	6	11	6	-0.92	-1.97	0.14
EAAT2	Cortex	Beckstrøm et al.(64)	10	10	6	-0.58	-1.48	0.32
EAAT2	Cortex	Hoshi et al.(70)	5	8	5	-1.76	-2.47	-1.05
EAAT2	Cortex	Poirel et al.(31)	63	64	4	-0.07	-0.42	0.28
Glutamate reuptake	Cortex	Masliah et al.(56)	7	16	9	-1.04	-1.99	-0.09
Glutamate reuptake	Cortex	Rothstein et al.(58)	8	6	8	-0.64	-1.73	0.45
Glutamate reuptake	Cortex	Simpson et al.(65)	8	6	8	-1.01	-1.44	-0.59
Glutamate reuptake	Cortex	Cowburn et al.(60)	6	5	8	-0.04	-0.30	0.21
Glutamate reuptake	Cortex	Cross et al.(57)	4	4	8	-0.60	-2.04	0.84
Glutamate reuptake	Cortex	Chalmers et al.(45)	6	6	7	-2.30	-3.24	-1.35

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
Glutamate reuptake	Cortex	Li et al.(63)	4	12	7	-1.52	-2.80	-0.23
Glutamate reuptake	Cortex	Beckstrøm et al.(64)	10	10	6	-0.38	-0.56	-0.20
Glutamate reuptake	Cortex	Cowburn et al.(59)	6	6	6	-2.01	-3.51	-0.52
Glutamate reuptake	Cortex	Hardy et al.(61)	7	5	6	-1.65	-2.35	-0.95
Glutamate reuptake	Hippocampus	Rothstein et al.(58)	4	4	8	0.03	-1.35	1.42
Glutamate reuptake	Hippocampus	Simpson et al.(65)	9	5	8	-0.95	-2.11	0.22
Glutamate reuptake	Hippocampus	Cowburn et al.(60)	6	5	8	-1.25	-2.61	0.10
Glutamate reuptake	Hippocampus	Hardy et al.(61)	7	8	6	-0.76	-1.82	0.30
GluK	Hippocampus	Geddes et al.(46)	8	10	12	-0.20	-0.37	-0.03
GluK	Hippocampus	Dewar et al.(81)	6	6	10	-0.48	-0.78	-0.18
GluK	Hippocampus	Jansen et al.(80)	9	8	8	0.00	-0.17	0.16
GluK	Hippocampus	Cowburn et al.(44)	5	5	8	0.71	-0.59	2.01
GluK	Hippocampus	Geddes et al.(43)	5	4	3	-0.39	-0.75	-0.03
NMDAR - GluN1	Cortex	Yeung et al.(66)	6	6	11	0.56	-0.61	1.72

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
NMDAR - GluN1	Cortex	Gong et al.(87)	10	12	10	-0.77	-1.65	0.10
NMDAR - GluN1	Cortex	Falsafi et al.(19)	24	24	7	0.40	-0.17	0.97
NMDAR - GluN1	Cortex	Wang et al.(20)	5	6	6	-2.27	-3.93	-0.60
NMDAR - GluN1	Cortex	Sze et al.(100)	6	5	6	-0.24	-1.43	0.95
NMDAR - GluN1	Cortex	Elnagar et al.(98)	8	7	6	-1.04	-2.14	0.06
NMDAR - GluN1	Entorhinal cortex	Ulas et al.(96)	6	6	14	-0.88	-1.30	-0.46
NMDAR - GluN1	Entorhinal cortex	Yeung et al.(66)	7	6	11	1.78	1.06	2.50
NMDAR - GluN1	Entorhinal cortex	Bi et al.(99)	9	9	8	0.18	0.01	0.34
NMDAR - GluN1	Entorhinal cortex	Wang et al.(20)	5	6	6	-1.23	-2.57	0.12
NMDAR - GluN1	Entorhinal cortex	Sze et al.(100)	6	5	6	-0.64	-1.01	-0.27
NMDAR - GluN1	Hippocampus	Ulas et al.(96)	6	6	14	-0.25	-0.50	0.00
NMDAR - GluN1	Hippocampus	Yeung et al.(66)	7	6	11	1.20	0.69	1.70
NMDAR - GluN1	Hippocampus	Berchtold et al.(97)	10	10	11	-0.63	-1.53	0.28
NMDAR - GluN1	Hippocampus	Bi et al.(99)	9	9	8	0.13	-0.80	1.05
NMDAR - GluN1	Hippocampus	Wang et al.(20)	5	6	6	-1.62	-3.06	-0.17
NMDAR - GluN1	Hippocampus	Sze et al.(100)	6	5	6	-1.55	-2.98	-0.12
NMDAR - GluN1	Hippocampus	Wakabayashi et al.(85)	7	7	6	-0.25	-1.31	0.80
NMDAR - GluN2A	Cortex	Yeung et al.(66)	6	6	11	0.05	-1.09	1.18

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
NMDAR - GluN2A	Cortex	Wang et al.(20)	5	6	6	-1.40	-2.79	-0.01
NMDAR - GluN2A	Cortex	Sze et al.(100)	6	5	6	-0.55	-1.77	0.67
NMDAR - GluN2A	Entorhinal cortex	Yeung et al.(66)	5	5	11	1.09	-0.29	2.47
NMDAR - GluN2A	Entorhinal cortex	Bi et al.(99)	9	9	8	-1.32	-2.37	-0.28
NMDAR - GluN2A	Entorhinal cortex	Wang et al.(20)	5	6	6	-0.44	-1.65	0.77
NMDAR - GluN2A	Entorhinal cortex	Sze et al.(100)	6	5	6	-1.37	-2.75	0.01
NMDAR - GluN2A	Hippocampus	Yeung et al.(66)	6	4	11	0.89	0.41	1.38
NMDAR - GluN2A	Hippocampus	Bi et al.(99)	9	9	8	-1.09	-2.09	-0.08
NMDAR - GluN2A	Hippocampus	Wang et al.(20)	5	6	6	-0.76	-2.00	0.49
NMDAR - GluN2A	Hippocampus	Sze et al.(100)	6	5	6	0.40	-0.81	1.60
NMDAR - GluN2B	Entorhinal cortex	Bi et al.(99)	9	9	8	-2.39	-3.67	-1.12
NMDAR - GluN2B	Entorhinal cortex	Wang et al.(20)	5	6	6	-0.61	-1.84	0.62
NMDAR - GluN2B	Entorhinal cortex	Sze et al.(100)	6	5	6	-1.50	-2.91	-0.08
NMDAR - GluN2B	Hippocampus	Marshal et al.(101)	3	8	9	-0.15	-1.48	1.18
NMDAR - GluN2B	Hippocampus	Bi et al.(99)	9	9	8	-0.91	-1.90	0.07
NMDAR - GluN2B	Hippocampus	Wang et al.(20)	5	6	6	-1.37	-2.75	0.01
NMDAR - GluN2B	Hippocampus	Sze et al.(100)	6	5	6	-1.43	-2.83	-0.03
Open-NMDAR	Cortex	Tsang et al.(89)	11	22	12	-0.79	-1.54	-0.04

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
Open-NMDAR	Cortex	Scheuer et al.(92)	20	21	8	-0.54	-0.67	-0.40
Open-NMDAR	Cortex	Ninomiya et al.(91)	10	8	8	-2.87	-4.27	-1.46
Open-NMDAR	Cortex	Simpson et al.(65)	8	6	8	-0.75	-1.09	-0.41
Open-NMDAR	Cortex	Palmer et al.(93)	8	8	8	0.25	0.05	0.44
Open-NMDAR	Cortex	Mouradian et al.(95)	11	9	5	-0.16	-0.31	-0.01
Open-NMDAR	Cortex	Shimohama et al.(94)	5	5	5	-4.82	-7.76	-1.88
Open-NMDAR	Hippocampus	Ulas et al.(90)	10	8	10	-0.58	-0.83	-0.34
Open-NMDAR	Hippocampus	Jansen et al.(80)	9	8	8	-1.19	-1.62	-0.76
Open-NMDAR	Hippocampus	Simpson et al.(65)	9	5	8	0.48	-0.64	1.59
Open-NMDAR	Hippocampus	Mouradian et al.(95)	9	7	5	-0.13	-1.12	0.86
VGluT1	Cortex	Garcia-Esparcia et al.(71)	39	20	8	0.91	0.34	1.47
VGluT1	Cortex	Sokolow et al.(35)	4	6	8	1.09	-0.31	2.49
VGluT1	Cortex	Kashani et al.(72)	5	4	7	-2.52	-4.54	-0.51
VGluT1	Cortex	Mitew et al.(33)	5	6	6	-3.91	-6.24	-1.58
VGluT1	Cortex	Kirvell et al.(32)	9	12	6	-0.83	-1.11	-0.54
VGluT1	Cortex	Poirel et al.(31)	63	64	4	-0.28	-0.63	0.07
VGluT2	Cortex	Sokolow et al.(35)	4	6	8	-0.26	-1.53	1.01
VGluT2	Cortex	Kirvell et al.(32)	10	11	6	-0.15	-0.28	-0.01

