DIE MIKROBIOTA-DARM-HIRN-ACHSE IM ZUSAMMENHANG MIT DER ALZHEIMER-DEMENZ AM BEISPIEL VON 5xFAD- UND WILDTYPISCHEN MÄUSEN

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Bei der vorliegenden kumulativen Dissertation handelt es sich um eine verkürzte Darstellung der Forschungsergebnisse. Die ausführlichen Ergebnisse wurden bereits in folgenden Fachzeitschriften veröffentlicht:

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2. **Impact of Acute and Chronic Amyloid-β Peptide Exposure on Gut Microbial Commensals in the Mouse.**

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Front Microbiol. 2020 May 20;11:1008. doi: 10.3389/fmicb.2020.01008. eCollection 2020.

3. **Impact of Gut Microbiome Manipulation in 5xFAD Mice on Alzheimer’s Disease-Like Pathology**

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Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die vorliegende Dissertation selbstständig verfasst habe und keine anderen Hilfsmittel/Quellen benutzt habe als die in dieser Arbeit angegebenen.

Weiterhin erkläre ich, dass kein anderes Prüfungsverfahren beantragt oder diese Arbeit anderweitig als Prüfungsarbeit in einer anderen Fakultät vorgelegt wurde.

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Danksagung

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Inhaltsverzeichnis

I. Abkürzungsverzeichnis

A-beta	Amyloid-beta
ACh	Acetylcholin
AChEI	Acetylcholinesterase-Inhibitoren
AD	„Alzheimer's disease“
ADAM10	„a disintegrin and metalloproteinase domain-containing protein 10“
AICD	„APP intracellular Domain”
ALS	Amyotrophe Lateralsklerose
ANS	autonomes Nervensystem
APH-1	„anterior pharynx-defective 1“
ApoA1	Apolipoprotein A1
APP	„Amyloid precursor protein“
aSyn	alpha-Synuclein
BACE1	“beta-site APP cleavage enzyme 1”
BBB	„blood-brain-barrier“
CDK5	„cyclin dependent protein kinase 5“
CED	chronisch-entzündliche Darmerkrankung
CSF	Cerebrospinalflüssigkeit
ENS	enterisches Nervensystem
FAD	familiäre AD
FMT	fäkaler Mikrobiomtransfer
FoxA2	Forkhead-Box-Protein A2
GIT	Gastrointestinaltrakt
GTT	„gut transition time“
GSK3-beta	„glycogen synthase kinase 3-beta“
HDL	„high density Lipoprotein”
HPA	„hypothalamic-pituitary-adrenal“
IFN-gamma	Interferon-gamma
IL	Interleukin
IPAN	intrinsisch primär afferente Nervenzellen
iPSC	„induced pluripotent stem cells"
KBE	koloniebildende Einheit
LPS	Lipopolysaccharide
MAPK	„mitogen activated protein kinase“

MAPT	Mikrotubuli-assoziiertes Tau-Protein
MS	Multiple Sklerose
mTOR	“mechanistic target of rapamycin”
NFT	„neurofibrillary tangles”
NMDA	*N*-Methyl-D-Aspartat
PD	“Parkinson’s disease”
PEN-2	Presenelin-Enhancer 2
PNS	peripheres Nervensystem
PP2A	Proteinphosphatase 2A
PSEN1	Presenelin-1
PSEN2	Presenelin-2
ROS	„reactive oxygen species”
sAPP-alpha	„soluble alpha-APP“
sAPP-beta	„soluble beta-APP“
TH1	T-Helferzellen vom Typ 1
TNF-alpha	Tumornekrosefaktor-alpha
ZNS	zentrales Nervensystem

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IV. Zusammenfassung

Die pathologischen Ursachen der Alzheimer-Demenz (AD) sind trotz jahrelanger Forschung weiterhin nicht vollständig aufgeklärt. Bislang gibt es keine kurativen Behandlungsmöglichkeiten, lediglich Symptom-mildernde Arzneimittel. Um die Erforschung der Erkrankung weiter voranzutreiben, ist es dringend notwendig, auch andere Wege einzuschlagen. Neue Therapieziele bieten alternative Behandlungsstrategien. Einen neuen Ansatz für Interventionen bieten der Darm und dessen Mikrobiom, die zum größten Teil das menschliche Immunsystem modulieren. Für die Parkinson-Demenz, einer mit der AD vergleichbaren neurodegenerativen Erkrankung, konnte bereits gezeigt werden, dass der Darm bei der Pathophysiologie eine große Rolle spielt. Der Zusammenhang des Darms mit der AD ist jedoch noch nicht vollständig bekannt und bedarf weiterer Erforschung.

In ersten Studien wurde die Darmmikrobiota von AD-Patienten mit der gesunder Personen verglichen und dabei wurden signifikante Unterschiede festgestellt. Inwiefern sich diese auf den Verlauf der Erkrankung auswirken, ist derzeit aber noch unklar.

Der Fokus dieser Dissertation wurde deshalb auf die Auswirkungen der AD auf den Darm und die intestinale Mikrobiota gelegt beziehungsweise auf das Mikrobiom als Akteur in der AD-Pathogenese. Dabei konnten wir Zusammenhänge der AD mit der Mikrobiota-Darm-Hirn-Achse am Beispiel von 5xFAD- (AD-Modellmäusen) und wildtypischen Mäusen nachweisen. Unter anderem verändert sich die Darmtransitzeit mit dem progressiven Verlauf der AD in 5xFAD-Mäusen. Amyloid-beta (A-beta)-Ablagerungen konnten im Darm der AD-Modellmäuse nachgewiesen und anschließend die daraus resultierenden Veränderungen in der mikrobiellen Zusammensetzung analysiert werden. Bei direkter Exposition von A-beta mit intestinalen Bakterien hat das Peptid antimikrobielle Eigenschaften. Darüber hinaus deuten die Ergebnisse auf einen Resilienzmechanismus der Darmmikrobiota bei langfristiger Exposition mit dem Peptid hin.

Umgekehrt hat auch ein moduliertes Mikrobiom Einfluss auf die AD: Eine 14-wöchige Behandlung von 5xFAD-Mäusen mit Antibiotika führte zu einer besseren Nestbaufähigkeit begleitet von einer Reduktion der A-beta-Ablagerungen im Hirn der 5xFAD-Mäuse.

Ausgehend von einer AD-assoziierten veränderten Genexpression von Apolipoprotein A1 (ApoA1) im Darm haben wir Lipidstoffwechselstörungen in 5xFAD-Mäusen festgestellt, manifestiert in einer erhöhten Ausscheidung von Lipiden über Fäzes einhergehend mit einer gestiegenen FoxA2-Genexpression, einem Transkriptionsfaktor im ApoA1-Stoffwechsel.

Die Ergebnisse stützen die Hypothese, dass die AD nicht nur eine Erkrankung des ZNS ist, und eröffnen den Blick auf neue Interventionsmöglichkeiten und therapeutische Maßnahmen.

V. Englischsprachige Zusammenfassung (Abstract)

The pathological causes of Alzheimer's dementia (AD) remain incompletely understood despite years of research. So far, there are no curative treatment options, only symptom-relieving drugs. To further advance research into the disease, there is an urgent need to explore other avenues. New therapeutic targets offer alternative treatment strategies. The gut and its microbiome, which largely modulate the human immune system, offer a new approach for intervention. For Parkinson's dementia, a neurodegenerative disease comparable to AD, it has already been shown that the gut plays a major role in pathophysiology. However, the relationship of the gut to AD is not yet fully understood and requires further investigation.

Initial studies compared the gut microbiota of AD patients with that of healthy individuals and found significant differences. However, the extent to which these affect the course of the disease is currently unclear.

Therefore, the focus of this dissertation was on the effects of AD on the gut and the intestinal microbiota, or controversely, on the microbiome as an actor in AD pathogenesis. In doing so, we were able to demonstrate connections of AD with the microbiota-gut-brain axis by using 5xFAD (AD model mice) and wild-type mice. Among others, gut transit time changes with the progressive course of AD in 5xFAD mice. Amyloid-beta (A-beta) deposition was detected in the intestines of AD model mice and the resulting changes in microbial composition were subsequently analyzed. Upon direct exposure of A-beta to intestinal bacteria, the peptide has antimicrobial properties. Furthermore, the results suggest a resilience mechanism of the intestinal microbiota upon long-term exposure to the peptide.

Conversely, a modulated microbiome also influences AD: 14-week treatment of 5xFAD mice with antibiotics resulted in improved nest-building ability accompanied by a reduction in A-beta deposition in the brains of 5xFAD mice.

Based on AD-associated altered gene expression of apolipoprotein A1 (ApoA1) in the intestine, we found lipid metabolic disturbances in 5xFAD mice, manifested in increased fecal excretion of lipids accompanied by increased FoxA2 gene expression, a transcription factor in ApoA1 metabolism.

The results support the hypothesis that AD is not only a disease of the CNS and open the view to new intervention possibilities and therapeutic measures.

1 Einleitung

1.1 Gesundes Altern und neurodegenerative Erkrankungen

Der Prozess des Alterns beschreibt fortwährende Änderungen mit der Zeit, die fast alle lebenden Organismen betreffen. Im Laufe des Lebens gehen diese mit einem Verlust der physiologischen Integrität einher und somit steigt das Risiko für Krankheiten und es mündet im Tod durch Akkumulation dieser Änderungen (Harman, 1991; López-Otín et al., 2013). Die Veränderungen finden auf molekularer und zellulärer Ebene statt.

Im Laufe der Jahre haben sich sowohl das Gesundheitssystem als auch die Lebensumstände der Menschen, wie z.B. Hygiene, Ernährung und die Wohnsituation, so weiterentwickelt und verbessert, dass die Bevölkerung durchschnittlich eine höhere Lebenserwartung hat. Seit den 60er Jahren ist diese in Deutschland um mehr als zehn Jahre gestiegen und liegt mittlerweile für Neugeborene bei 78,6 (Jungen) bzw. 83,4 (Mädchen) Jahren (Statistisches Bundesamt, 2021). Sie ist damit weltweit gesehen auf Platz vier bezogen auf das höchste Durchschnittsalter. Zudem ist davon auszugehen, dass durch die stetigen technologischen und vor allem medizinischen Fortschritte die Lebenserwartung in den nächsten Jahren nochmals um bis zu acht Jahre steigen wird (Statistisches Bundesamt, 2019). Schätzungen nach zu urteilen, wird im Jahre 2050 jeder Dritte über 60 Jahre alt sein. Dies führt wiederum zu einer höheren Belastung des Gesundheitssystems und zu einem steigenden Bedarf an Betreuung der älteren Generationen, z.B. durch nahe Angehörige. Dies geht wiederum mit einem erhöhten psychosozialen Stress sowohl für den Patienten als auch die Angehörigen einher.

Es gibt multiple Forschungsansätze, um den Alterungsprozess mit dem Ziel einer gesunden Alterung zu untersuchen. Levine und Kollegen z.B. konnten zeigen, dass epigenetische Biomarker im Zusammenhang mit Alter und Gesundheit stehen (Levine et al., 2018). Dieser sogenannte „DNAm PhenoAge“-Biomarker korreliert mit dem Alter und dem Morbiditäts- bzw. Mortalitätsrisiko. Es konnte zudem ein Zusammenhang des Biomarkers mit Kennzeichen der Alzheimer Krankheit („Alzheimer’s Disease“, AD) gezeigt werden, wie z. B. Amyloid-Plaques und fibrillären Ablagerungen. Auf Basis solcher Studien kann das Krankheitsrisiko im Alter abgeschätzt und für Interventionsstudien genutzt werden. Andere Ansätze beschäftigen sich mit der Verlangsamung des Alterns. So konnte gezeigt werden, dass eine Inhibition von mTOR („mechanistic target of rapamycin“) - eine cytoplasmatische Kinase, die das Zellwachstum und den Metabolismus beeinflusst - zu einer verlängerten Lebensdauer von *Drosophila melanogaster* und Mäusen führt (Bitto et al., 2016; Bjedov et al., 2010; Hurez et al., 2015; Kapahi et al., 2004). Eine präventive und langfristige pharmakologische Behandlung oder sogar eine

genetische Intervention ist zum jetzigen Zeitpunkt noch keine alltäglich anwendbare Möglichkeit für den Menschen. Deshalb bieten Diäten oder eine restriktive Kalorienzufuhr eine realisierbare Alternative, die ebenfalls zu einer verlängerten Lebensspanne und verbesserter Gesundheit führen (Fontana et al., 2010; Hwangbo et al., 2020).

Je nach Konstitution und persönlicher Verfassung reagieren Menschen unterschiedlich auf Krankheiten wie z.B. aktuell COVID-19, oder psychischen Stress (Chan, 1977; Moradi et al., 2021). Ein Begriff, der sich in diesem Zusammenhang geprägt hat, ist „Resilienz“. Dieser beschreibt die Widerstandsfähigkeit von Individuen (Übersicht in (Wiig et al., 2020)(Afek et al., 2021)). Resilienz spielt in vielen aktuellen Forschungen, z.B. auch im Zusammenhang mit „Lockdowns“ während der Corona-Pandemie, eine große Rolle (Kimhi et al., 2020; Veer et al., 2021). Ziel ist es, Resilienzfaktoren zu finden und vor allem für psychologisch gesundes Altern zu nutzen.