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
VGluT2	Cortex	Poirel et al.(31)	63	64	4	0.12	-0.22	0.47
Aspartate	Cortex	Sasaki et al.(74)	10	9	12	-0.82	-1.12	-0.52
Aspartate	Cortex	Gueli et al.(17)	13	13	12	-1.48	-2.36	-0.60
Aspartate	Cortex	D`Aniello et al.(101)	5	5	10	-0.90	-1.38	-0.42
Aspartate	Cortex	Seidl et al.(23)	8	8	10	-0.21	-0.40	-0.02
Aspartate	Cortex	Gramsbergen et al.(76)	12	12	8	0.45	-0.36	1.27
Aspartate	Hippocampus	Sasaki et al.(74)	10	8	12	-1.08	-2.09	-0.07
Aspartate	Hippocampus	D`Aniello et al.(101)	5	5	10	-1.14	-2.53	0.25
Aspartate	Hippocampus	Gramsbergen et al.(76)	12	12	8	-0.61	-1.44	0.21
Glutamate	Cortex	Tommaso et al.(22)	10	10	13	-1.17	-2.14	-0.21
Glutamate	Cortex	Gueli et al.(17)	13	13	12	-1.23	-2.08	-0.38
Glutamate	Cortex	Sasaki et al.(74)	10	9	12	-1.31	-1.75	-0.87
Glutamate	Cortex	Seidl et al.(23)	8	8	10	-0.22	-0.40	-0.03
Glutamate	Cortex	Gramsbergen et al.(76)	12	12	8	0.01	-0.79	0.81
Glutamine	Cortex	Tommaso et al.(22)	10	10	13	-0.51	-1.40	0.39
Glutamine	Cortex	Gueli et al.(17)	13	13	12	0.92	0.11	1.74
Glutamine	Cortex	Seidl et al.(23)	8	8	10	-0.14	-0.32	0.04
Glutamine	Cortex	Gramsbergen et al.(76)	12	12	8	-0.11	-0.91	0.69

Outcome	Region	Study (reference n. from main text)	CN	AD	Bias score	SMD	ci_low	ci_high
AMPAR - GluA1	Entorhinal cortex	Wang et al.(20)	5	6	6	-0.65	-1.89	0.58
AMPAR - GluA1	Entorhinal cortex	Wakabayashi et al.(85)	7	7	6	-1.57	-2.82	-0.32
AMPAR - GluA1	Entorhinal cortex	Yasuda et al.(84)	6	8	3	-1.03	-2.18	0.12
AMPAR - GluA1	Hippocampus	Pellegrini-Giampietro et al.(82)	10	9	10	-0.88	-1.83	0.08
AMPAR - GluA1	Hippocampus	Wang et al.(20)	5	6	6	-1.03	-2.34	0.27
AMPAR - GluA1	Hippocampus	Wakabayashi et al.(85)	7	7	6	-1.81	-3.12	-0.50
AMPAR - GluA1	Hippocampus	González et al.(83)	8	5	5	-1.34	-2.61	-0.07
AMPAR - GluA2/3	Cortex	Yeung et al.(66)	7	6	11	-0.55	-1.67	0.57
AMPAR - GluA2/3	Cortex	Gong et al.(87)	10	12	10	-3.46	-4.86	-2.06
AMPAR - GluA2/3	Cortex	Falsafi et al.(19)	24	24	9	0.42	0.32	0.52
AMPAR - GluA2/3	Cortex	Wang et al.(20)	5	6	6	-1.19	-2.52	0.15
AMPAR - GluA2/3	Entorhinal cortex	Yeung et al.(66)	7	6	11	-0.51	-1.63	0.60
AMPAR - GluA2/3	Entorhinal cortex	Wang et al.(20)	5	6	6	-0.71	-1.95	0.53

Abbreviations: AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor); AMPAR-GluA (AMPAR-subunit); EAAT (excitatory amino acid transporter); GS (glutamine synthetase); GluK (kainate receptor); mGlu (metabotropic glutamate receptor); open-NMDAR (N-methyl-D-aspartate receptor). NMDAR-GluN (NMDAR-subunit). VGluT (vesicular glutamate transporter). SMD (standard mean difference). CI (confidence interval). CN (cognitively normal). AD (Alzheimer`s disease).

Supplementary Table S4. Results from the Jackknife method

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
AMPAR - GluA1_Entorhinal cortex	Wakabayashi et al.(85)	-0.86	0.43	-1.99	0.046	-1.70	-0.02	0.00	0.00	0.046
AMPAR - GluA1_Entorhinal cortex	Wang et al.(20)	-1.28	0.43	-2.96	0.003	-2.12	-0.43	0.00	0.00	0.009
AMPAR - GluA1_Entorhinal cortex	Yasuda et al.(84)	-1.11	0.46	-2.42	0.016	-2.01	-0.21	0.02	4.51	0.023
AMPAR - GluA1_Hippocampus	González et al.(87)	-1.16	0.34	-3.42	0.001	-1.82	-0.49	0.00	0.00	0.001
AMPAR - GluA1_Hippocampus	Pellegrini-Giampietro et al.(83)	-1.39	0.38	-3.65	0.000	-2.14	-0.65	0.00	0.00	0.001
AMPAR - GluA1_Hippocampus	Wakabayashi et al.(85)	-1.04	0.34	-3.10	0.002	-1.70	-0.38	0.00	0.00	0.002
AMPAR - GluA1_Hippocampus	Wang et al.(20)	-1.24	0.34	-3.68	0.000	-1.90	-0.58	0.00	0.00	0.001
AMPAR - GluA1_Whole brain	Falsafi et al.(21)	-1.12	0.18	-6.19	0.000	-1.47	-0.76	0.02	11.41	0.000
AMPAR - GluA1_Whole brain	González et al.(87)	-0.46	0.67	-0.69	0.491	-1.78	0.85	2.08	94.07	0.595
AMPAR - GluA1_Whole brain	Pellegrini-Giampietro et al.(83)	-0.54	0.70	-0.77	0.441	-1.92	0.84	2.27	94.16	0.595
AMPAR - GluA1_Whole brain	Wakabayashi et al.(85)	-0.37	0.69	-0.53	0.595	-1.72	0.99	2.16	92.76	0.595

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
AMPAR - GluA1_Whole brain	Wang et al.(20)	-0.55	0.83	-0.66	0.510	-2.17	1.08	3.18	93.94	0.595
AMPAR - GluA1_Whole brain	Yasuda et al.(84)	-0.51	0.68	-0.75	0.451	-1.85	0.82	2.15	94.15	0.595
AMPAR - GluA2/3_Cortex	Falsafi et al.(21)	-1.70	0.86	-1.96	0.050	-3.39	0.00	1.81	80.90	0.198
AMPAR - GluA2/3_Cortex	Gong et al.(88)	-0.28	0.53	-0.53	0.597	-1.31	0.75	0.61	76.05	0.597
AMPAR - GluA2/3_Cortex	Wang et al.(20)	-1.10	1.05	-1.05	0.293	-3.15	0.95	3.02	93.76	0.390
AMPAR - GluA2/3_Cortex	Yeung et al.(21)	-1.33	1.20	-1.11	0.266	-3.68	1.02	4.00	94.23	0.390
AMPAR - GluA2/3_Entorhinal cortex	Thorns et al.(87)	-0.90	0.35	-2.53	0.011	-1.59	-0.20	0.00	0.00	0.015
AMPAR - GluA2/3_Entorhinal cortex	Wang et al.(20)	-0.86	0.30	-2.90	0.004	-1.45	-0.28	0.00	0.00	0.007
AMPAR - GluA2/3_Entorhinal cortex	Yasuda et al.(84)	-0.68	0.30	-2.29	0.022	-1.26	-0.10	0.00	0.00	0.022
AMPAR - GluA2/3_Entorhinal cortex	Yeung et al.(21)	-0.93	0.31	-3.04	0.002	-1.53	-0.33	0.00	0.00	0.007
AMPAR - GluA2/3_Hippocampus	Thorns et al.(87)	-0.57	0.67	-0.86	0.392	-1.88	0.74	0.69	72.48	0.392
AMPAR - GluA2/3_Hippocampus	Wang et al.(20)	-0.20	0.11	-1.80	0.071	-0.41	0.02	0.01	57.74	0.214
AMPAR - GluA2/3_Hippocampus	Yeung et al.(21)	-0.65	0.54	-1.20	0.228	-1.70	0.40	0.41	61.56	0.343

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
AMPAR - GluA2/3_Whole brain	Falsafi et al.(21)	-0.80	0.25	-3.21	0.001	-1.30	-0.31	0.24	84.10	0.009
AMPAR - GluA2/3_Whole brain	Gong et al.(88)	-0.36	0.27	-1.36	0.174	-0.89	0.16	0.33	95.46	0.174
AMPAR - GluA2/3_Whole brain	Thorns et al.(87)	-0.73	0.35	-2.07	0.038	-1.43	-0.04	0.58	93.85	0.067
AMPAR - GluA2/3_Whole brain	Tsamis et al.(88)	-0.73	0.30	-2.46	0.014	-1.31	-0.15	0.42	96.29	0.048
AMPAR - GluA2/3_Whole brain	Wang et al.(20)	-0.53	0.29	-1.81	0.070	-1.10	0.04	0.37	95.63	0.082
AMPAR - GluA2/3_Whole brain	Yasuda et al.(84)	-0.53	0.29	-1.85	0.065	-1.09	0.03	0.39	96.10	0.082
AMPAR - GluA2/3_Whole brain	Yeung et al.(21)	-0.77	0.34	-2.27	0.023	-1.43	-0.10	0.52	96.15	0.054
AMPAR_Hippocampus	Dewar et al.(83)	-0.64	0.52	-1.23	0.219	-1.65	0.38	0.51	94.94	0.219
AMPAR_Hippocampus	Geddes et al.(43)	-0.89	0.26	-3.38	0.001	-1.41	-0.38	0.10	72.00	0.002
AMPAR_Hippocampus	Jansen et al.(80)	-0.37	0.25	-1.46	0.145	-0.86	0.13	0.11	85.18	0.217
AMPAR_Whole brain	Chalmers et al.(45)	-0.68	0.32	-2.12	0.034	-1.30	-0.05	0.27	91.33	0.134
AMPAR_Whole brain	Dewar et al.(83)	-0.28	0.33	-0.85	0.397	-0.93	0.37	0.31	94.32	0.500
AMPAR_Whole brain	Geddes et al.(43)	-0.51	0.50	-1.02	0.307	-1.48	0.47	0.70	95.50	0.500

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
AMPAR_Whole brain	Jansen et al.(80)	-0.19	0.28	-0.67	0.500	-0.72	0.35	0.21	91.79	0.500
Aspartate_Cortex	Sasaki et al.(71)	-0.51	0.31	-1.62	0.106	-1.12	0.11	0.30	82.38	0.106
Aspartate_Cortex	D'Aniello et al.(103)	-0.50	0.28	-1.75	0.080	-1.05	0.06	0.24	86.15	0.099
Aspartate_Cortex	Gramsbergen et al.(22)	-0.76	0.25	-3.02	0.003	-1.25	-0.27	0.19	85.66	0.013
Aspartate_Cortex	Gueli et al.(10)	-0.44	0.24	-1.82	0.069	-0.92	0.03	0.18	85.07	0.099
Aspartate_Cortex	Seidl et al.(23)	-0.71	0.28	-2.52	0.012	-1.26	-0.16	0.22	74.11	0.029
Aspartate_Hippocampus	Sasaki et al.(71)	-0.75	0.36	-2.07	0.038	-1.46	-0.04	0.00	0.00	0.038
Aspartate_Hippocampus	D'Aniello et al.(103)	-0.80	0.33	-2.45	0.014	-1.44	-0.16	0.00	0.00	0.021
Aspartate_Hippocampus	Gramsbergen et al.(22)	-1.10	0.42	-2.63	0.008	-1.91	-0.28	0.00	0.00	0.021
Aspartate_Whole brain	Sasaki et al.(71)	-0.62	0.24	-2.59	0.010	-1.10	-0.15	0.18	89.02	0.012
Aspartate_Whole brain	D'Aniello et al.(103)	-0.56	0.22	-2.58	0.010	-0.99	-0.13	0.15	90.50	0.012
Aspartate_Whole brain	Gramsbergen et al.(22)	-0.74	0.14	-5.41	0.000	-1.01	-0.47	0.03	48.85	0.000
Aspartate_Whole brain	Gueli et al.(10)	-0.52	0.20	-2.59	0.010	-0.92	-0.13	0.14	90.50	0.012
Aspartate_Whole brain	Seidl et al.(23)	-0.70	0.28	-2.53	0.012	-1.25	-0.16	0.25	91.29	0.012
EAAT2_Cortex	Beckstrøm et al.(66)	-0.21	0.37	-0.56	0.573	-0.94	0.52	0.78	87.59	0.727

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
EAAT2_Cortex	Garcia-Esparcia et al.(73)	-0.52	0.27	-1.95	0.051	-1.04	0.00	0.32	71.56	0.406
EAAT2_Cortex	Hoshi et al.(72)	-0.01	0.29	-0.04	0.966	-0.58	0.56	0.42	77.96	0.966
EAAT2_Cortex	Lee et al.(102)	-0.35	0.36	-0.98	0.328	-1.05	0.35	0.74	87.51	0.727
EAAT2_Cortex	Poirel et al.(34)	-0.29	0.44	-0.65	0.518	-1.16	0.58	1.16	87.79	0.727
EAAT2_Cortex	Tian et al.(69)	-0.17	0.36	-0.47	0.636	-0.88	0.54	0.74	87.28	0.727
EAAT2_Cortex	Woltjer et al.(67)	-0.25	0.41	-0.62	0.537	-1.06	0.55	0.97	87.77	0.727
EAAT2_Cortex	Yeung et al.(66)	-0.23	0.37	-0.63	0.528	-0.95	0.49	0.77	87.77	0.727
EAAT2_Whole brain	Beckstrøm et al.(66)	-0.19	0.23	-0.83	0.404	-0.64	0.26	0.33	92.42	0.475
EAAT2_Whole brain	Garcia-Esparcia et al.(73)	-0.43	0.17	-2.56	0.010	-0.76	-0.10	0.16	88.62	0.094
EAAT2_Whole brain	Hoshi et al.(72)	-0.09	0.19	-0.50	0.620	-0.46	0.27	0.21	91.41	0.620
EAAT2_Whole brain	Kobayash et al.(68)	-0.20	0.22	-0.92	0.359	-0.62	0.22	0.28	92.59	0.475
EAAT2_Whole brain	Lee et al.(102)	-0.29	0.19	-1.52	0.128	-0.67	0.08	0.24	92.50	0.383
EAAT2_Whole brain	Poirel et al.(34)	-0.27	0.21	-1.29	0.196	-0.69	0.14	0.27	92.39	0.440
EAAT2_Whole brain	Tian et al.(69)	-0.20	0.20	-1.01	0.311	-0.58	0.19	0.25	92.61	0.475

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
EAAT2_Whole brain	Woltjer et al.(67)	-0.21	0.26	-0.80	0.422	-0.72	0.30	0.44	91.56	0.475
EAAT2_Whole brain	Yeung et al.(66)	-0.34	0.19	-1.85	0.064	-0.71	0.02	0.19	88.20	0.289
Glutamate reuptake_Cortex	Beckstrøm et al.(66)	-1.15	0.31	-3.73	0.000	-1.75	-0.54	0.61	83.80	0.000
Glutamate reuptake_Cortex	Chalmers et al.(45)	-0.83	0.20	-4.07	0.000	-1.23	-0.43	0.21	78.40	0.000
Glutamate reuptake_Cortex	Cowburn et al.(100)	-0.93	0.22	-4.20	0.000	-1.36	-0.49	0.28	83.12	0.000
Glutamate reuptake_Cortex	Cowburn et al.(65)	-1.17	0.26	-4.59	0.000	-1.68	-0.67	0.37	77.97	0.000
Glutamate reuptake_Cortex	Cross et al.(67)	-1.02	0.23	-4.45	0.000	-1.47	-0.57	0.31	84.47	0.000
Glutamate reuptake_Cortex	Hardy et al.(67)	-0.88	0.22	-4.08	0.000	-1.31	-0.46	0.24	80.15	0.000
Glutamate reuptake_Cortex	Lee et al.(102)	-0.95	0.23	-4.22	0.000	-1.39	-0.51	0.29	83.66	0.000
Glutamate reuptake_Cortex	Masliah et al.(56)	-0.99	0.23	-4.26	0.000	-1.44	-0.53	0.31	84.04	0.000
Glutamate reuptake_Cortex	Rothstein et al.(64)	-1.03	0.23	-4.41	0.000	-1.48	-0.57	0.31	84.45	0.000
Glutamate reuptake_Cortex	Simpson et al.(67)	-0.99	0.24	-4.12	0.000	-1.47	-0.52	0.32	82.11	0.000
Glutamate reuptake_Hippocampus	Cowburn et al.(65)	-0.63	0.35	-1.81	0.071	-1.31	0.05	0.00	0.00	0.071