Grundsätzlich ist mit fortschreitendem Alter eine Zunahme an Beschwerden zu beobachten, wie z.B. Seh- und Hörverlust, Herz-Kreislauf-Beschwerden oder Einschränkungen des Bewegungsapparates. Auch kognitive Beeinträchtigungen und eine höhere Wahrscheinlichkeit, an einer neurodegenerativen Krankheit zu entwickeln, stehen im direkten Zusammenhang mit dem Alter (Hou et al., 2019). 2020 lebten in Deutschland ca. 1,6 Millionen Menschen mit einer Demenzerkrankung, die meisten darunter litten unter der AD (E.V., 2020). Die Inzidenz liegt bei 300.000 Neuerkrankungen pro Jahr, gleichzeitig sinken die Sterbefälle der Erkrankten durch die zuvor genannten Veränderungen im Lebensumfeld und Gesundheitssektor. Demnach ist mit einem stetigen Wachstum der Zahl der Erkrankten zu rechnen (bis 2050 auf ca. 2,3-2,6 Millionen in Deutschland).

Neurodegenerative Erkrankungen zeichnen sich durch einen fortschreitenden Verlauf aus, der zum Verlust von Nervenzellen führt. Je nach Schwere des Verlaufs manifestieren sich klinische Symptome früher oder später und können nicht zuletzt zur Vollzeitpflege des Patienten führen. Umso wichtiger ist es, die Mechanismen und Ursachen der AD zu verstehen und potenzielle neue Behandlungsmethoden für diese verheerende Erkrankung zu finden.

1.1.1 Die Alzheimer-Demenz

Die AD wurde erstmals 1907 vom deutschen Neurologen Alois Alzheimer, nach dem die Erkrankung benannt wurde, beschrieben (Alzheimer, 1907). Seine 51-jährige Patientin Auguste Deter beschrieb er als desorientiert, vergesslich und verwirrt. *Post mortem* stellte er histopathologische Veränderungen wie Protein-basierte Ablagerungen in der Hirnrinde fest. Diese Ablagerungen wurden erst in den 1960er Jahren mittels Elektronenmikroskopie weiter

untersucht, um die Strukturen näher aufzuklären (Kidd, 1964; Terry, 1963). Sie sind geprägt von neurofibrillären Bündeln („neurofibrillary tangles“, NFT) aus Tau-Proteinen, sowie senilen Plaques, die hauptsächlich aus A-beta-Peptiden bestehen (Brion et al., 1985; Glenner & Wong, 1984; Grundke-Iqbal et al., 1986; Ihara et al., 1986; Kosik et al., 1986; Wood et al., 1986).

Mittlerweile unterscheidet man zwei Formen der AD: die familiäre AD (FAD) und die sporadische AD. Bei der FAD treten klinische Symptome deutlich früher auf („early onset“), durchschnittlich in einem Alter von 56 Jahren (Koedam et al., 2010). Grund für die FAD sind Mutationen in den Genen des Amyloid-Vorläuferproteins („Amyloid precursor protein“, APP), Presenelin-1 (PSEN1) und Presenelin-2 (PSEN2), die an der Bildung und Prozessierung des A-beta beteiligt sind (siehe Kapitel 1.1.2). Diese Form ist jedoch nur etwa einem Prozent der AD-Fälle zuzuordnen (Bekris et al., 2010). In den meisten Fällen tritt die sporadische, sich spät manifestierende AD im mittleren Alter von 74 Jahren auf („late onset“, (Koedam et al., 2010)). Zusätzlich wird zwischen leichter, mittelschwerer und schwerer AD unterschieden. Als Hauptrisikofaktor gilt das Alter (Brookmeyer et al., 2007). Im Jahr 2020 litten 3% der 65- bis 74-Jährigen, 17% der 75- bis 84-Jährigen und 32% der 85 Jahre alten und älteren Menschen an der AD (“2020 Alzheimer’s Disease Facts and Figures.,” 2020). Die Prävalenz der AD steigt demnach exponentiell mit dem Alter und verdoppelt sich etwa alle fünf Jahre ab einem Alter von 65 (Kukull et al., 2002). Aber auch das Apolipoprotein E (ApoE)-Allel ε4 wurde bereits 1993 mit einem höheren AD-Risiko assoziiert (Corder et al., 1993). ApoE wird im Gehirn hauptsächlich von Astrozyten produziert, ist die Hauptkomponente von Lipoprotein hoher Dichte („high density lipoprotein“, HDL) und sorgt damit für den Transport von Cholesterin und Phospholipiden ((Y. Chen et al., 2021; Kockx et al., 2018)). Abhängig von der ApoE-Isoform wird die A-beta-Akkumulation und -Eliminierung unterschiedlich beeinflusst (Castellano et al., 2011). Demnach führt das ApoE-ε4 Allel zu einer erhöhten Dichte von A-beta-Ablagerungen (Castellano et al., 2011; Christensen et al., 2010; Hashimoto et al., 2012; Koffie et al., 2012; Youmans et al., 2012) und steigert das Risiko, an der AD zu erkranken um den Faktor 2,8 pro Allel. Der Beginn der Erkrankung verschiebt sich pro ε4-Allel um etwa fünf Jahre nach vorne (Corder et al., 1993; Pastor et al., 2003). Dem ApoE-ε2-Allel werden dagegen protektive Eigenschaften zugeschrieben (Chartier-Harlin et al., 1994).

Erste Symptome der AD sind Gedächtnisverlust oder z. B. Probleme mit der Sprache, die hervorgerufen werden durch eine Schädigung der dafür verantwortlichen Neurone (“2020 Alzheimer’s Disease Facts and Figures.,” 2020). Zur Diagnose werden verschiedene Tests wie das „Mini-Mental State Exam“ (MMSE, (Folstein et al., 1975)) und der „Clock drawing Test“ (CDT, (Brodaty & Moore, 1997)) mit dem Patienten durchgeführt. Die Veränderungen im Gehirn der AD-Patienten beginnen vor der klinischen Manifestation der Erkrankung, weshalb die Diagnose erst gestellt werden kann, wenn bereits irreversible Schäden vorhanden sind (Sperling et al.,

2011; S. J. Vos et al., 2013). Im progressiven Verlauf der Krankheit werden die Patienten meist Vollzeit pflegebedürftig, alltägliche Körperfunktionen wie Gehen und Schlucken werden beeinträchtigt ("2020 Alzheimer's Disease Facts and Figures.," 2020). Zuletzt steigt die Anfälligkeit für z. B. eine Sepsis oder Pneumonie. Eine AD endet schließlich immer mit dem Tod des Patienten. *Post mortem* kann an Gehirnen von AD-Patienten eine ausgeprägte Atrophie festgestellt werden, die durch die neurodegenerativen Prozesse verursacht wurde (Adduru et al., 2020; ten Kate et al., 2018).

1.1.2 Molekulare Grundlagen der Alzheimer Demenz

Die genauen Ursachen der AD sind bis heute nicht vollständig aufgeklärt. Wie bereits Alois Alzheimer 1907 beschrieben hat, gibt es jedoch zwei charakteristische, pathologische Merkmale der AD: die NFT und die senilen Plaques (Alzheimer, 1907).

Bei den NFT handelt es sich um Aggregate aus hyperphosphorylierten Tau-Proteinen (Grundke-Iqbal et al., 1986). Physiologisch gibt es sechs Tau-Isoformen, die durch alternatives Spleißen entstehen (Andorfer et al., 2003; M. Goedert et al., 1989; Miguel et al., 2019). Diese werden fast ausschließlich in Neuronen und in geringem Maß in Gliazellen exprimiert und sind dort am Aufbau, der Funktion und Aggregation der Mikrotubuli beteiligt (G. Lee et al., 1989; Mandelkow et al., 1996; Weingarten et al., 1975). Zudem interagiert Tau mit der DNA und kann zu deren Stabilität beitragen (Camero et al., 2014). Im Prozess der post-translationalen Modifikation wird Tau phosphoryliert, wodurch die Mikrotubuli-Assemblierung und somit der axonale Transport gestört wird. Im Falle der AD liegt Tau in einem pathologisch hyperphosphorylierten Zustand vor, welcher zur Aggregation des Proteins und damit zur Bildung der NFT innerhalb der Nervenzellen führt (Grundke-Iqbal et al., 1986). Tau-Kinasen, wie z. B. GSK3β („glycogen synthase kinase 3-beta"), MAPK („mitogen activated protein kinase") und CDK5 („cyclin dependent protein kinase 5"), konnten in NFT nachgewiesen werden, was auf einen direkten Zusammenhang zwischen Phosphorylierung und Krankheitsverlauf schließen lässt (Flaherty et al., 2000; Yamaguchi et al., 1996). Zusätzlich ist die Konzentration an Phosphatasen, wie der Proteinphosphatase 2A (PP2A), im Gehirn von AD-Patienten reduziert, wodurch gleichzeitig der hyperphosphorylierte Zustand gefördert wird (F. Liu et al., 2005). Studien zufolge können sich NFT, vergleichbar zu Prion-Proteinen, trans-synaptisch in verbundene Neurone ausbreiten, zum Rückgang dendritischer Ausläufer führen und damit zum progressiven Verlauf der Erkrankung beitragen (Dejanovic et al., 2018; DeVos et al., 2018; Hoover et al., 2010; Jucker & Walker, 2013; Messing et al., 2013). Dafür spricht auch, dass die Anzahl der NFT im Gehirn und die Konzentration an Tau-Proteinen in der Cerebrospinalflüssigkeit (CSF) mit dem kognitiven

Rückgang der AD-Patienten in Zusammenhang gebracht werden kann (Ingelsson et al., 2004; Wallin et al., 2010).

Die senilen Plaques bestehen aus akkumulierten A-beta-Peptiden und sind Grundlage der Amyloid-Kaskaden-Hypothese, welche früh die zentrale Rolle vieler Forschungsansätze bildete (J. Hardy, 2006; J. A. Hardy & Higgins, 1992). Kern der Hypothese ist eine durch A-beta ausgelöste Kaskade an zellulären Prozessen, welche zur unvermeidbaren Pathogenese der AD führen. In einer Studie von Holmes und Kollegen konnte festgestellt werden, dass auch durch eine Reduktion der A-beta-Last das progressive Fortschreiten der AD, sprich Symptome wie z. B. der Rückgangz der kognitiven Fähigkeiten der Patienten, nicht aufgehalten werden kann (Holmes et al., 2008). Demnach ist eine frühzeitige Intervention von großer Bedeutung. A-beta entsteht durch proteolytische Spaltung des APP (Haass & Selkoe, 1993; Sisodia, 1992; X. Sun et al., 2005). APP ist ein Typ-I-Transmembranprotein und besitzt eine kleine intrazelluläre und eine größere extrazelluläre Domäne. Es kommt in acht verschiedenen Isoformen vor und kann durch α- und β-Sekretasen prozessiert werden (Hampel et al., 2021; Sandbrink et al., 1996; Sisodia, 1992). Je nachdem, welche Sekretase aktiv ist, unterscheidet man zwischen dem amyloidogenen und dem nicht-amyloidogenen Weg (Abbildung 1).

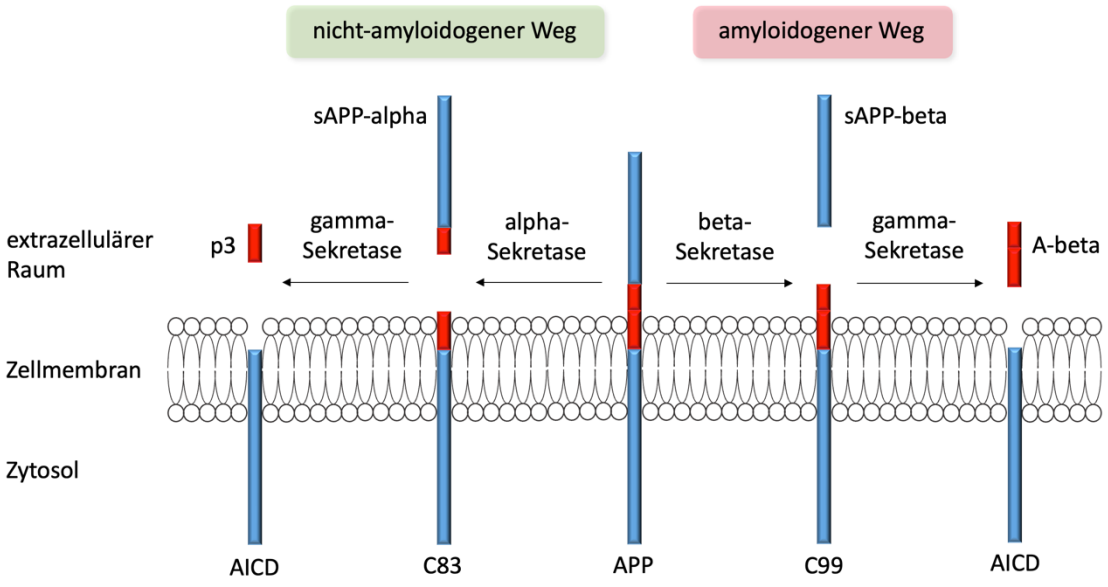

Abbildung 1: Proteolytische Spaltung des Amyloid-Vorläuferproteins (APP)

APP kann auf zwei verschiedenen Wegen prozessiert werden: dem amyloidogenen und dem nicht-amyloidogenen Weg. Im Falle des nicht-amyloidogenen Weges wird APP zunächst von der alpha-Sekretase gespalten, wodurch sAPP-alpha im extrazellulären Raum frei wird. Anschließend wird p3 vom verbleibenden C-terminalen, Membran-gebundenen Fragment C83 mittels gamma-Sekretase abgespalten, wodurch das Fragment AICD entsteht. Beim amyloidogenen Weg wird APP zunächst von der beta-Sekretase und anschließend von der gamma-Sekretase prozessiert. Es entstehen die extrazellulären Fragmente sAPP-beta und A-beta, sowie die membrangebundenen Fragmente C99 und AICD.