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
Glutamate reuptake_Hippocampus	Hardy et al.(67)	-0.76	0.38	-1.99	0.046	-1.50	-0.01	0.00	0.00	0.071
Glutamate reuptake_Hippocampus	Rothstein et al.(64)	-0.95	0.35	-2.74	0.006	-1.63	-0.27	0.00	0.00	0.025
Glutamate reuptake_Hippocampus	Simpson et al.(67)	-0.69	0.36	-1.88	0.060	-1.40	0.03	0.00	0.00	0.071
Glutamate reuptake_Whole brain	Beckstrøm et al.(66)	-0.85	0.23	-3.67	0.000	-1.31	-0.40	0.38	84.54	0.000
Glutamate reuptake_Whole brain	Chalmers et al.(45)	-0.60	0.17	-3.64	0.000	-0.93	-0.28	0.17	79.54	0.000
Glutamate reuptake_Whole brain	Cowburn et al.(100)	-0.69	0.18	-3.82	0.000	-1.04	-0.34	0.22	83.54	0.000
Glutamate reuptake_Whole brain	Cowburn et al.(65)	-0.80	0.21	-3.84	0.000	-1.22	-0.39	0.29	84.68	0.000
Glutamate reuptake_Whole brain	Cross et al.(67)	-0.76	0.19	-4.05	0.000	-1.12	-0.39	0.24	84.73	0.000
Glutamate reuptake_Whole brain	Hardy et al.(67)	-0.69	0.19	-3.70	0.000	-1.06	-0.32	0.22	82.44	0.000
Glutamate reuptake_Whole brain	Lee et al.(102)	-0.70	0.18	-3.84	0.000	-1.06	-0.34	0.23	84.00	0.000
Glutamate reuptake_Whole brain	Masliah et al.(56)	-0.73	0.19	-3.86	0.000	-1.09	-0.36	0.24	84.32	0.000
Glutamate reuptake_Whole brain	Rothstein et al.(64)	-0.82	0.20	-3.99	0.000	-1.22	-0.42	0.28	84.69	0.000
Glutamate reuptake_Whole brain	Simpson et al.(67)	-0.75	0.20	-3.75	0.000	-1.15	-0.36	0.26	83.54	0.000

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
Glutamate reuptake_Whole brain	Xuereb et al.(64)	-0.85	0.16	-5.34	0.000	-1.16	-0.54	0.14	71.31	0.000
Glutamate_Cortex	Gueli et al.(10)	-0.66	0.36	-1.84	0.065	-1.36	0.04	0.41	87.21	0.065
Glutamate_Cortex	Sasaki et al.(71)	-0.56	0.29	-1.92	0.055	-1.13	0.01	0.21	66.75	0.065
Glutamate_Cortex	Gramsbergen et al.(22)	-0.94	0.38	-2.47	0.014	-1.68	-0.19	0.47	88.41	0.034
Glutamate_Cortex	Tommaso et al.(73)	-0.68	0.36	-1.90	0.057	-1.38	0.02	0.42	87.83	0.065
Glutamate_Cortex	Seidl et al.(23)	-0.95	0.31	-3.04	0.002	-1.56	-0.34	0.24	63.78	0.012
Glutamate_Whole brain	Gueli et al.(10)	-0.77	0.19	-4.13	0.000	-1.13	-0.40	0.10	76.82	0.000
Glutamate_Whole brain	Sasaki et al.(71)	-0.66	0.16	-4.03	0.000	-0.97	-0.34	0.06	60.62	0.000
Glutamate_Whole brain	Hyman et al.(72)	-0.74	0.16	-4.57	0.000	-1.06	-0.43	0.08	73.20	0.000
Glutamate_Whole brain	Gramsbergen et al.(22)	-1.05	0.28	-3.73	0.000	-1.60	-0.50	0.24	76.14	0.000
Glutamate_Whole brain	Tommaso et al.(73)	-0.79	0.19	-4.19	0.000	-1.15	-0.42	0.10	77.90	0.000
Glutamate_Whole brain	Seidl et al.(23)	-1.05	0.28	-3.79	0.000	-1.60	-0.51	0.24	77.49	0.000
Glutamine_Cortex	Gramsbergen et al.(22)	0.06	0.35	0.18	0.858	-0.62	0.74	0.25	71.65	0.858
Glutamine_Cortex	Gueli et al.(10)	-0.16	0.09	-1.77	0.077	-0.33	0.02	0.00	0.00	0.309
Glutamine_Cortex	Tommaso et al.(73)	0.14	0.31	0.46	0.642	-0.46	0.75	0.19	68.07	0.858
Glutamine_Cortex	Seidl et al.(23)	0.11	0.42	0.27	0.788	-0.72	0.94	0.36	66.25	0.858

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
Glutamine_Whole brain	Gramsbergen et al.(22)	0.07	0.35	0.19	0.850	-0.61	0.74	0.25	71.39	0.951
Glutamine_Whole brain	Gueli et al.(10)	-0.21	0.05	-3.79	0.000	-0.31	-0.10	0.00	0.00	0.001
Glutamine_Whole brain	Tommaso et al.(73)	-0.09	0.14	-0.61	0.544	-0.36	0.19	0.04	74.96	0.951
Glutamine_Whole brain	Seidl et al.(23)	0.02	0.37	0.06	0.951	-0.69	0.74	0.29	75.02	0.951
GS_Whole brain	Burbaeva et al.(78)	-0.19	0.77	-0.24	0.807	-1.71	1.33	1.11	91.89	0.890
GS_Whole brain	Hensley et al.(78)	0.93	0.34	2.72	0.007	0.26	1.60	0.02	9.47	0.020
GS_Whole brain	Jørgensen et al.(83)	0.16	1.12	0.14	0.890	-2.04	2.35	2.39	94.56	0.890
GluK_Hippocampus	Cowburn et al.(44)	-0.23	0.10	-2.21	0.027	-0.44	-0.03	0.03	69.03	0.067
GluK_Hippocampus	Dewar et al.(83)	-0.14	0.10	-1.37	0.171	-0.34	0.06	0.02	54.06	0.212
GluK_Hippocampus	Geddes et al.(46)	-0.17	0.12	-1.43	0.154	-0.41	0.06	0.03	69.32	0.212
GluK_Hippocampus	Geddes et al.(43)	-0.21	0.17	-1.25	0.212	-0.55	0.12	0.07	73.08	0.212
GluK_Hippocampus	Jansen et al.(80)	-0.30	0.11	-2.70	0.007	-0.52	-0.08	0.02	42.08	0.035
GluK_Whole brain	Chalmers et al.(45)	-0.15	0.11	-1.38	0.167	-0.37	0.06	0.04	77.28	0.961
GluK_Whole brain	Cowburn et al.(44)	-0.07	0.16	-0.45	0.650	-0.39	0.24	0.11	87.57	0.961

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
GluK_Whole brain	Dewar et al.(83)	0.08	0.13	0.57	0.572	-0.19	0.34	0.07	83.77	0.961
GluK_Whole brain	Geddes et al.(46)	0.04	0.15	0.25	0.805	-0.26	0.33	0.10	87.77	0.961
GluK_Whole brain	Geddes et al.(43)	-0.02	0.19	-0.10	0.918	-0.40	0.36	0.16	89.05	0.961
GluK_Whole brain	Jansen et al.(80)	-0.01	0.19	-0.05	0.961	-0.38	0.36	0.15	88.91	0.961
mGlu_Whole brain	Albasanz et al.(38)	0.29	0.57	0.50	0.617	-0.84	1.41	1.13	88.89	0.972
mGlu_Whole brain	Dewar et al.(83)	0.02	0.71	0.03	0.972	-1.37	1.42	1.73	86.68	0.972
mGlu_Whole brain	Gong et al.(88)	-0.17	0.73	-0.23	0.821	-1.60	1.27	1.89	91.31	0.972
mGlu_Whole brain	Lee et al.(102)	-0.55	0.46	-1.19	0.236	-1.46	0.36	0.63	77.08	0.972
mGlu_Whole brain	Tsamis et al.(88)	-0.37	0.63	-0.59	0.556	-1.60	0.86	1.38	90.29	0.972
NMDAR - GluN1_Cortex	Elnagar et al.(99)	-0.31	0.41	-0.75	0.451	-1.12	0.50	0.56	69.10	0.542
NMDAR - GluN1_Cortex	Falsafi et al.(21)	-0.66	0.39	-1.68	0.093	-1.43	0.11	0.41	54.43	0.424
NMDAR - GluN1_Cortex	Gong et al.(88)	-0.37	0.45	-0.83	0.409	-1.24	0.51	0.66	70.26	0.542
NMDAR - GluN1_Cortex	Sze et al.(100)	-0.49	0.45	-1.09	0.276	-1.36	0.39	0.70	74.41	0.542
NMDAR - GluN1_Cortex	Wang et al.(20)	-0.18	0.33	-0.56	0.574	-0.82	0.45	0.29	56.64	0.574
NMDAR - GluN1_Cortex	Yeung et al.(21)	-0.62	0.42	-1.47	0.141	-1.46	0.21	0.61	71.30	0.424