Beim nicht-amyloidogenen Weg wird APP zunächst durch eine alpha-Sekretase geschnitten, wobei das extrazelluläre Produkt sAPP-alpha und ein C-terminaler, Membran-gebundener Rest mit 83 Aminosäuren (C83) entsteht (Sisodia, 1992). ADAM10 („a disintegrin and metalloproteinase domain-containing protein 10“) ist dabei die wichtigste, physiologisch vorkommende APP prozessierende alpha-Sekretase (Endres & Fahrenholz, 2012; Lundgren et al., 2020; Postina et al., 2004). Anschließend wird C83 durch einen gamma-Sekretasekomplex geschnitten, wodurch das Fragment p3 in den extrazellulären Raum freigesetzt wird. Die intrazelluläre Domäne des APP bleibt in der Membran gebunden („APP intracellular domain“, AICD). Der gamma-Sekretasekomplex besteht aus Homodimeren aus PSEN-1- und PSEN-2, Nicastrin, dem Stabilisationsfaktor APH-1 („anterior pharynx-defective 1“) und dem Presenelin-Enhancer 2 (PEN-2) (Kimberly et al., 2003; Lu et al., 2014; R. Zhou et al., 2019). Er wird im endoplasmatischen Retikulum (ER) durch Proteolyse gebildet und anschließend in das späte ER, aber auch in Mitochondrien und den Golgi-Apparat transportiert.

Im Falle des amyloidogenen Weges wird APP zunächst durch eine beta-Sekretase (BACE1, „beta-site APP cleavage enzyme 1“) gespalten, wodurch sAPP-beta und das C-terminale, 99 Aminosäuren lange Fragment C99 entsteht (Hampel et al., 2021; Vassar et al., 1999). Die Prozessierung des C99 durch die gamma-Sekretase resultiert anschließend in der Sekretion des 4 kDa großen A-beta in den Extrazellularraum. Dabei variiert die Länge des A-beta zwischen 37-42 Aminosäuren.

Bei vorliegender AD verschiebt sich das Gleichgewicht in Richtung des amyloidogenen Weges, sodass vermehrt A-beta produziert wird (Endres & Fahrenholz, 2012). A-beta40 und vor allem A-beta42 haben eine starke Tendenz zu aggregieren (Stine et al., 2003). Dabei entstehen Oligomere, welche sich zu Fibrillen zusammenlagern und anschließend die für die AD charakteristischen Plaques im Gehirn bilden. Diese induzieren unter anderem die Hyperphosphorylierung von Tau, wodurch neurotoxische Prozesse der AD initiiert werden (de Felice et al., 2008; Jin et al., 2011). Interessanterweise wird A-beta jedoch nicht nur im Gehirn gebildet, sondern konnte bereits peripher, z. B. in der Leber und im Darm, nachgewiesen werden (Brandscheid et al., 2017; Buniatian et al., 2020; Selkoe et al., 1988). Über die Blut-Hirn-Schranke („blood-brain-barrier“, BBB) oder transcytotische Prozesse kann dieses wiederum in das Hirnparenchym gelangen (Pflanzner et al., 2011; Tamaoka et al., 1992). A-beta aktiviert dort unter anderem Mikroglia und dadurch inflammatorische Prozesse (Frautschy et al., 1998). Mikroglia steuern Immunreaktionen und Prozesse wie Phagozytose und Pinozytose im zentralen Nervensystem (ZNS) und tragen damit zur neuronalen Plastizität bei (P. T. Nguyen et al., 2020). Eine Aktivierung dieser Zellen führt zum Abbau von A-beta, was vorrangig zu Beginn der Erkrankung eine wichtige protektive Eigenschaft darstellt. Eine chronische Aktivierung der

Mikroglia induziert jedoch die Sekretion von z. B. reaktiven Sauerstoffspezies („reactive oxygen species“, ROS) und Cytokinen wie Interleukin 6 (IL-6) und dem Tumornekrosefaktor-alpha (TNF-α) (Ding et al., 2017; Matsumoto et al., 2018; Ren et al., 2020). Dies führt wiederum zu oxidativem Stress und Neurotoxizität.

1.1.3 Therapie der Alzheimer Demenz

Bis zum jetzigen Zeitpunkt gibt es keine Therapie der AD, die den Verlauf verlangsamen oder gar stoppen kann. Die zur Therapie zugelassenen Arzneistoffe helfen symptomatisch, indem sie die Gedächtnisleistung verbessern. Dazu gehören die Acetylcholinesterase-Inhibitoren (AChEI) Rivastigmin, Galantamin und Donepezil und der NMDA-Rezeptor-Antagonist (NMDA: *N*-Methyl-*D*-Aspartat-Rezeptor) Memantin (Übersicht in (Vaz & Silvestre, 2020)). Neben den bereits erwähnten pathologischen Merkmalen, den NFT und senilen Plaques, ist mit fortschreitender Erkrankung auch die cholinerge Reizweiterleitung beeinträchtigt (Tohgi et al., 1994). Der *Nucleus basalis* von Meynert ist eines der wichtigsten Acetylcholin (ACh)-produzierenden Kerngebiete im Telencephalon, welcher durch die neurodegenerativen Prozesse der AD stark beeinträchtigt wird (Arendt et al., 1983; Hanna Al-Shaikh et al., 2020; Whitehouse et al., 1981). Eine reduzierte ACh-Ausschüttung, hervorgerufen durch die AD, konnte auch im Neocortex, dem Hippocampus und der Amygdala festgestellt werden, die vorrangig für Lernen, Gedächtnis und Verhalten verantwortlich sind (Shinotoh et al., 2000). Donepezil wurde 1996 als erstes Medikament zur Behandlung der leichten bis mittelschweren AD und 2010 für die mittelschwere bis schwere AD zugelassen, Rivastigmin und Galantamin im Jahr 2000 für die Behandlung von leichter bis mittelschwerer AD (Übersicht in (Sharma, 2019)). Die Wirkung der AChEI ist jedoch gering bis mäßig und von kurzer Dauer, da der Fortschritt der Neurodegeneration nicht aufgehalten wird (Fink et al., 2020; Knight et al., 2018; Y. Sun et al., 2008). Zudem führt die Einnahme zu unerwünschten Nebenwirkungen, wie z.B. Beschwerden im Bereich des Gastrointestinaltrakts (GIT) (Marucci et al., 2021). Memantin ist ein nicht-kompetitiver NMDA-Rezeptor-Antagonist. NMDA-Rezeptoren werden durch Glycin, Glutamat oder NMDA aktiviert und spielen eine zentrale Rolle bei der Langzeitpotenzierung. Memantin verhindert eine durch A-beta ausgelöste übermäßige Stimulation durch Glutamat und somit eine pathologisch hohe Calcium-Konzentration in neuronalen Zellen, die zu apoptotischen Prozessen führt (Dreyer et al., 1995; Harkany et al., 2000). Es wurde 2003 von der FDA zur Behandlung der mittelschweren bis schweren AD zugelassen, allerdings konnte in neueren Studien lediglich ein geringer Nutzen, bzw. bei der Behandlung von leichter AD kein Unterschied zur Placebo-Kontrollgruppe festgestellt werden (Knapp et al., 2017; McShane et al., 2019). Nebenwirkungen von Memantin

sind unter anderem Kopfschmerzen, Müdigkeit, motorische Unruhe und GIT-Beschwerden wie Obstipation (Alva & Cummings, 2008).

Für andere Symptome, vor allem psychologischen Ursprungs, wie Aggressionen, Unruhe und Halluzinationen, können durch ärztliche Empfehlung Antipsychotika verschrieben werden. Diese erhöhen jedoch das Schlaganfall-Risiko und können bis zum Tod des AD-Patienten führen, weshalb der Nutzen und das Risiko stets im Verhältnis gesehen werden muss (Maust et al., 2015; Ralph & Espinet, 2018). Darüber hinaus können verschiedene nicht-pharmakologische Therapien, wie z.B. Gedächtnistraining mit Hilfe von Computern oder Musiktherapie, unterstützend bei AD-Patienten eingesetzt werden. Studien ergaben zudem, dass Bewegung und aerobes Training die kognitive Leistung verbessern können (J. K. Morris et al., 2017; Palleschi et al., 1996).

1.2 Das Gastrointestinale System

Der GIT, oder auch Magen-Darm-Trakt genannt, ist die größte Schnittstelle des Körpers zur Außenwelt und bezeichnet den Verdauungsapparat vom Ösophagus bis zum Anus (Abbildung 2). Er besitzt beim Menschen durchschnittlich eine Länge von 796 cm mit einer Oberfläche von 32 m^2 (Helander & Fändriks, 2014; Hounnou et al., 2002). Zum GIT gehören der Ösophagus, der Magen, der Dünndarm, welcher eingeteilt wird in Duodenum, Jejunum und Ileum, sowie der Dickdarm mit Caecum, Kolon, Sigmoid und Rektum und zuletzt der Anus (Greenwood-Van Meerveld et al., 2017). Zudem wird unterschieden zwischen dem oberen GIT, von Ösophagus bis Duodenum, und dem unteren GIT, von Jejunum bis Rektum. Die aufgenommene, mechanisch zerkleinerte Nahrung wird im GIT verarbeitet und dabei mit Hilfe der Darmperistaltik zum jeweils nächsten zuständigen Abschnitt weiter transportiert (Huizinga & Lammers, 2009). Nachdem der Nahrungsbrei den Ösophagus passiert hat, wird dieser im Magen mittels Wasserzugabe und Magensäure zum Chymus verarbeitet (Boland, 2016; Goodman, 2010). Dieser wird anschließend im Dünndarm chemisch zerteilt, z. B. durch Gallensäuren und Bikarbonat, aber auch durch Enzyme wie Peptidasen oder Lipasen. Diese werden von anhängenden Drüsen wie dem Pankreas sezerniert. Makronährstoffe wie Proteine, Fette und Zucker werden somit in ihre Spaltprodukte (Aminosäuren, Fettsäuren und Monosaccharide) zerteilt und können anschließend resorbiert und dem Körper zur Verfügung gestellt werden (Goodman, 2010). Die restlichen, unverdaulichen Bestandteile werden im Kolon durch Mikroben prozessiert. Zuletzt wird der nicht verwertbare Rest nach Eindickung durch Wasserresorption im Kolon und Sigmoid gelagert und über das Rektum und den Anus als Fäzes ausgeschieden (Bhatnagar et al., 2004).