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
NMDAR - GluN1_Entorhinal cortex	Bi et al.(100)	-0.21	0.56	-0.38	0.705	-1.30	0.88	1.09	93.11	0.957
NMDAR - GluN1_Entorhinal cortex	Sze et al.(100)	0.03	0.49	0.05	0.957	-0.93	0.98	0.81	93.55	0.957
NMDAR - GluN1_Entorhinal cortex	Ulas et al.(96)	0.10	0.43	0.23	0.814	-0.74	0.94	0.61	92.72	0.957
NMDAR - GluN1_Entorhinal cortex	Wang et al.(20)	0.05	0.40	0.14	0.892	-0.73	0.84	0.58	94.69	0.957
NMDAR - GluN1_Entorhinal cortex	Yeung et al.(21)	-0.54	0.34	-1.58	0.115	-1.21	0.13	0.38	91.40	0.575
NMDAR - GluN1_Hippocampus	Berchtold et al.(98)	-0.23	0.40	-0.58	0.562	-1.01	0.55	0.70	85.57	0.749
NMDAR - GluN1_Hippocampus	Bi et al.(100)	-0.36	0.40	-0.90	0.368	-1.15	0.43	0.73	86.11	0.749
NMDAR - GluN1_Hippocampus	Sze et al.(100)	-0.12	0.36	-0.33	0.738	-0.83	0.58	0.58	84.36	0.749
NMDAR - GluN1_Hippocampus	Ulas et al.(96)	-0.34	0.49	-0.71	0.481	-1.30	0.61	1.14	83.56	0.749
NMDAR - GluN1_Hippocampus	Wakabayashi et al.(85)	-0.29	0.39	-0.74	0.457	-1.07	0.48	0.71	86.13	0.749
NMDAR - GluN1_Hippocampus	Wang et al.(20)	-0.11	0.36	-0.32	0.749	-0.82	0.59	0.58	84.23	0.749
NMDAR - GluN1_Hippocampus	Yeung et al.(21)	-0.45	0.21	-2.09	0.037	-0.87	-0.03	0.09	34.05	0.259
NMDAR - GluN1_Whole brain	Berchtold et al.(98)	-0.25	0.22	-1.13	0.259	-0.67	0.18	0.35	87.49	0.346

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
NMDAR - GluN1_Whole brain	Bi et al.(100)	-0.34	0.26	-1.34	0.181	-0.85	0.16	0.51	85.13	0.346
NMDAR - GluN1_Whole brain	Elnagar et al.(99)	-0.22	0.21	-1.03	0.301	-0.64	0.20	0.34	87.21	0.346
NMDAR - GluN1_Whole brain	Falsafi et al.(21)	-0.35	0.22	-1.57	0.116	-0.79	0.09	0.37	87.33	0.346
NMDAR - GluN1_Whole brain	Gong et al.(88)	-0.23	0.22	-1.07	0.284	-0.66	0.19	0.35	87.30	0.346
NMDAR - GluN1_Whole brain	Sze et al.(100)	-0.22	0.22	-1.01	0.314	-0.65	0.21	0.35	85.44	0.346
NMDAR - GluN1_Whole brain	Tsamis et al.(88)	-0.31	0.22	-1.41	0.159	-0.73	0.12	0.36	87.74	0.346
NMDAR - GluN1_Whole brain	Ulas et al.(96)	-0.27	0.24	-1.10	0.271	-0.75	0.21	0.45	86.47	0.346
NMDAR - GluN1_Whole brain	Wakabayashi et al.(85)	-0.28	0.22	-1.27	0.203	-0.70	0.15	0.36	87.74	0.346
NMDAR - GluN1_Whole brain	Wang et al.(20)	-0.13	0.20	-0.67	0.500	-0.52	0.25	0.26	83.62	0.500
NMDAR - GluN1_Whole brain	Yeung et al.(21)	-0.43	0.19	-2.24	0.025	-0.81	-0.05	0.24	81.66	0.278
NMDAR - GluN2A_Cortex	Sze et al.(100)	-0.62	0.72	-0.86	0.391	-2.04	0.80	0.63	60.25	0.586
NMDAR - GluN2A_Cortex	Wang et al.(20)	-0.23	0.42	-0.54	0.586	-1.06	0.60	0.00	0.00	0.586
NMDAR - GluN2A_Cortex	Yeung et al.(21)	-0.92	0.47	-1.97	0.048	-1.84	-0.01	0.00	0.00	0.145

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
NMDAR - GluN2A_Entorhinal cortex	Bi et al.(100)	-0.24	0.69	-0.36	0.722	-1.59	1.10	0.97	68.06	0.722
NMDAR - GluN2A_Entorhinal cortex	Sze et al.(100)	-0.28	0.69	-0.41	0.679	-1.63	1.06	1.04	73.34	0.722
NMDAR - GluN2A_Entorhinal cortex	Wang et al.(20)	-0.57	0.78	-0.73	0.467	-2.09	0.96	1.40	77.19	0.722
NMDAR - GluN2A_Entorhinal cortex	Yeung et al.(21)	-1.05	0.35	-3.01	0.003	-1.74	-0.37	0.00	0.00	0.011
NMDAR - GluN2A_Hippocampus	Bi et al.(100)	0.31	0.49	0.62	0.533	-0.66	1.27	0.48	66.79	0.862
NMDAR - GluN2A_Hippocampus	Sze et al.(100)	-0.25	0.73	-0.34	0.734	-1.69	1.19	1.39	87.26	0.862
NMDAR - GluN2A_Hippocampus	Wang et al.(20)	0.11	0.64	0.17	0.862	-1.14	1.36	1.00	83.52	0.862
NMDAR - GluN2A_Hippocampus	Yeung et al.(21)	-0.52	0.45	-1.15	0.252	-1.41	0.37	0.27	44.11	0.862
NMDAR - GluN2A_Whole brain	Bi et al.(100)	-0.15	0.45	-0.33	0.739	-1.02	0.73	0.56	93.18	0.739
NMDAR - GluN2A_Whole brain	Sze et al.(100)	-0.40	0.61	-0.66	0.510	-1.59	0.79	1.06	95.53	0.739
NMDAR - GluN2A_Whole brain	Wang et al.(20)	-0.27	0.53	-0.52	0.604	-1.31	0.76	0.79	95.14	0.739
NMDAR - GluN2A_Whole brain	Yeung et al.(21)	-0.80	0.23	-3.43	0.001	-1.26	-0.34	0.12	76.45	0.002
NMDAR - GluN2B_Entorhinal cortex	Bi et al.(100)	-0.99	0.47	-2.10	0.036	-1.92	-0.07	0.00	0.00	0.054

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
NMDAR - GluN2B_Entorhinal cortex	Sze et al.(100)	-1.49	0.89	-1.68	0.093	-3.24	0.25	1.18	74.29	0.093
NMDAR - GluN2B_Entorhinal cortex	Wang et al.(20)	-1.99	0.48	-4.12	0.000	-2.94	-1.04	0.00	0.00	0.000
NMDAR - GluN2B_Hippocampus	Bi et al.(100)	-0.96	0.42	-2.27	0.023	-1.79	-0.13	0.05	9.71	0.023
NMDAR - GluN2B_Hippocampus	Marshal et al.(101)	-1.16	0.35	-3.27	0.001	-1.85	-0.46	0.00	0.00	0.004
NMDAR - GluN2B_Hippocampus	Sze et al.(100)	-0.82	0.35	-2.35	0.019	-1.51	-0.14	0.00	0.00	0.023
NMDAR - GluN2B_Hippocampus	Wang et al.(20)	-0.83	0.35	-2.38	0.017	-1.52	-0.15	0.00	0.00	0.023
NMDAR - GluN2B_Whole brain	Bi et al.(100)	-0.92	0.17	-5.46	0.000	-1.25	-0.59	0.00	0.17	0.000
NMDAR - GluN2B_Whole brain	Marshal et al.(101)	-1.14	0.18	-6.25	0.000	-1.50	-0.78	0.04	36.74	0.000
NMDAR - GluN2B_Whole brain	Sze et al.(100)	-1.16	0.26	-4.42	0.000	-1.68	-0.65	0.09	42.81	0.000
NMDAR - GluN2B_Whole brain	Wang et al.(20)	-1.00	0.30	-3.35	0.001	-1.59	-0.42	0.15	60.80	0.001
open-NMDAR_Cortex	Mouradian et al.(95)	-0.80	0.29	-2.80	0.005	-1.37	-0.24	0.34	93.06	0.016
open-NMDAR_Cortex	Ninomiya et al.(92)	-0.42	0.19	-2.17	0.030	-0.80	-0.04	0.16	92.13	0.030
open-NMDAR_Cortex	Palmer et al.(94)	-0.73	0.20	-3.67	0.000	-1.12	-0.34	0.14	87.44	0.002

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
open-NMDAR_Cortex	Scheuer et al.(92)	-0.66	0.26	-2.53	0.012	-1.17	-0.15	0.27	90.87	0.016
open-NMDAR_Cortex	Shimohama et al.(94)	-0.49	0.19	-2.53	0.012	-0.87	-0.11	0.17	92.58	0.016
open-NMDAR_Cortex	Simpson et al.(67)	-0.54	0.23	-2.39	0.017	-0.98	-0.10	0.20	92.77	0.020
open-NMDAR_Cortex	Tsang et al.(89)	-0.55	0.22	-2.53	0.011	-0.98	-0.12	0.19	93.32	0.016
open-NMDAR_Hippocampus	Jansen et al.(80)	-0.27	0.30	-0.88	0.378	-0.86	0.33	0.15	48.77	0.489
open-NMDAR_Hippocampus	Mouradian et al.(95)	-0.61	0.32	-1.90	0.058	-1.24	0.02	0.23	80.25	0.116
open-NMDAR_Hippocampus	Simpson et al.(67)	-0.73	0.26	-2.77	0.006	-1.24	-0.21	0.14	71.88	0.022
open-NMDAR_Hippocampus	Ulas et al.(90)	-0.38	0.54	-0.69	0.489	-1.44	0.69	0.69	79.72	0.489
open-NMDAR_Whole brain	Jansen et al.(80)	-0.49	0.16	-3.03	0.002	-0.81	-0.17	0.14	90.99	0.002
open-NMDAR_Whole brain	Mouradian et al.(95)	-0.72	0.20	-3.60	0.000	-1.11	-0.33	0.23	91.84	0.001
open-NMDAR_Whole brain	Ninomiya et al.(92)	-0.50	0.16	-3.19	0.001	-0.80	-0.19	0.14	91.38	0.002
open-NMDAR_Whole brain	Palmer et al.(94)	-0.70	0.15	-4.67	0.000	-0.99	-0.41	0.11	86.62	0.000
open-NMDAR_Whole brain	Scheuer et al.(92)	-0.65	0.20	-3.28	0.001	-1.04	-0.26	0.22	91.36	0.001

Outcome_region	Study (reference n. from main text)	Estimate	SE	Zval	Pval	CI_low	CI_high	Tau^2	I^2	Padj
open-NMDAR_Whole brain	Shimohama et al.(94)	-0.54	0.16	-3.47	0.001	-0.85	-0.24	0.15	91.75	0.001
open-NMDAR_Whole brain	Simpson et al.(67)	-0.67	0.19	-3.46	0.001	-1.04	-0.29	0.21	92.52	0.001
open-NMDAR_Whole brain	Tsang et al.(89)	-0.58	0.17	-3.42	0.001	-0.92	-0.25	0.17	92.42	0.001

The contribution of each study to the result was evaluated using a leave-one-out method (Jackknife' test) and the p-values were adjusted (Padj) by false discovery rate considering other synthesis with same outcome in different regions. Abbreviations: AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor); AMPAR-GluA (AMPAR-subunit); EAAT (excitatory amino acid transporter); GS (glutamine synthetase); GluK (kainate receptor); mGlu (metabotropic glutamate receptor); open-NMDAR (N-methyl-D-aspartate receptor). NMDAR-GluN (NMDAR-subunit). VGluT (vesicular glutamate transporter). SE (Standard error). CI (Confidence interval). P-values adjusted by false discovery rate.

Supplementary Table S5. Results from the Egger's regression test

Outcome_region	Coefficient	SE	Zvalue	Pvalue	CI_low	CI_high
GS_Whole brain	-1.68	0.23	-7.34	0.000	-2.13	-1.23
NMDAR - GluN2B_Whole brain	-1.63	0.53	-3.11	0.002	-2.67	-0.60
AMPAR_Hippocampus	0.43	0.17	2.46	0.014	0.09	0.76
open-NMDAR_Hippocampus	-1.24	0.61	-2.04	0.042	-2.44	-0.05
Glutamate_Whole brain	-0.26	0.14	-1.85	0.065	-0.53	0.02
GluK_Hippocampus	-0.32	0.19	-1.63	0.103	-0.70	0.06
Glutamine_Whole brain	-0.28	0.17	-1.59	0.113	-0.62	0.07
NMDAR - GluN2A_Cortex	6.36	4.38	1.45	0.146	-2.22	14.95
NMDAR - GluN1_Cortex	1.36	1.03	1.32	0.187	-0.66	3.37
NMDAR - GluN2A_Hippocampus	1.54	1.40	1.10	0.273	-1.21	4.28
NMDAR - GluN1_Hippocampus	1.02	0.95	1.08	0.279	-0.83	2.88
NMDAR - GluN2A_Entorhinal cortex	-4.76	5.09	-0.94	0.350	-14.75	5.22
Aspartate_Cortex	-0.47	0.53	-0.90	0.369	-1.50	0.56
Glutamate reuptake_Cortex	-0.26	0.30	-0.85	0.397	-0.85	0.34
EAAT2_Whole brain	-0.29	0.35	-0.84	0.399	-0.97	0.39
AMPAR_Whole brain	0.84	1.05	0.80	0.422	-1.22	2.91
VGluT1_Cortex	0.54	0.68	0.79	0.431	-0.80	1.88
AMPAR - GluA2_3_Cortex	0.80	1.16	0.69	0.491	-1.48	3.08
Glutamate reuptake_Hippocampus	-1.78	2.85	-0.63	0.531	-7.36	3.79
VGluT1_Whole brain	0.35	0.65	0.54	0.586	-0.91	1.62
NMDAR - GluN2A_Whole brain	-1.96	3.67	-0.54	0.593	-9.15	5.22
Glutamate_Cortex	-0.40	0.75	-0.53	0.596	-1.86	1.07

Outcome_region	Coefficient	SE	Zvalue	Pvalue	CI_low	CI_high
AMPAR - GluA2_3_Whole brain	0.17	0.33	0.52	0.605	-0.48	0.82
GluK_Whole brain	-0.21	0.46	-0.47	0.638	-1.11	0.68
VGluT2_Cortex	-0.08	0.19	-0.44	0.662	-0.46	0.30
Aspartate_Whole brain	0.06	0.15	0.39	0.699	-0.24	0.36
open-NMDAR_Cortex	0.09	0.24	0.37	0.712	-0.38	0.55
NMDAR - GluN1_Whole brain	0.15	0.47	0.31	0.757	-0.78	1.07
mGlu_Whole brain	0.54	1.88	0.29	0.774	-3.15	4.23
AMPAR - GluA1_Hippocampus	0.60	2.13	0.28	0.779	-3.57	4.76
Glutamate reuptake_Whole brain	-0.07	0.28	-0.24	0.807	-0.61	0.47
NMDAR - GluN2B_Hippocampus	-0.43	2.18	-0.20	0.845	-4.70	3.84
Glutamine_Cortex	-0.15	0.79	-0.19	0.851	-1.70	1.40
NMDAR - GluN2B_Entorhinal cortex	1.56	11.87	0.13	0.896	-21.71	24.82
AMPAR - GluA1_Entorhinal cortex	1.21	9.52	0.13	0.898	-17.45	19.88
AMPAR - GluA1_Whole brain	0.22	2.14	0.10	0.917	-3.97	4.41
NMDAR - GluN1_Entorhinal cortex	0.07	0.78	0.10	0.924	-1.45	1.60
AMPAR - GluA2_3_Entorhinal cortex	-0.14	1.47	-0.09	0.926	-3.01	2.74
AMPAR - GluA2_3_Hippocampus	-0.02	0.23	-0.09	0.928	-0.46	0.42

Egger's regression test was used to assess small-study effect as a method to examine publication bias. Abbreviations: AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor); AMPAR-GluA (AMPAR-subunit); EAAT (excitatory amino acid transporter); GS (glutamine synthetase); GluK (kainate receptor); mGlu (metabotropic glutamate receptor); open-NMDAR (N-methyl-D-aspartate receptor). NMDAR-GluN (NMDAR-subunit). VGluT (vesicular glutamate transporter). SE (standard error). CI (confidence interval).