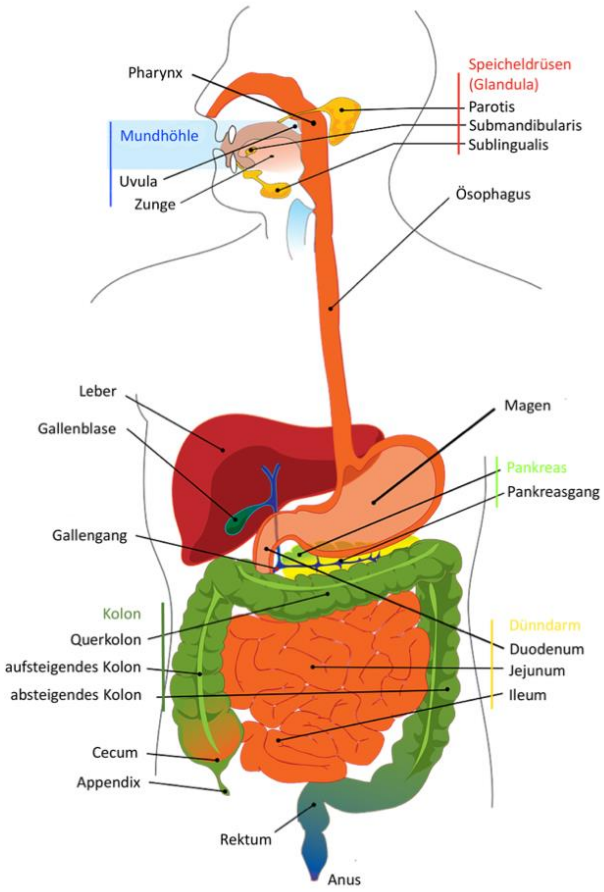

Abbildung 2: Grafische Darstellung des Gastrointestinaltrakts

Der GIT erstreckt sich vom Ösophagus bis zum Anus. Aufgenommene Nahrung gelangt über die Mundhöhle in den oberen GIT mit Ösophagus, Magen und Duodenum und anschließend zum unteren GIT, der aus Jejunum, Ileum, Kolon, Rektum und Anus besteht. Das Kolon wird zudem aufgeteilt in Querkolon, aufsteigendes Kolon und absteigendes Kolon. (Modifizierte Abbildung von Pixabay)

Neben der Verwertung der Nährstoffe kommt dem GIT eine protektive Funktion zu. Als einer der wichtigsten Akteure des Immunsystems spielt er bis heute eine zentrale Rolle bei vielen Forschungsansätzen (Matteoli & Boeckxstaens, 2013; Schuijt et al., 2016). Schwerpunkte sind chronisch-entzündliche Darmerkrankungen (CED) wie Morbus Crohn oder Colitis ulcerosa, aber auch Bluthochdruck oder Diabetes mellitus (Meng et al., 2020; Yano & Niiranen, 2021; Y. Zhang et al., 2020). Aber auch bei Erkrankungen wie der AD als Krankheit des ZNS hat sich heute der Blickwinkel geändert: Einige neue Studien setzen den Fokus auf eine ganzheitliche, den Darm einschließende, Untersuchung. Komorbiditäten der AD, wie z. B. Diabetes mellitus und eine Gewichtsabnahme der Patienten unterstützen diesen Standpunkt (G. Ho et al., 2020; Santos et al., 2018).

1.2.1 Morphologie des Gastrointestinaltrakts

Im Querschnitt ist der Darm grob in vier Schichten aufgeteilt (siehe Abbildung 3, (Schünke et al., 2012)). Die innere Lage bildet die Mukosa, auch Schleimhaut genannt, welche aus Epithel, *Lamina propria* und *Muscularis mucosae* besteht. Das Epithel ist maßgeblich an der Resorption von Nahrungsbestandteilen beteiligt, primär durch zahlreiche Membranproteine („tight junctions“, (Pearce et al., 2018)). Zudem bildet es eine protektive Barriere. Direkt verbunden mit dem Epithel ist die *Lamina propria* (Basalmembran), ein Bindegewebe, das über Blut- und Lymphgefäße den Transfer von Substanzen aus dem Darm in den Blutkreislauf ermöglicht (Pelaseyed et al., 2014; Toyoda et al., 1997). Zusätzlich bildet die *Lamina propria* über Lymphknoten mit Immunzellen eine Barriere für Pathogene, die über den GIT aufgenommen werden. Die äußere Schicht der Mukosa bildet die *Muscularis mucosae*, eine Muskelschicht, welche die Bewegung der Mikrovilli ermöglicht. Mikrovilli sind fadenförmige Ausstülpungen der Epithelzellen, die eine Vergrößerung der Zelloberfläche bewirken und dadurch die Aufnahme von Substanzen und Nährstoffen verbessern (McConnell et al., 2009; Oda et al., 1969). Die Mikrovilli liegen auf der apikalen Seite der Epithelzellen und sind ca. 1-2 μm lang. Sie bestehen aus Aktinfilamenten, die über Fimbrin und Villin stabilisiert werden und innerhalb der Epithelschicht das Terminalgeflecht bilden. Dieses wird wiederum durch Tropomyosin und Myosin verstärkt. An den Mikrovilli befindet sich die Glykokalix aus Glykoproteinen und -lipiden, welche den Kontakt zu anderen Zellen und somit auch den Transport von z. B. Proteinen ermöglichen (Y. Zhao et al., 2001). Angrenzend an die Mukosa befindet sich die Submukosa mit dem Meissner Plexus (*Plexus submucosus*) (Brehmer et al., 2010; Wedel et al., 1999). Dieser bildet über Ganglien ein großes Nervenfasergeflecht und damit einen Teil des enterischen Nervensystems (ENS) und steht im direkten Kontakt zur Muskularis. Die Muskularis bildet über glatte Muskelzellen die innere Schicht der ringförmigen Muskulatur mit dem Auerbach’schen Plexus (*Plexus myentericus*) und die äußere Schicht der Längsmuskulatur (Brehmer et al., 2010). Durch Kontraktionen der zwei Muskelschichten wird die Peristaltik und Motilität des Darms gesteuert (Sanders et al., 2016). Die äußerste Schicht bildet die Serosa, eine zusätzliche Barriere für entzündliche Prozesse, gebildet aus Plattenepithelzellen, dem Mesothel.

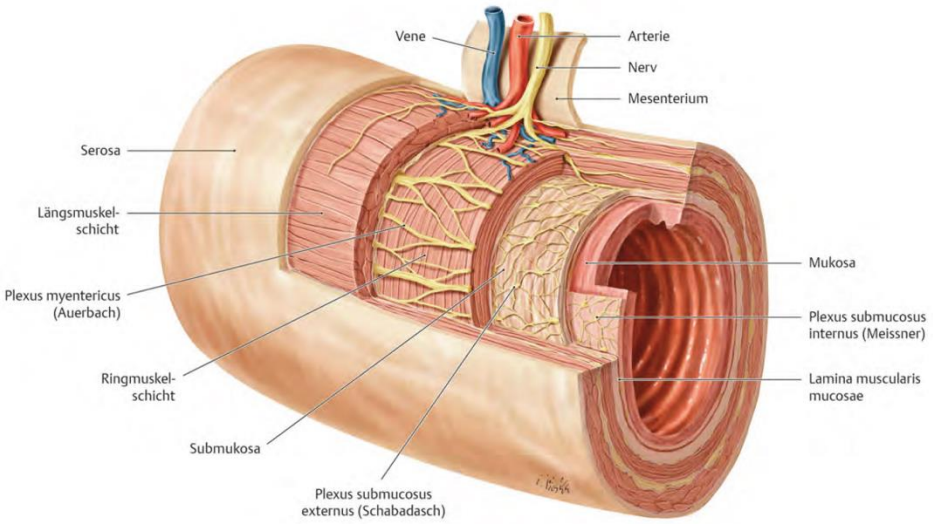

Abbildung 3: Querschnitt des menschlichen Gastrointestinaltrakts

Der GIT besitzt vom Ösophagus bis zum Anus einen grundlegend ähnlichen Aufbau. Das Lumen wird begrenzt von der Schleimhaut (Mukosa), bestehend aus Epithelzellen, der *Lamina propria* und einer abschließenden Muskelschicht der *Lamina muscularis mucosae*. Daran anschließend folgt die Submukosa mit einem ausgedehnten Netz von Neuronen, den *Plexus submucosus internus* und *externus* (Meißner- und Schabadasch-Plexus). Diese verbinden die Schleimhaut mit der Muskularis, bestehend aus Ringmuskel- und Längsmuskelschicht. Über Kontraktionen der Muskelschichten wird die Persistaltik und Motilität der Magen-Darm-Wand gesteuert. Zwischen den beiden Muskelschichten befindet sich der *Plexus myentericus* (Auerbach-Plexus). Die äußerste Barriere, die Serose, wird aus Plattenepithelzellen und Mesothel gebildet. (Abbildung übernommen aus (Schünke et al., 2012) Illustration von M. Voll und K. Wesker)

Der GIT wird innerviert von seinem eigenen Nervensystem, dem ENS. Es bildet den größten Teil des peripheren Nervensystems (PNS) und besitzt ca. 400 bis 600 Millionen Neurone (Meißner-Plexus in der Submukosa und Auerbach'scher Plexus in der Muskularis) (Furness, 2012). Die Neurone des ENS können aufgeteilt werden in intrinsisch afferente Neurone, Interneurone und Motorneurone, welche über Neurotransmitter wie Acetylcholin oder Serotonin gesteuert werden (Furness et al., 2004). Sie bilden netzartige Strukturen, die enterischen Ganglien, und Bündel aus Nervenfasern. Das ENS koordiniert unabhängig vom ZNS die Motilität des Darms durch Steuerung der glatten Muskelzellen, die endokrine und exokrine Sekretion im GIT, sowie die Bewegung der Mukosa, die Darmdurchblutung und das gastrointestinale Immunsystem (McKeown et al., 2013; Rao & Gershon, 2016; Smith et al., 2013). Die Nervenzellen, die das ENS aufbauen, stammen von Vorläuferzellen der Neuralleiste ab. Während der Embryogenese durchlaufen multipotente vagale Vorläuferzellen zunächst den Darm rostrokaudal und differenzieren sich nach Verlassen des Zellzyklus in verschiedene Neurone des ENS (Burns et al., 2004; Kapur et al., 2004). Anschließend können Neuralleistenzellen kranial in den Darm eindringen und kaudal den GIT durchwandern (le Douarin et al., 1994). Diese differenzieren sich danach zu Gliazellen und etwa 16 verschiedenen Neuronenpopulationen, die sich durch Morphologie, Neurotransmitter, Rezeptoren und Ionenkanäle unterscheiden (Furness, 2012; McKeown et al., 2013; Saffrey, 2013).

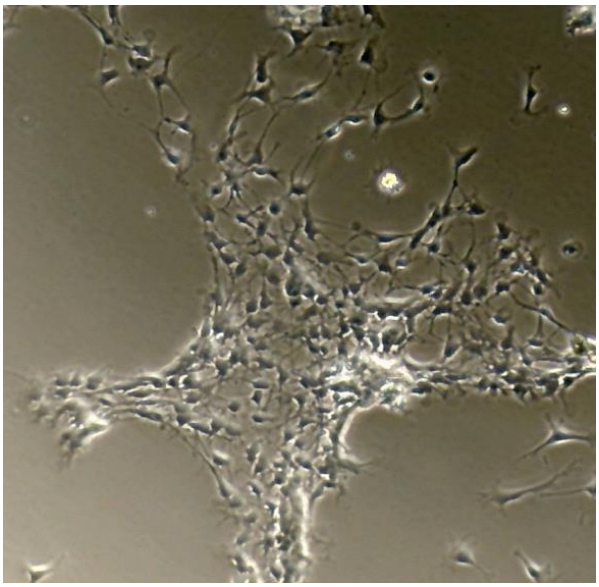

Abbildung 4: Mikroskopische Aufnahme enterischer Neurone aus dem Darmgewebe einer Maus

Enterische Neurone wurden aus dem Darmgewebe von Mäusen isoliert und drei Tage lang kultiviert. Anschließend sind die charakteristischen netzartigen Strukturen des Plexus erkennbar.

Der Aufbau des ENS macht es zum komplexesten Nervensystem im menschlichen Körper, obgleich die Neurotransmitter, Zelltypen und Rezeptoren des ENS mit denen des ZNS vergleichbar sind (Kuwahara et al., 2020). Da es unabhängig von äußeren Signalen arbeiten kann, wird es auch als „zweites Gehirn“ bezeichnet (Schneider et al., 2019). Gleichwohl steht es in bidirektionalem Kontakt mit dem Hirn über die sogenannte „Darm-Hirn-Achse“. Diese umfasst das autonome Nervensystem (ANS), den *Nervus vagus*, Immunzellen und das Mikrobiom (Mayer & Tillisch, 2011). Das autonome Nervensystem (ANS) besteht aus dem parasympathischen und dem sympathischen Nervensystem sowie dem ENS. Parasympathikus und Sympathikus besitzen eine antagonistische Wirkung, wobei der Parasympathikus für die Regeneration und den Aufbau von Energiereserven zuständig ist und eine Aktivierung des Sympathikus den Abbau von Energiereserven mit dem Ziel einer erhöhten Leistungsbereitschaft zur Folge hat. Über den *Nervus vagus* wird eine Kommunikation über afferente und efferente Fasern möglich (Quan & Banks, 2007). Informationen des Parasympathikus zum GIT werden über den *Nervus vagus* mittels Efferenzen übertragen. Jejunum und Ileum werden sympathisch über den *Nervus splanchnicus minor* innerviert, das Kolon über *Nervus splanchnicus major* und *minor* (Schünke et al., 2012). Eine Aktivierung des Sympathikus hemmt z.B. die Acetylcholinausschüttung und damit die Magen-Darm-Aktivität. Über viszerale Afferenzen gelangen Informationen vom ENS zum ZNS. Diese Informationsweiterleitung kann z. B. auch die Stimmung und Emotionen beeinflussen, das sogenannte „Bauchgefühl“ (Liang et al., 2018). Insgesamt verläuft die Signalweiterleitung über den *Nervus vagus* zu 80% über afferente Signale zum Gehirn, was zeigt,

welche essenzielle Rolle der GIT spielt. Hierüber wird z. B. ebenfalls die Hypothalamus-Hypophysen-Nebennieren-Achse („hypothalamic-pituitary-adrenal“, HPA) moduliert. Stresssignale werden über die HPA-Achse verarbeitet mit dem Resultat der Ausschüttung von Glucocorticoiden, hauptsächlich Cortisol (Palma-Gudiel et al., 2021). Dieses wirkt u. a. immunsuppressiv und erhöht z. B. die Glukoseaufnahme über den Darm (Matsumura et al., 2017).

Auch das Immunsystem des GIT steht im direkten Zusammenhang mit dem ZNS. Die wichtigsten Immunzellen des GIT sind unter anderem die T-Zellen, mononukleäre Phagozyten und angeborene lymphoide Zellen (Goto et al., 2014; Neurath, 2014; Zimmerman et al., 2008). Patienten mit einer CED leiden oftmals an Angststörungen und Depressionen (Byrne et al., 2017; Navabi et al., 2018). In Mäusen konnte außerdem gezeigt werden, dass Stressreize die Anfälligkeit für Darmentzündungen, die Darmmotilität und -permeabilität, die viszerale Empfindlichkeit und die Sekretionsfunktion modulieren (Larauche et al., 2010; Lennon et al., 2013; O’Malley et al., 2013; Reber et al., 2006). Zudem führte ein übermäßiger Salzkonsum bei Mäusen zu einer Expansion von T-Helferzellen vom Typ 17 (TH17) im Dünndarm, woraufhin ein Anstieg an IL-17 im Plasma erfolgte (Faraco et al., 2018). Daraus resultierte eine reduzierte Stickstoffmonoxid-Produktion in Endothelzellen des Gehirns.

1.2.2 Das gastrointestinale Mikrobiom

Als humanes Mikrobiom bezeichnet man die Gesamtheit aller Mikroorganismen und ihrer Gene im und auf dem menschlichen Körper. Es umfasst ca. drei Millionen Gene und damit 150-mal mehr als humane Gene (W. M. de Vos & de Vos, 2012; Lozupone et al., 2012). Der Darm wird von Mikroorganismen wie Pilzen, Viren, Protozoen und Archaeen aber vor allem Bakterien besiedelt, wie z. B. in einem großen Projekt des „Institutes of Health“ der Vereinigten Staaten mit dem Namen „Human Microbiome Project“ festgestellt wurde (Gevers et al., 2012; Kasai et al., 2016). Demnach machen Bakterien ca. 99% der mikrobiellen Zusammensetzung aus. Die meisten Bakterien gehören den Phyla der Gram-negativen Bacteroidetes und der Gram-positiven Firmicutes, gefolgt von Actinobacteria, Proteobacteria, Fusobacteria, Tenericutes und Verrucomicrobia an (Gomaa, 2020; Sender et al., 2016). Insgesamt wird der Darm von 10^{13} bis 10^{14} mikrobiellen Zellen bewohnt, die insgesamt bis zu 2 kg Gewicht einnehmen können (Mischke & Plösch, 2016; Sender et al., 2016). Analog zum genetischen Fingerabdruck besitzt jeder Mensch ein charakteristisches Mikrobiom, das unter dem ständigen Einfluss von z. B. dem menschlichen Lebensraum und der Umwelt, Ernährung und Diäten oder pharmakologischen Behandlungen steht (Ahmad et al., 2020; Gupta et al., 2020; Rocca et al., 2019). Das eigene

Mikrobiom wird zu Beginn des Lebens von der Mutter geprägt. Im Mutterleib wächst der Fötus in einer bakterienarmen Umgebung auf (Neu, 2016). Mit der Geburt beginnt die initiale Besiedelung mit Bakterien. Dabei spielt bereits die Art der Entbindung eine Rolle. Das Mikrobiom des Fötus wird bei einer vaginalen Geburt durch die mütterliche perineale und vaginale Mikroflora geprägt, wohingegen ein Kaiserschnitt bereits zu einer Dysbiose des Mikrobioms führen kann (Dominguez-Bello et al., 2010; J. J. Goedert et al., 2014; Montoya-Williams et al., 2018). Langfristig führt dies zu einer bakteriellen Zusammensetzung, die sich von Personen mit vaginaler Geburt unterscheidet. Wird das Neugeborene gestillt, so resultiert dies in einem komplexeren Milieu an Bifidobakterien, was wiederum zu einer besseren neurologischen Entwicklung führt im Vergleich zu einer Ernährung mit Ersatznahrung (Roger et al., 2010). Auch das Geschlecht und z. B. geschlechtsspezifische Hormone wie Östrogen und Testosteron wirken modulierend auf die bakterielle Zusammensetzung (Valeri & Endres, 2021). Dies sind nur wenige Beispiele dafür, wie Faktoren das Mikrobiom prägen bzw. modulieren können.

Die intestinale Mikrobiota hat verschiedene Aufgaben, wie z. B. die Verdauung von Nahrungsbestandteilen, die durch humane Enzyme nicht verwertet werden können. Die Bakterien im Kolon spalten Ballaststoffe wie Lignin, resistente Stärke und Oligosaccharide (z. B. Fructo-Oligosaccharide und Galacto-Oligosaccaride) mittels mikrobieller Enzyme in Energielieferanten, wie kurzkettige Fettsäuren („short chain fatty acids“, SCFA), gespalten (Vital et al., 2018; R. Zhang et al., 2021). Zu den SCFA gehören unter anderem Essigsäure, Propionsäure und Buttersäure, die vor allem von Firmicutes und Bacteroidetes produziert werden (Louis & Flint, 2017). Neben der Funktion als Energielieferant dient Essigsäure als Ausgangsstoff für die Lipogenese und Cholesterinbiosynthese in der Leber. Buttersäure fördert z. B. die Produktion von Leptin aus Adipozyten und wirkt, wie auch Propionsäure, protektiv, indem es die Darmbarriere stabilisiert (L. Liu et al., 2021; Mueller et al., 2020; S. Zhao et al., 2020; D. Zhou et al., 2017). Propionsäure senkt zudem den Cholesterinspiegel und verbessert die Insulinsensitivität (Chambers et al., 2019; Haghikia et al., 2021). Eine hohe Konzentration an SCFA schützt außerdem vor Allergien, ausgelöst durch Lebensmittel oder durch Allergene, die über die Atemluft aufgenommen werden (Roduit et al., 2019; Tan et al., 2016). Bei Säuglingen konnte die Gabe von präbiotischen Oligosacchariden z. B. das Risiko einer infantilen atopischen Dermatitis signifikant reduzieren (Moro et al., 2006). Zudem produzieren Mikrobiota essenzielle Vitamine wie Riboflavin, Biotin sowie Vitamin B und K (LeBlanc et al., 2013).

Einige Bakterien bilden protektive Bakteriozine, welche das Wachstum von pathogenen Bakterien hemmen (Jia et al., 2018; Lakshminarayanan et al., 2013). Bakteriozine aktivieren über die Darm-Hirn-Achse das Immunsystem des Wirts im ZNS. Die Mikrobiota setzen außerdem Neurotransmitter, wie Serotonin oder auch gamma-Aminobuttersäure („gamma amino butyric

acid“, GABA), den wichtigsten inhibitorischen Neurotransmitter, und Mediatoren wie Tryptophan frei (de Vadder et al., 2018; Duranti et al., 2020; Hata et al., 2017; Krishnan et al., 2018; Louis & Flint, 2017). Diese können wie auch SCFA über Afferenzen des *Nervus vagus* Informationen an das ZNS weiterleiten. Die Metaboliten können zudem über die Mukosa den zirkulären Blutstrom erreichen und teilweise die BBB passieren (Parker et al., 2020; Wenzel et al., 2020).

Bereits in vielen Versuchen mit Mäusen konnte gezeigt werden, dass eine Manipulation des Mikrobioms, sei es mit Antibiotika oder Probiotika bzw. durch keimfreie Aufzucht der Mäuse, das Verhalten beeinflusst (Chu et al., 2019; Neufeld et al., 2011; Yang et al., 2020). Keimfreie Mäuse zeigten z. B. ein reduziertes Angstverhalten im Vergleich zu SPF-Mäusen („specific-pathogen-free“, spezifisch pathogenfrei) (Heijtz et al., 2011; Neufeld et al., 2011). Durch eine Rekolonisation des Darms mit Bakterien lässt sich der Phänotyp revertieren (Clarke et al., 2013). Dies lässt auf einen direkten Zusammenhang des Mikrobioms mit dem ZNS schließen, da Verhalten direkt über die Funktionsweise des Gehirns gesteuert wird.

Eine Dysbalance der intestinalen Homöostase kann das Wohlbefinden stark beeinflussen und wird mit CED, aber z. B. auch Fettleibigkeit und Insulinresistenzen in Verbindung gebracht (Amabebe et al., 2020; Jiao et al., 2018; C. J. Lee et al., 2020). Ein resilientes Mikrobiom kann Störungen kompensieren und eine gesunde Mikrobiota-Zusammensetzung aufrechterhalten.

1.3 Das gastrointestinale System und neurodegenerative Erkrankungen

Neurodegenerative Erkrankungen sind aus symptomatischer Sicht in erster Linie Erkrankungen des ZNS. Gedächtnisverlust, kognitive Defizite und motorische Störungen gehen auf einen Rückgang der neuronalen Konnektivität im Gehirn zurück. In den letzten Jahren rückte das gastrointestinale System und dessen mikrobiologische Kommensalen immer mehr in den Vordergrund der Forschung, auch in Bezug auf neurodegenerative Erkrankungen. Da das autonome ENS des Darms mehr Neurone enthält als das Rückenmark und dem ZNS sehr ähnelt, liegt die Vermutung nahe, dass das ENS wie das ZNS bei neurodegenerativen Pathologien eine Rolle spielt (Hall, 2016).

Multiple Sklerose (MS) ist die häufigste neurodegenerative Erkrankung des ZNS bei jungen Erwachsenen (Oh et al., 2018). Dabei treten sowohl entzündliche als auch neurodegenerative Prozesse auf. Diese werden durch Immunzellen ausgelöst, die über die BBB ins Gehirn eintreten und dort die Myelinscheiden von Axonen angreifen (Bell et al., 2019; Correale et al., 2016). Als Folge dessen sterben Nervenzellen im progressiven Verlauf ab. Ein direkter Zusammenhang der MS mit dem intestinalen Mikrobiom konnte mit Hilfe von Modellmäusen gezeigt werden. Durch einen fäkalen Mikrobiomtransfer (FMT) entwickelten keimfreie MS-Modellmäuse eine Resistenz

gegenüber der Autoimmunenzephalomyelitis (Berer et al., 2011; Y. K. Lee et al., 2011). In einer Pilotstudie aus dem Jahre 2016 konnte außerdem gezeigt werden, dass Patienten mit schubförmiger, remittierender MS eine erhöhte intestinale Permeabilität aufweisen (Buscarinu et al., 2017). Darüber hinaus wurde in einer Längsschnittstudie mit pädiatrischen MS-Patienten festgestellt, dass die Zusammensetzung der Darmmikrobiota mit dem zukünftigen Rückfallrisiko zusammenhängt (Tremlett et al., 2016). Eine geringere Menge bzw. das Fehlen von Fusobacteria wurde mit einem 76%igen Risiko eines früheren Rückfalls in Verbindung gebracht.

Die amyotrophe Lateralsklerose (ALS) ist eine neurodegenerative Erkrankung, bei der Neurone des Gehirns und des Rückenmarks betroffen sind. Die Krankheit führt in der Regel innerhalb von 3 bis 5 Jahren zum Tod durch respiratorische Lähmung (Alonso et al., 2009). Auch im Falle der ALS konnte ein Zusammenhang mit dem Darm und dessen Mikrobiota gezeigt werden. Manipuliert man die Darmmikrobiota von ALS-Modellmäusen mittels Antibiotika oder keimfreier Aufzucht, reduzieren sich Symptome wie motorische Defizite (Blacher et al., 2019). Zudem liegt bei ALS-Modellmäusen eine Schädigung der „tight junctions“-Struktur, eine erhöhte Darmpermeabilität und eine Dysbiose der Darmmikrobiota im Vergleich zu wildtypischen Mäusen vor (Wu et al., 2015). Insbesondere wurde eine verminderte Anzahl an Buttersäure-produzierenden Bakterien gefunden. Die Darmmikrobiota von ALS-Patienten unterscheidet sich signifikant im Vergleich zu Kontrollgruppen: Sie ist geprägt durch eine erhöhte Anzahl an *Escherichia coli* (*E. coli*) sowie Enterobakterien und eine Reduktion von Clostridien als auch Buttersäure-produzierenden Bakterien, wie Oscillibacter, Anaerostipes und Lachnospira (Fang et al., 2016; Mazzini et al., 2018). Eine Nahrungsergänzung von ALS-Modellmäusen mit Buttersäure (2% in Trinkwasser) führte zu einer verbesserten Darmintegrität und einer gesteigerten Überlebensrate (Y. Zhang et al., 2017). Dies ist in Einklang mit den Ergebnissen von Bacher und Kollegen, die herausfanden, dass *Akkermansia muciniphila*, welches neben *Faecalibacterium prausnitzii* der Hauptproduzent von Buttersäure ist, die Symptome der ALS in transgenen Mäusen verbessert (Blacher et al., 2019). Die Ergebnisse lassen darauf schließen, dass die Pathogenese der ALS maßgeblich durch die Dysbiose der Buttersäure-produzierende Bakterien beeinflusst wird.