Supplementary Table S6. Results from the sensitivity analysis

Outcome	Region	SMD	CI_lower	CI_upper	Pvalue	Padj.all	Padj_within	Zvalue	I^2	Tau^2	Deviance
AMPAR	CA1	-0.78	-1.34	-0.22	0.006	0.023	0.030	-2.72	37.8	0.1	4.52
AMPAR	CA3	-0.31	-1.08	0.45	0.418	0.523	0.418	-0.81	65.1	0.3	6.76
AMPAR	DG	-0.38	-0.99	0.22	0.216	0.355	0.270	-1.24	49.3	0.2	5.72
AMPAR	Hippocampus	-0.63	-1.23	-0.03	0.041	0.103	0.068	-2.05	91.5	0.3	9.63
AMPAR	Whole brain	-0.68	-1.30	-0.05	0.034	0.090	0.068	-2.13	91.3	0.3	9.26
AMPAR - GluA2/3	Cortex	-1.32	-3.03	0.40	0.133	0.259	0.177	-1.50	88.7	2.0	9.42
AMPAR - GluA2/3	Whole brain	-1.07	-2.78	0.64	0.221	0.355	0.221	-1.22	90.3	2.0	11.51
Aspartate	Cortex	-0.58	-1.05	-0.11	0.016	0.051	0.016	-2.41	84.4	0.2	14.50
Aspartate	Hippocampus	-0.86	-1.44	-0.28	0.004	0.018	0.006	-2.90	0.0	0.0	0.68
Aspartate	Whole brain	-0.64	-1.04	-0.23	0.002	0.011	0.006	-3.11	89.7	0.2	14.75
EAAT2	Cortex	0.25	-0.96	1.47	0.685	0.802	0.746	0.41	89.3	1.0	8.39
EAAT2	Whole brain	0.10	-0.51	0.72	0.746	0.824	0.746	0.32	95.8	0.4	18.35
Glutamate	Cortex	-0.76	-1.38	-0.13	0.018	0.051	0.018	-2.37	85.2	0.4	12.37
Glutamate	Whole brain	-0.74	-1.06	-0.43	0.000	0.000	0.000	-4.57	73.2	0.1	10.81

Outcome	Region	SMD	CI_lower	CI_upper	Pvalue	Padj.all	Padj_within	Zvalue	I^2	Tau^2	Deviance
Glutamate reuptake	Cortex	-0.77	-1.35	-0.19	0.010	0.035	0.015	-2.59	78.6	0.4	11.08
Glutamate reuptake	Hippocampus	-0.76	-1.50	-0.01	0.046	0.109	0.046	-1.99	0.0	0.0	1.88
Glutamate reuptake	Whole brain	-0.67	-0.93	-0.40	0.000	0.000	0.000	-4.91	33.2	0.0	7.71
Glutamine	Cortex	0.01	-0.47	0.49	0.971	0.993	0.971	0.04	57.5	0.1	8.19
Glutamine	Whole brain	-0.12	-0.37	0.13	0.343	0.454	0.686	-0.95	64.7	0.0	10.27
GluK	Hippocampus	-0.17	-0.41	0.06	0.154	0.277	0.308	-1.43	69.3	0.0	8.49
GluK	Whole brain	-0.11	-0.34	0.13	0.363	0.467	0.363	-0.91	79.7	0.0	11.42
mGlu	Whole brain	-0.55	-1.46	0.36	0.236	0.366		-1.19	77.1	0.6	10.97
NMDAR - GluN1	Entorhinal cortex	0.31	-0.75	1.37	0.565	0.687	0.993	0.58	95.3	0.8	13.60
VGluT1	Cortex	NA									
VGluT1	Whole brain	NA									
VGluT2	Cortex	NA									
GS	Whole brain	NA									
AMPAR - GluA1	Entorhinal cortex	NA									

Outcome	Region	SMD	CI_lower	CI_upper	Pvalue	Padj.all	Padj_within	Zvalue	I^2	Tau^2	Deviance
AMPAR - GluA1	Hippocampus	NA									
AMPAR - GluA1	Whole brain	NA									
AMPAR - GluA2/3	Entorhinal cortex	NA									
AMPAR - GluA2/3	Hippocampus	NA									
NMDAR - GluN2B	Entorhinal cortex	N									
NMDAR - GluN2B	Hippocampus	NA									
NMDAR - GluN2B	Whole brain	NA									
NMDAR - GluN2A	Cortex	NA									
NMDAR - GluN2A	Entorhinal cortex	NA									
NMDAR - GluN2A	Hippocampus	NA									
NMDAR - GluN1	Cortex	NA									

Studies assigned as “high-risk” of bias were removed from each synthesis to evaluate their contribution to the results. The p-values were adjusted by false discovery rate considering all synthesis (padj_all) or only considering the same outcome in different regions (padj_within). NA = If less than three studies remained after removal of high-risk studies, the sensitivity analysis was not performed. Abbreviations: AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor); AMPAR-GluA (AMPAR-subunit); EAAT (excitatory amino acid transporter); GS (glutamine synthetase); GluK (kainate receptor); mGlu (metabotropic glutamate receptor); Not available (NA). open-NMDAR (N-methyl-D-aspartate receptor). NMDAR-GluN (NMDAR-subunit). VGluT (vesicular glutamate transporter). Standard mean difference (SMD). Confidence interval (CI). Correction by FDR was performed within same outcome when at least two regions were available. Otherwise, outcomes were adjusted considering all outcomes.

CAPÍTULO 2

ESTUDO CLÍNICO DE BIOMARCADORES SINÁPTICOS NA DOENÇA DE ALZHEIMER

(A ser submetido no periódico Alzheimer's & Dementia)

Este capítulo investiga a hipótese de que tau-total (t-tau) no líquido cefalorraquidiano (LCR) está mais associado a biomarcadores de degeneração sináptica do que de degeneração neuronal. Analisamos dados de 1.825 indivíduos de três coortes independentes (TRIAD, ADNI, WRAP), utilizando biomarcadores de t-tau no LCR, volume hipocampal (HCV), proteína neurofilamentar de cadeia leve (NfL), neurogranina (Ng) e SNAP25. Observamos uma correlação mais forte entre t-tau no LCR e os biomarcadores sinápticos Ng e SNAP25 em comparação com os biomarcadores de neurodegeneração HCV e NfL. A análise revelou que os biomarcadores de degeneração sináptica explicam uma maior proporção da variância de t-tau do que os biomarcadores de degeneração neuronal. Ademais, níveis elevados de t-tau foram observados em indivíduos positivos para degeneração sináptica, independentemente do status de degeneração neuronal.

Este estudo demonstra que t-tau está mais relacionado à disfunção sináptica do que à degeneração neuronal, oferecendo implicações para estudos de biomarcadores e potencial refinamento na compreensão e uso clínico de t-tau no monitoramento da progressão da DA.

CAPÍTULO 3

ESTUDO PRÉ-CLÍNICO DE DENSIDADE SINÁPTICA EM MODELO ANIMAL DA DOENÇA DE ALZHEIMER

(Manuscrito em preparação para submissão no periódico PNAS como um “brief report”)

Neste capítulo, investigamos a densidade sináptica cerebral em ratos TgF344-AD envelhecidos utilizando PET-SV2A com o marcador [^{18}F]SDM8. Curvas tempo-atividade ao longo do escaneamento foram geradas a partir de regiões cerebrais de interesse e valores de captação padronizados (SUVs) foram analisados. Análises voxel-a-voxel foram conduzidas para comparar a expressão de SV2A entre os grupos transgênico e controle. Observamos que os SUVs atingiram o pico logo após a injeção do traçador e em seguida se estabilizaram. A análise voxel-a-voxel revelou reduções significativas no SUVr (ponte) de [^{18}F]SDM8 em ratos transgênicos, particularmente no córtex e hipocampo, consistentes com a perda sináptica observada na DA humana.

Em conclusão, nossos achados demonstram uma redução na densidade sináptica em regiões cerebrais chave de ratos TgF344-AD envelhecidos utilizando imagens de PET-SV2A. Este estudo apoia a utilidade do modelo TgF344-AD para pesquisas pré-clínicas sobre mudanças sinápticas na DA e destaca o potencial de biomarcadores sinápticos utilizando plataformas de imagem para monitoramento da progressão da doença e eficácia de tratamentos.