1.3.1 Einfluss der Darm-Hirn-Achse auf neurodegenerative Erkrankungen am Beispiel der Parkinson-Demenz

Ein Zusammenhang des Darms und dessen Mikrobiom mit neurodegenerativen Erkrankungen wurde in zahlreichen Studien für die Parkinson-Erkrankung („Parkinson disease“, PD) gezeigt. Diese ist in der klinischen Ausprägung und den Pathomechanismen mit der AD verwandt. Pathologische Merkmale der PD sind Aggregate von alpha-Synuclein (aSyn) als Hauptbestandteil

der Lewy-Körperchen im Gehirn sowie Neurodegeneration von dopaminergen Neuronen (Spillantini et al., 1997). Die aggregierten Proteine wurden 1912 erstmals von Fritz Heinrich Lewy beschrieben, nach dem sie benannt wurden, und 1919 von Konstantin Nikolaevich Trétiakoff mit der PD in Verbindung gebracht (Holdorff, 2002). Klinische Symptome manifestieren sich in Form von Tremor, Bradykinese und Rigor (Sveinbjornsdottir, 2016). Neben den neuronalen Symptomen sind gastrointestinale Störungen die hauptsächlichen Beschwerden von PD-Patienten, die meist schon viele Jahre vor den neuronalen Störungen beginnen (Lubomski et al., 2020; Ou et al., 2021; Schrag et al., 2015). Dies impliziert einen direkten Zusammenhang der PD mit dem GIT.

In mehreren Studien wurde das Darmmikrobiom von PD-Patienten mit dem von Kontrollpersonen verglichen und dabei wurden signifikante Unterschiede festgestellt, wie z.B. eine reduzierte Vielfalt der bakteriellen Zusammensetzung (Scheperjans et al., 2015). Insbesondere anti-inflammatorisch wirkende Bakterien wie Prevotella, Faecalibacterium, Blautia und Bifidobacterium liegen in PD-Patienten reduziert vor (Bedarf et al., 2017; Hasegawa et al., 2015; Nishiwaki et al., 2020; Petrov et al., 2017; Unger et al., 2016). Dadurch fehlen essenzielle Buttersäure-produzierende Bakterien, die sowohl die Mukosa schützen und ernähren als auch die Darmbarriere aufrechterhalten. Keshavarzian und Kollegen fanden eine Reduktion der Gattung Faecalibacterium in Kolon-Biopsien von PD-Patienten im Vergleich zu gesunden Kontrollen (Keshavarzian et al., 2015). Vertreter dieser Gattung wie *Faecalibacterium prausnitzii* metabolisieren L-Dopa zu Dopamin (Maini Rekdal et al., 2019). Im Einklang dazu fanden Unger und Kollegen reduzierte SCFA-Konzentrationen im Stuhl von PD-Patienten im Vergleich zu altersentsprechenden Kontrollen (Unger et al., 2016). Eine Metaanalyse von 2017 korrelierte zudem ein höheres Risiko für PD mit einer erhöhten Menge an *Helicobacter pylori* (X. Shen et al., 2017). Studien weisen darauf hin, dass *Helicobacter pylori* die Absorption von L-Dopa reduziert und zu einer Verschlechterung motorischer Symptome der PD führt (Lyte, 2010; McGee et al., 2018). Mit Hilfe von PD-Modellmäusen, die aSyn überexprimieren, konnte zusätzlich gezeigt werden, dass die Entwicklung von motorischen Defiziten und Neuroinflammation durch Antibiotika-Behandlung verlangsamt werden kann. Diese entwickelten sich erst deutlich nach FMT des Mikrobioms von PD-Patienten in die PD-Modellmäuse (Sampson et al., 2016).

Neben der Dysbiose der Bakterien konnten bei der Analyse von 34 Stuhlproben von PD-Patienten im Vergleich zu 28 Kontrollen erhöhte Konzentrationen von alpha-1-Antitrypsin und Zonulinfestgestellt werden (Schwiertz et al., 2018). Beide sind charakteristische Marker für die intestinale Permeabilität. Zudem war Calprotectin, ein kalziumbindendes Protein, signifikant erhöht. Dieses wird bei inflammatorischen Prozessen von neutrophilen Granulozyten,

Monozyten und Epithelzellen ausgeschüttet. Calprotectin ist ebenfalls bei CED (Morbus Crohn oder Colitis ulcerosa) erhöht und kann in Stuhlproben der Patienten nachgewiesen werden (Guardiola et al., 2014).

Bereits im Frühstadium der PD kann fehlgefaltetes aSyn in Biopsien der Mukosa nachgewiesen werden (Hilton et al., 2014). Auch Lewy-Körperchen treten gehäuft im ENS auf, weshalb die Hypothese aufgestellt wurde, dass sich die Krankheit zu Beginn im GIT manifestiert (Aldecoa et al., 2015; del Tredici et al., 2010; Klingelhoefer & Reichmann, 2015; Wakabayashi et al., 1990). aSyn könnte anschließend prionenähnlich über vagale Bahnen zum Gehirn gelangen und dort die charakteristischen Aggregate bilden. Dieser Hypothese gingen bereits einige Forschergruppen nach und konnten mit Hilfe von Tiermodellen den Weg von aSyn sowohl vom Gehirn in den GIT als auch umgekehrt nachvollziehen (Holmqvist et al., 2014; Pan-Montojo et al., 2012; Ulusoy et al., 2017). Ulusoy und Kollegen z. B. injizierten aSyn in den *Nervus vagus* von Ratten und konnten anschließend die PD-charakteristischen Lewy-Körperchen sowohl in der Magenwand als auch in der *Medulla oblongata* mikroskopisch nachweisen (Ulusoy et al., 2017). Das Risiko, an PD zu erkranken, ist außerdem für Patienten, die sich einer trunkalen Vagotomie unterzogen haben, erniedrigt (Svensson et al., 2015).

Dies sind nur einige Beispiele für die gefundenen Zusammenhänge des GIT und des gastrointestinalen Mikrobioms mit der PD, wobei die Manifestation der Erkrankung im Darm zunächst noch bewiesen werden muss. Auch im Falle der AD gibt es erste Hinweise auf eine gegenseitige Beeinflussung von Darm und Gehirn, wenngleich die Datenlage unzureichend ist verglichen mit der PD.

1.3.2 Die Mikrobiota-Darm-Hirn-Achse im Zusammenhang mit der Alzheimer Demenz

Der größte Risikofaktor, an der AD zu erkranken, ist das Alter. Dieses wiederum beeinflusst die Immunantwort, das Mikrobiom und die Permeabilität der Darmbarriere (Hopkins et al., 2001; Man et al., 2015; Ouwehand et al., 2008). Vergleicht man das Mikrobiom von älteren Personen mit dem von Kindern und Jugendlichen, so ist mit zunehmendem Alter z. B. eine Reduktion von Bifidobakterien und protektiven Molekülen wie SCFA und eine Zunahme von Enterobakterien zu beobachten (Caracciolo et al., 2014; Hopkins et al., 2001; Woodmansey et al., 2004). Zudem korrelieren die Serumspiegel von TNF-alpha negativ mit der Konzentration an Bifidobakterien, was auf eine Modulation der Immunantwort hinweist (Ouwehand et al., 2008). Aufgrund der erhöhten Permeabilität der Darmbarriere im fortgeschrittenen Alter ist davon auszugehen, dass der mikrobielle Austausch zwischen Darmlumen und Blutbahn erhöht ist, wodurch wiederum inflammatorische Prozesse, z. B. durch Lipopolysaccharide (LPS) aus der Zellwand von Gram-

negativen Bakterien, ausgelöst werden können (Ghosh et al., 2020; Guo et al., 2015). Demnach liegt die Vermutung nahe, dass auch im Falle der AD - vergleichbar zur PD - Veränderungen der Darmphysiologie und der intestinalen Kommensalen stattfinden, bzw. *vice versa* das Darmmikrobiom im Zusammenhang mit der Pathogenese der AD steht.

Bereits 1989 konnten durch Selkoe und Kollegen A-beta-Ablagerungen in Geweben außerhalb des Gehirns nachgewiesen werden, unter anderem *post mortem* im Darmgewebe einer AD-Patientin (Joachim et al., 1989). Dennoch wurde das Mikrobiom erst in den letzten Jahren im Zusammenhang mit der AD untersucht. Dabei ist die Anzahl an Publikationen mit Vergleichen des Mikrobioms von AD-Patienten mit gesunden Kontrollen und AD-Modellmäusen mit wildtypischen Wurfgeschwistern immer noch gering (Zusammenfassung in Tabelle 1, moduliert aus (dos Santos Guilherme & Endres, 2020; Endres & Schäfer, 2018)). Auffällig ist zudem, dass die taxonomische Ebene, auf der die Mikrobiota jeweils untersucht wurde, sehr unterschiedlich ist, weshalb die Vergleichbarkeit der Daten untereinander schwierig ist. In einer auf 16sRNA basierenden Untersuchung von *post mortem* Hirngewebeproben wurde ein genereller Anstieg der Zellzahl der Mikrobiota und eine signifikante Erhöhung der Immunsystem-stimulierenden Spezies *Propionibacterium acnes* in Proben von AD-Patienten im Vergleich zu Kontrollen gefunden (Emery et al., 2017). Im Zusammenhang mit einem Anstieg von LPS im Gehirn spricht dies für eine generelle Aktivierung des Immunsystems (Emery et al., 2017). Eine chronische Infektion von AD-Patienten mit *Helicobacter pylori* führt zusätzlich zu erhöhten Entzündungsreaktionen und niedrigeren Punktzahlen im Mini-Mental-Status-Test (MMST) im Vergleich zu nicht infizierten AD-Patienten (Roubaud-Baudron et al., 2012). Ein pro-inflammatorisches Darmmikrobiom wurde ebenfalls bei Patienten mit Hirnamyloidose und kognitiven Beeinträchtigungen gefunden, wobei ein Anstieg von Escherichia/Shigella (pro-inflammatorisch) und eine Reduktion von *Eubacterium rectale* identifiziert wurden (Cattaneo et al., 2017).

Tabelle 1: Auswirkungen der AD auf das intestinale Mikrobiom

Zusammenfassung der Unterschiede des intestinalen Mikrobioms in Modellmäusen der AD im Vergleich zu wildtypischen Mäusen und AD-Patienten im Vergleich zu gesunden Kontrollen auf Ebene des bakteriellen Stammes, der Familie und der Gattung bzw. Spezies. (↑: erhöht, ↓: erniedrigt, →: keine Veränderungen, n.e.: nicht ermittelt; Tabelle modifiziert nach *(dos Santos Guilherme & Endres, 2020)*)

Referenz	Model	Stamm		Familie		Gattung/Spezies	
Harach et al., 2017	AD Modellmaus	Firmicutes	↓	Erysipelotrichidae		Allobaculum	↓
		Bacteroidetes	↑	Rikenellaceae	↑	n.d.	
				S24-7	↑		
		Verrucomicrobia	↓	Verrucomicrobiales		Akkermansia	↓
		Proteobacteria	↓	n.d.		n.d.	
		Actinobacteria	↓				
		Tenericutes	↑				
Brandscheid et al., 2017	AD Modellmaus	Frimicutes	↑	Clostridiaceae		Clostridium (C. leptum)	↑
		Bacteroidetes	↓	n.d.		n.d.	
		Verrucomicrobia	→	Verrucomicrobiaceae		Akkermansia	→
		Proteobacteria	→	Enterobacteriaceae		Escherichia (E.coli)	→
		Actinobacteria	→	Bifidobacteriaceae		Bifidobacterium	→
Bäuerl et al., 2018	AD Modellmaus	Frimicutes		Erysipelotrichidae	↑	n.d.	
				Ruminococcaceae		Ruminococcus	↓
						Oscillospira	↓
		Proteobacteria	↑	Sutterellaceae		Sutterella	↑
		Bacteroidetes		Rikenellaceae	↓	n.d.	
Shen et al., 2017	AD Modellmaus	Proteobacteria		Helicobacteraceae	↑	Helicobacter	↑
				Desulfovibrionaceae	↑	n.d.	
		Bacteroidetes		Porphyromonoadaceae		Odoribacter	↑
				Prevotellaceae		Prevotella	↓
Li et al., 2019	AD Modellmaus	Bacteroidetes		Bacteroidaceae		Bacteroides acidifaciens	↑
						Bacteroides coprocola	↑
				Rikenellaceae		Tidjanibacter massiliensis	↑
						Alistipes obesi	↑
						Alistipes finegoldii	↓
				Tannerellaceae		Parabacteroides distasonis	↓
		Proteobacteria		Moraxellaceae		Acinetobacter guillouiae	↑
				Helicobacteraceae		Helicobacter ganmani	↑
				Gammaproteobacteriaceae		Flintibacter butyricus	↓
		Firmicutes		Clostridiaceae		Clostridium methylpentosum	↓
				Eubacteriaceae		Aminipila butyrica	↓
				Oscillospiraceae		Lawsonibacter asaccharolyticus	↓
				Lachnospiraceae		Kineothrix alysoides	↓
				Ruminococcaceae		Faecalibacerium prausnitzii	↑
Chen et al., 2020	AD Modellmaus	Proteobacteria		Enterobacteriaceae	↑	Escherichia/Shigella	↑
		Verrucomicrobia		Verrucomicrobiaceae	↑	Akkermansia	↑
		Bacteroidetes		Prevotellaceae	↑	Alloprecotella	↑
				Bacteroidaceae	↓	Bifidobacterium	↑
				Rikenellaceae	↓	n.d.	
		Actinobacteria		Bifidobacteriaceae	↑	n.d.	
		Firmicutes		Erysipelotrichaceae	↑	Erysipelatoclostridium	↑
						Allobaculum	↑
				Lachnospiraceae	↓	Blautia	↑

Tabelle 1: Auswirkungen der AD auf das intestinale Mikrobiom

Referenz	Model	Stamm		Familie		Gattung/Spezies	
Vogt et al., 2017	Mensch	Firmicutes	↓	Ruminococcaceae	↓	n.d.	
				Turicibacteraceae	↓		
				Peptostreptococcaceae	↓		
				Clostridiaceae	↓	Clostridium	↓
				Mogibacteriaceae	↓	n.d.	
				Veillonellaceae		Dialister	↓
				Erysipelotrichaceae		Turicibacter	↓
				Gemellaceae	↑	Gemella	↑
				Lachnospiraceae		Blautia	↑
		Bacteroidetes	↑	Bacteroidaceae	↑	Bacteroides	↑
				Rikenellaceae	↑	Alistipes	↑
		Actinobacteria	↓	Bifidobacteriaceae	↓	Bifidobacterium	↓
				Coriobacteriaceae		Adlercreutzia	↓
		Proteobacteria		Desulfovibrionaceae		Bilophila	↑
Cattaneo et al., 2017	Mensch	Firmicutes		Eubacteriaceae		Eubacterium (E. rectale)	↓
		Proteobacteria		Enterobacteriaceae		Escherichia/Shigella	↑
Zhou et al., 2021	Mensch	Actinobacteria		Bifidobacteriaceae		Bifidobacterium	↑
		Proteobacteria		Sphingomondaceae		Sphingomonas	↑
		Firmicutes		Lactobacillaceae		Lactobacillus	↑
				Lachnospiraceae		Blautia	↑
				Ruminococcaceae		Anaerobacterium	↓
						Papillibacter	↓
		Bacteroidetes		Porphyromonoadaceae		Odoribacter	↓

Wodurch die Veränderungen der Mikrobiota zustande kommen, konnte noch nicht klar nachvollzogen werden. In Studien konnte gezeigt werden, dass AD-Patienten Veränderungen im Essverhalten und der Transitzeit der Darmpassage zeigen (Ikeda et al., 2002; C. H. Morris et al., 1989; Mungas et al., 1990; Priefer & Robbins, 1997). Zusätzlich ist bereits einige Jahre vor der klinischen Manifestation der Krankheit ein Gewichtsverlust der Patienten zu verzeichnen (Jimenez et al., 2017). Gleiche Ergebnisse lieferten Versuche mit AD-Modellmäusen, die eine veränderte Gewichtszunahme und eine längere Essensperiode zeigten (Pugh et al., 2007). Zusammen mit der geringeren Gewichtszunahme konnte in AD-Modellmäusen eine erhöhte Firmicutes/Bacteroidetes-Ratio mit einem einhergehenden Trypsinmangel gefunden werden (Brandscheid et al., 2017).

Bakterielle Produkte wie Proteinasen könnten die Progression der AD umgekehrt ebenfalls beeinflussen (Übersicht in (dos Santos Guilherme & Endres, 2020)). Proteine aus *E. coli* und *Bacillus subtilis natto* besitzen z. B. eine A-beta-abbauende enzymatische Aktivität (Hsu et al., 2009; Sharoar et al., 2013). Auch LPS, welches sich in der Zellmembran Gram-negativer Bakterien befindet, interagiert direkt mit A-beta und kann, wie auch andere bakterielle Toxine, die A-beta-Aggregation modulieren (Hsu et al., 2009; Sharoar et al., 2013). LPS beeinflusst zudem die BBB, woraus ein erhöhter Einstrom an A-beta ins Gehirn resultiert (Jaeger et al., 2009). In *in-vitro* Studien waren außerdem SCFA dazu in der Lage, die A-beta-Aggregation zu inhibieren (L. Ho et al., 2018).

Das langfristige Ziel ist es, mittels Ernährungsumstellung oder z. B. pro- oder präbiotischer Nahrungsergänzung das Mikrobiom so zu modulieren, dass die Pathogenese der AD positiv beeinflusst wird. In ersten Studien wurden einzelne probiotische Bakterienspezies auf eine Verbesserung der AD-Symptome untersucht. *Bifidobacterium longum* konnte z. B. der Dysbiose der Mikrobiota entgegen wirken und führte damit zu einem verlangsamten kognitiven Rückgang von AD-Modellmäusen (H.-J. Lee et al., 2019). Ob sich diese Ergebnisse auch auf den Menschen übertragen lassen, konnte bis zum jetzigen Zeitpunkt nicht geklärt werden.

1.4 5xFAD-Mäuse als Modellorganismus für die Alzheimer-Demenz

Die der Arbeit zugrundeliegenden Publikationen adressieren verschiedene Ansätze, um den Zusammenhang der Mikrobiota-Darm-Hirn-Achse mit der AD zu untersuchen. Gearbeitet wurde hierzu mit AD-Modellmäusen des B6SJL-Tg(APPSvFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax-Stammes, bekannt unter der Bezeichnung 5xFAD. Diese überexprimieren humanes A-beta aufgrund von drei Mutationen des humanen APP (Swedish (K670N, M671L), Florida (I716V), London (V717l)) und zwei Mutationen des humanen PSEN1 (M146L, L286V) (Oakley et al., 2006). Die Transgene werden über den Thy1-Promotor gesteuert. Mithilfe der 5xFAD-Mäuse können A-beta-induzierte neurodegenerative Prozesse und Auswirkungen der Formation der AD-typischen amyloiden Plaques untersucht werden. Letztere können bereits in einem Alter von 6 bis 8 Wochen auf Proteinebene im Gehirn nachgewiesen werden, mit 25 Wochen sind große Mengen Plaques im Hippocampus und im Cortex zu finden (Oakley et al., 2006). Auf Ebene der mRNA kann A-beta bereits nach 4 Wochen im Hirn- und Darmgewebe identifiziert werden (Brandscheid et al., 2017). Defizite im Verhalten der 5xFAD-Mäuse, z.B. im räumlichen Lernen, zeigen sich ab einem Alter von 17 bis 22 Wochen (Oakley et al., 2006). Zusammenfassend stellen 5xFAD-Mäuse somit ein relativ aggressives Modell für die AD dar, worauf bei Versuchen mit den Mäusen geachtet werden muss.

2 Zielsetzung der Arbeit

Die Zusammenhänge zwischen dem Darm, dem Mikrobiom und der AD sind nach wie vor kaum bekannt bzw. die Ergebnisse auf dem Gebiet sind divers und erlauben keine klare Aussage. Ziel der Arbeit ist es deshalb, die Thematik möglichst ganzheitlich zu betrachten, um neue Erkenntnisse auf diesem Gebiet zu gewinnen. Hierzu sollen verschiedene Ansätze verfolgt werden.

Zum einen soll die Darmphysiologie anhand der Darmtransitzeit von AD-Modellmäusen im Vergleich zu wildtypischen Mäusen in unterschiedlichen Altersstadien, d.h. vergleichbar zu verschiedenen Schweregraden der Erkrankung, untersucht werden. Diese gibt erste Hinweise auf Störungen der Darmfunktion. Mit Hilfe des Mausmodells können somit Schlüsse gezogen werden, ob Veränderungen der Darmmotilität im direkten Zusammenhang mit der fortschreitenden Pathogenese stehen. Im gleichen Modell soll die Expression von A-beta im neuronalen Darmgewebe der Maus analysiert werden. Im Falle, dass A-beta im neuronalen Darmgewebe der AD-Modellmäuse exprimiert wird, kann davon ausgegangen werden, dass die intestinale Mikrobiota in Kontakt mit diesem tritt.

Inwiefern die zuvor beschriebenen Veränderungen der Mikrobiota zustande kommen, ob diese eine der Ursachen der AD oder eine Folge der Erkrankung und der damit einhergehenden A-beta-Aggregation im Darm sind, soll nach positivem Nachweis von A-beta im Darm der Mäuse untersucht werden. Hierzu sollen Bakterien aus murinen Fäzes mit aktivem A-beta inkubiert werden und anschließend die Viabilität im Vergleich zu einer Inkubation mit inaktivem A-beta bestimmt werden. Zusätzlich soll das Bakterienwachstum zweier ausgewählter Bakterienfamilien, Lactobacillaceae und Enterobacteriaceae, näher betrachtet werden. A-beta und das inaktive Peptid als Vergleich sollen zudem wildtypischen Mäusen oral verabreicht werden. So können die Auswirkungen auf die Mikrobiota nach einer Magenpassage anhand einer 16S-rRNA-Analyse des anschließend entnommenen Chymus erforscht werden.

Eine veränderte Darmphysiologie, ein Mikrobiom in Dysbiose, A-beta-Aggregation im Darm – all diese Faktoren könnten ebenfalls die Genexpression im Darm beeinflussen. Eine durch [redacted] durchgeführte Analyse betrachtete diesen Ansatz mittels Expressionsanalyse von 84 AD-relevanten Genprodukten im Darmgewebe von 5xFAD-Mäusen. Daraus resultierte ApoA1 als stärkstes differentiell exprimiertes Gen in allen Darmabschnitten. Hierauf basierend sollen Veränderungen im Lipidstoffwechsel, abhängig von unterschiedlichen Altersstadien und somit Schweregraden der Erkrankung, z.B. anhand vom Lipidgehalt der Fäzes und Expression relevanter Transkriptionsfaktoren, untersucht werden.

Um dem Henne-Ei-Problem näher auf die Spur zu kommen, soll auf der anderen Seite das Mikrobiom von 5xFAD-Mäusen mit Hilfe von Antibiotika und Probiotika über einen Zeitraum von 14 Wochen moduliert werden. Hierbei werden zwei Extreme betrachtet, um anschließend Mikrobiota-assoziierte Auswirkungen auf z.B. die senilen Plaques im Hirn der Mäuse, aber auch die kognitive Leistung der AD-Modellmäuse zu ergründen. Zusätzlich sollen auch physiologische Parameter wie der Blutzuckerspiegel am Ende der Versuchsdurchführung bestimmt werden.

3 Publikationen

Die vorliegende Dissertation wurde in kumulativer Form angefertigt. Die Ergebnisse, die dieser Arbeit zugrunde liegen, wurden in Fachjournalen veröffentlicht. Nachfolgend werden die Veröffentlichungen, dessen Ergebnisse für diese Arbeit verwendet wurden, genannt und kurz zusammengefasst. Der eigene wissenschaftliche Beitrag zu den Arbeiten wird dargelegt. Die Originalarbeiten finden sich im Anhang in Kapitel 7.1.

3.1 Alzheimer's disease in the gut – Major changes in the gut of 5xFAD model mice with ApoA1 as potential key player.

Autoren: Nicolai Maximilian Stoye, **Malena dos Santos Guilherme**, Kristina Endres

Publiziert in: FASEB Journal, 2020 Sep; 34(9):11883-11899

doi: 10.1096/fj.201903128RR

Zusammenfassung: In der Publikation wurde der Einfluss der AD auf den Darm untersucht. Dazu wurde die Darmphysiologie mittels Messung der Transitzeit des Darms und Expression von A-beta im Darmgewebe der AD Modellmäuse analysiert. Außerdem wurde die Expression von AD-relevanten Genen im Darm untersucht und dabei ApoA1 als mögliches Schlüsselprotein und Verursacher von Lipidstoffwechselveränderungen festgestellt.