PARTE III

3. CONCLUSÕES

Esta tese de doutorado foi focada em alterações sinápticas que ocorrem na DA. Primeiramente, apresentamos uma revisão sistemática com metanálise de diversos componentes do sistema de neurotransmissão glutamatérgico em tecido post-mortem de indivíduos com DA. Depois, investigamos a relação do biomarcador tau-total com biomarcadores de degeneração sináptica e neurodegeneração. Finalmente, estudamos densidade sináptica em modelo animal de DA.

No **Capítulo 1**, integramos décadas de dados da literatura sobre a expressão/níveis/função de glutamato, aspartato, glutamina, receptores e transportadores glutamatérgicos, e proteínas de reciclagem de glutamato. Nossos achados evidenciam uma disfunção generalizada nesse sistema de neurotransmissão, sustentando a necessidade de estudos posteriores focados em medicamentos existentes e novos que possam modular essa via na DA.

Especificamente, nossa análise evidenciou uma redução na captação de glutamato em diversas regiões cerebrais, sugerindo um possível acúmulo desse neurotransmissor na fenda sináptica, contribuindo para a excitotoxicidade e a morte neuronal. Observamos também uma diminuição nos níveis de glutamato e aspartato no tecido cerebral de pacientes com DA, indicando uma perda geral, possivelmente por morte neuronal. No entanto, estudos revelaram níveis inalterados de glutamato no LCR de indivíduos com DA. Portanto, nosso estudo ressalta uma variabilidade regional que não pode ser negligenciada, ainda mais no atual cenário de largo uso de biomarcadores de fluidos.

Quanto aos marcadores pré-sinápticos VGluT1 e VGluT2, embora existam evidências consistentes de perda de sinapses na DA (CAMPORESI et al., 2020), detectamos níveis normais de ambos. Além disso, nossos achados sugerem que esses marcadores podem não estar diretamente envolvidos na disfunção vesicular na DA.

Notoriamente, encontramos uma hipoativação generalizada de NMDAR sugerindo que os indivíduos já se encontravam em uma fase tardia e mais severa da doença, quando há evidências de excitotoxicidade. Também observamos uma diminuição seletiva da subunidade GluN2B, sugerindo uma montagem compensatória. Juntos, esses resultados reforçam o papel no NMDAR na DA e sugerem uma barreira para a ação da memantina, destacando a necessidade de compreender melhor a dinâmica da excitotoxicidade na doença.

Os pontos fortes do nosso estudo incluem a síntese de uma grande diversidade de componentes do sistema glutamatérgico, possibilitando uma visão geral do sistema, utilizando

a mesma metodologia. Para fortalecer a metanálise nós utilizamos critérios rigorosos e adotamos um limiar conservador para análises de risco de viés. Devido à falta de dados para regiões cerebrais específicas, agrupamos regiões corticais e subcorticais, realizando metanálises individuais quando possível. Assim, os resultados gerais podem ocultar diferenças sutis, mas servem para examinar alterações glutamatérgicas robustas que ocorrem no cérebro como um todo. Contudo, a baixa quantidade de estudos e a alta heterogeneidade afetam o poder estatístico, consistindo em limitações da nossa pesquisa.

No **Capítulo 2**, demonstramos que o biomarcador t-tau quantificado no LCR reflete melhor a degeneração sináptica do que a degeneração axonal no espectro da DA, ao comparar as relações entre os níveis de t-tau e HCV, NfL, Ng e SNAP25, advindos de três coortes.

No contexto da abordagem conceitual ATN, o t-tau tem sido tradicionalmente interpretado como biomarcador de neurodegeneração, na mesma categoria de atrofia cerebral medida por ressonância magnética e níveis de NfL medidos no LCR. No entanto, nossos achados indicam que este biomarcador está mais intimamente ligado à disfunção sináptica do que à degeneração neuronal pronunciada. Ainda, observamos essa relação em indivíduos com e sem comprometimento cognitivo. Consequentemente, aumento dos níveis de t-tau pode ser interpretado como um evento precoce e sugere que está presente antes do comprometimento cognitivo. Nesse sentido, no contexto da busca por biomarcadores que possam detectar a patologia antes do aparecimento dos sintomas, a t-tau demonstra potencial relevante.

Embora estudos anteriores tenham observado uma associação robusta entre t-tau e marcadores sinápticos, nosso estudo é o primeiro, para nosso conhecimento, a demonstrar que a t-tau está mais fortemente associada à degeneração sináptica do que à neurodegeneração, ou degeneração axonal. O fato de que indivíduos com comprometimento cognitivo mostraram duas vezes maior contribuição da neurodegeneração para a variância de t-tau sugere que o dano cognitivo pode estar mais fortemente associado com o prejuízo axonal do que sináptico. Nesse sentido, nossos resultados reforçam as evidências de que a sinaptopatia ocorre antes da neurodegeneração (DAVIES et al., 1987; HOOVER et al., 2010; JACKSON et al., 2017; QIANG et al., 2022; TERRY et al., 1991).

Além disso, nosso estudo utilizou a estrutura conceitual ATN para classificar os participantes em N+ e S+, revelando uma distribuição que reflete a severidade da doença. Indivíduos com degeneração sináptica anormal exibiram níveis mais elevados de t-tau, independentemente de apresentarem ou não degeneração neuronal concomitante.

O fato de nossos resultados corroborarem com estudos anteriores demonstrando baixa correlação entre biomarcadores de neurodegeneração e alta correlação entre os de perda

sináptica sugere que a degeneração sináptica engloba um processo mais consistente do que os mecanismos subjacentes à atrofia e lesão neuronal na DA. Assim, nossos achados adicionam evidências que destacam a concordância limitada entre biomarcadores de neurodegeneração para a classificação ATN e monitoramento da doença. Isso enfatiza ainda mais a importância da exploração de biomarcadores sinápticos, incluindo t-tau, como ferramentas potencialmente mais robustas para o estadiamento da DA.

Um ponto forte do nosso estudo clínico inclui a replicação geral dos resultados em três coortes independentes e um grande tamanho amostral ao todo. As limitações consistem nas diferenças metodológicas e de ensaios para quantificação dos biomarcadores entre as coortes, bem como a indisponibilidade de todos os biomarcadores para todos os participantes. Essas limitações restringem algumas das análises de correlação. Além disso, o uso de pontos de corte para classificação binária consiste em uma ferramenta valiosa para a pesquisa e comumente utilizada na pesquisa clínica. Entretanto, eles são influenciados inerentemente pelo método utilizado.

No **Capítulo 3**, nossas análises demonstram robusta perda de densidade sináptica cerebral no modelo TgF344-AD. O uso do PET[^{18}F]SDM8 permitiu uma visualização global, *in vivo*, de perda da proteína SV2A nos ratos transgênicos envelhecidos. As curvas de atividade mostraram uma rápida captação e transferência do traçador para o tecido, consistente com dados de estudos clínicos e em primatas não-humanos.

Além disso, nossas observações refletem a redução de PET-SV2A em pacientes com DA bem como as regiões tipicamente afetadas na doença, como o córtex frontal e o hipocampo, consistentes com os primeiros e os mais recentes relatos de redução de densidade sináptica na DA. Dessa forma, nossa pesquisa confirma o potencial translacional do modelo TgF344-AD para estudo de sinaptopatia.

Dado que as evidências de perda sináptica nesse modelo ainda são escarças, nosso estudo pode ser considerado bastante relevante. Ainda, o uso de PET que possibilita a visualização do cérebro inteiro oferece uma excelente oportunidade para a melhor compreensão das alterações sinápticas sem o viés do uso de tecido post-mortem. Assim, nossos dados experimentais reforçam a centralidade da sinaptopatia na DA e destacam o modelo como uma ferramenta valiosa para o estudo dos mecanismos subjacentes à patologia.

Em conclusão, nossos estudos oferecem evidências relevantes para o entendimento da sinaptopatia na DA. Primeiramente, nossa revisão sistemática com metanálise concluiu que indivíduos com DA exibem uma depleção abrangente do sistema glutamatérgico no cérebro, além de revelar diferenças regionais não detectáveis no LCR. Além disso, em nosso estudo

clínico descobrimos que t-tau quantificado no LCR reflete melhor a degeneração sináptica do que a degeneração axonal em indivíduos no espectro da DA, sugerindo uma nova interpretação e uso mais precoce desse biomarcador. Finalmente, nossos resultados experimentais apontam para uma redução na densidade sináptica no modelo TgF344-AD, incentivando o uso do mesmo para o estudo da sinaptopatia na DA.

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