Eigener Beitrag: Für diese Veröffentlichung habe ich folgende Versuche durchgeführt: Lipid-Extraktion aus Fäzes, Genexpressionsanalyse von FoxA2, Abca1 und Arp-1 aus Darmproben sowie von ApoA1 aus Leberproben, Fütterungsversuche der Mäuse zur Analyse der Darmtransitzeit sowie der quantitative Nachweis von GFAP und A-beta mittels Western Blots. Außerdem habe ich die Interaktion von A-beta und ApoA1 mittels Fluroszenz-basierten Assays über 4 Tage gemessen. Bei der Erstellung des Manuskripts habe ich durch Kommentierung mitgewirkt.

3.2 Impact of Acute and Chronic Amyloid-β Peptide Exposure on Gut Microbial Commensals in the Mouse.

Autoren: **Malena dos Santos Guilherme**, Hristo Todorov, Carina Osterhof, Anton Möllerke, Kristina Cub, Thomas Hankeln, Susanne Gerber, Kristina Endres

Publiziert in: Frontiers in Microbiology, 2020 May 20; 11:1008

doi: 10.3389/fmicb.2020.01008

Zusammenfassung: In der Publikation wurden antimikrobielle Eigenschaften von A-beta auf intestinale Mikrobiota von wildtypischen und AD-Modellmäusen untersucht. Zudem wurde der akute und chronische Einfluss von A-beta auf Darmbakterien *in-vivo* analysiert. Dazu wurde zum

einen wildtypischen Mäusen das Peptid oral verabreicht und anschließend Chymus-Proben genommen und zum anderen Chymus von wildtypischen im Vergleich zu AD-Modellmäusen gesammelt. Nach 16S-rRNA-Analyse der mikrobiellen Gene konnten kurzfristige Auswirkungen des A-betas und langfristige Veränderungen des Mikrobioms durch die AD gezeigt werden.

Eigener Beitrag: Für diese Arbeit habe ich die Inkubationsversuche der Mikrobiota mit A-beta durchgeführt und anschließend das Bakterienwachstum sowie die Viabilität der Bakterien untersucht. Zudem habe ich die Mäuse mit A-beta bzw. dem inaktiven Peptid für die Analyse der Auswirkungen auf das Mikrobiom gefüttert. Das Manuskript habe ich zusammen mit Hristo Todorov geschrieben und bei der Bearbeitung mitgewirkt.

3.3 Impact of Gut Microbiome Manipulation in 5xFAD Mice on Alzheimer's Disease-Like Pathology

Autoren: **Malena dos Santos Guilherme**, Vu Thu Thuy Nguyen, Christoph Reinhardt, Kristina Endres

Publiziert in: Microorganisms, 2021 Apr 13; 9(4):815

doi: 10.3390/microorganisms9040815

Zusammenfassung: In dieser Publikation sollten die Auswirkungen eines modifizierten Mikrobioms mittels Antibiotika und ausgewählter Probiotika auf die Pathologie der AD untersucht werden. Dazu wurden die Mäuse über 14 Wochen über das Trinkwasser mit Antibiotika bzw. Probiotika behandelt. Der Verlauf der Behandlung wurde über das Bakterienwachstum ausgewählter Stämme beobachtet. Nach Ende der Behandlung wurde die kognitive Leistung mittels Nesting-Tests untersucht. Bei der Sektion wurden Gewebe-Proben genommen und der Blutzuckerspiegel gemessen. Die A-beta Ablagerungen im Gehirn wurden analysiert sowie Veränderungen im Glukosestoffwechsel näher untersucht.

Eigener Beitrag: Für diese Veröffentlichung habe ich die AD-Modellmäuse mit Antibiotika bzw. Probiotika über 14 Wochen behandelt und wöchentlich die Futteraufnahme, das Trinkverhalten und die Gewichtszunahme ermittelt. Der Verlauf der Behandlung wurde mittels Bakterienanalyse aus Fäzes anhand von zwei Stämmen, Lactobacillaceae und Enterobacteriaceae, zu Beginn der Behandlung, nach zwei Wochen und am Ende des Versuchs von mir untersucht. Zudem habe ich überprüft, ob die verwendeten Probiotika die Magenpassage überstehen, in dem ich diese mit Mageninhalt von keimfreien Mäusen inkubiert und anschließend das Bakterienwachstum analysiert habe. Die Nestbauversuche wurden durch mich durchgeführt, sowie die Analyse der A-beta Ablagerungen im Gehirn der Mäuse. Die Sektionen wurden in Zusammenarbeit mit [redacted]

durchgeführt. Anschließend habe ich die Gewebegewichte ermittelt. Zudem habe ich bei der Bearbeitung und Überprüfung des Manuskripts mitgewirkt.

4 Diskussion und Ausblick

Selkoe und Kollegen konnten bereits 1989 A-beta-Ablagerungen im Darmgewebe einer AD-Patientin nachweisen (Joachim et al., 1989). Zudem wurden Unterschiede in der Dichte des ENS und Veränderungen in der Trypsin-Aktivität im Darm in AD-Mausmodellen beschrieben (Brandscheid et al., 2017; Semar et al., 2013). Auch die Morphologie des Darms ändert sich bei vorhandener AD: In elf Wochen alten 5xFAD-Mäusen konnte unter anderem eine Verdickung der Muskularis im Vergleich zu wildtypischen Mäusen festgestellt werden (V. T. T. Nguyen et al., 2021). Diese Veränderungen können unter anderem die physiologischen Eigenschaften des Darms beeinträchtigen. Einen ersten Hinweis auf eine gestörte Darmfunktion kann die Transitzeit geben (Kim & Rhee, 2012). Eine Veränderung der Darmtransitzeit („gut transition time“, GTT) wurde bereits mit psychischen Krankheiten wie Ängsten und Depressionen in Verbindung gebracht (Gorard et al., 1996). In drei verschiedenen Altersstufen der 5xFAD-Mäuse wurde die GTT im Vergleich zu wildtypischen Geschwistertieren untersucht. Acht Wochen alte Mäuse stellten dabei ein frühes AD-Stadium dar, 21 Wochen alte ein fortgeschrittenes Stadium, in dem die Mäuse bereits kognitive Beeinträchtigungen, jedoch keinen Funktionsverlust des Kurzzeitgedächtnisses zeigten, und 40 Wochen alte Mäuse ein spätes pathologisches Stadium der AD mit kognitiven und funktionellen Einschränkungen (Kimura & Ohno, 2009; Oakley et al., 2006). Im frühen Stadium mit acht Wochen konnten keine Unterschiede der GTT gemessen werden, jedoch zeigten die gealterten 5xFAD-Mäuse sowohl bereits mit 21 Wochen als auch mit 40 Wochen eine signifikant schnellere Darmpassage im Vergleich zu den gleichaltrigen Wurfgeschwistern. Dies deutet auf eine Veränderung der Darmfunktion mit dem Fortschreiten der Krankheit hin. Mit dem progressiven Verlauf der AD zeichnen sich auch Dysbiosen der intestinalen Mikrobiota ab (siehe Kapitel 1.3.2). Bakterien wie *Akkermansia muciniphila* und *Faecalibacterium prausnitzii* sind wichtige Lieferanten für SCFA (Ferreira-Halder et al., 2017; Ottman et al., 2017). SCFAs, vor allem Buttersäure, interagieren mit dem ENS, indem sie die cholinerge Weiterleitung verbessern und damit die Darmmotilität steigern (Tian et al., 2020; L. Wang et al., 2020). Eine Dysbalance der Mikrobiota kann demnach einen direkten Einfluss auf die Darmmotilität haben. Eine veränderte GTT kann wiederum das Mikrobiom ebenfalls direkt verändern (Tottey et al., 2017).

Die Zusammensetzung der Mikrobiota kann auch auf Grund der antimikrobiellen Eigenschaft von A-beta verändert werden. Wir konnten zeigen, dass A-beta im Darm der 5xFAD-Mäuse exprimiert wird. Daraus resultiert, dass die Mikrobiota chronisch mit dem Peptid in Kontakt sind. Die Wachstums-hemmende und toxische Beeinflussung von A-beta wurde bereits bei einzelnen Mikroben, wie *Enterococcus faecalis* oder *Candida albicans*, nachgewiesen (Soscia et al., 2010).

Die Untersuchungen wurden *in-vitro* unter aeroben Verhältnissen durchgeführt, welche nicht den vorwiegenden physiologischen Bedingungen im Darm entsprechen (Soscia et al., 2010; Spitzer et al., 2016). Der Einfluss des verwendeten A-betas auf die intestinale Mikrobiota von AD-Modellmäusen und wildtypischen Mäusen wurde mittels Inkubationsversuchen unter anaeroben Bedingungen untersucht. Eine kurze Expositionszeit von zehn Minuten reichte aus, um die Viabilität der Bakterien von wildtypischen Mäusen signifikant zu reduzieren. Interessanterweise konnte diese Beobachtung nicht bei Bakterien aus Fäzes von 5xFAD-Mäusen gemacht werden. Weiterhin wurden zwei ausgewählte Bakterienfamilien, Lactobacillaceae und Enterobacteriaceae, näher untersucht. Das Wachstum beider Bakterienfamilien wurde im Vergleich zur Kontrollinkubation mit dem inaktiven Peptid signifikant reduziert, wenn die Bakterien aus Fäzes von wildtypischen Mäusen stammten. Wie zuvor konnte diese Beobachtung nicht bei Fäzesbakterien von AD-Modellmäusen gemacht werden. Die Ergebnisse deuten darauf hin, dass die lange Exposition der Mikrobiota von 5xFAD-Mäuse mit A-beta eine adaptive Reaktion auslöst. Unterstützend zu dieser Annahme fanden Soscia und Kollegen ein reduziertes Wachstum von *Enterococcus faecalis* zu Beginn der Exposition, das Wachstum setzte jedoch während des Versuchsverlaufs zu einem späteren Zeitpunkt wieder ein (Soscia et al., 2010).

In-vitro Studien können lediglich im Ansatz das widerspiegeln, was sich *in-vivo* tatsächlich abspielt. Um die physiologischen Umstände nachzuahmen, wurde das Peptid wildtypischen Mäusen oral verabreicht und die Veränderungen der Mikrobiota im Vergleich zur Fütterung mit dem inaktiven Peptid mittels 16S-rRNA-Analyse untersucht. Die 16S-rRNA-Analyse wurde von Hristo Todorov (AG Gerber, Johannes Gutenberg-Universität Mainz) durchgeführt. Über die gleiche Analyse wurde die Mikrobiota von wildtypischen im Vergleich zu 5xFAD-Mäusen im gleichen Alter untersucht. Weder zwischen der Fütterung mit A-beta und der Kontrollfütterung mit dem inaktiven Peptid noch zwischen wildtypischen und AD-Modellmäusen konnte ein Unterschied in der alpha-Diversität festgestellt werden. Dies ist im Einklang mit vorher publizierten Ergebnissen über Versuche mit Mäusen im vergleichbaren Alter (Harach et al., 2017; L. Shen et al., 2017; L. Zhang et al., 2017). Widersprüchliche Ergebnisse wurden über die alpha-Diversität mit fortschreitendem Alter der Mäuse berichtet: Es konnte sowohl eine Zunahme in APP/PS1 Mäusen, als auch eine Abnahme in unterschiedlichen Studien gefunden werden (Bäuerl et al., 2018; L. Shen et al., 2017; L. Zhang et al., 2017). Aufgrund der kontroversen Forschungsergebnisse muss der Zusammenhang der alpha-Diversität des Darmmikrobioms mit der AD in zukünftigen Studien noch näher aufgeklärt werden. Wie die alpha-Diversität zeigte auch die beta-Diversität keinerlei Unterschiede im Vergleich der wildtypischen mit den transgenen Mäusen. Auch dies ist im Einklang mit der Literatur (Bäuerl et al., 2018; Harach et al., 2017; L. Zhang et al., 2017). Interessanterweise differenzierte sich die beta-Diversität in zwei

klar voneinander getrennte Gruppen beim Vergleich der wildtypischen Mäuse, die mit A-beta bzw. dem inaktiven Peptid gefüttert wurden. Wie in den vorherigen Ergebnissen weist dies auf einen Anpassungs- oder möglicherweise Resilienzmechanismus der Mikrobiota, die länger mit A-beta exponiert sind, hin. Die Ergebnisse bündeln sich in der Hypothese, dass A-beta vor allem im frühen Stadium der AD ein Treiber einer Dysbiose der Mikrobiota sein könnte.

Ein moduliertes Mikrobiom, eine veränderte Darmmotilität aber auch die AD an sich können sich auf die Genexpression im Darmgewebe auswirken. Auf Grund dieser Annahme untersuchte [redacted] 84 AD-assoziierte Gene. Wie zuvor bei der Analyse der GTT wurden dafür Proben von einem Monat alten Mäusen ohne Beeinträchtigung durch die AD, zwei Monate alten Mäusen, als Referenz für den Beginn der Manifestation der AD, und von acht Monate alten Mäusen, mit nachweislichem Verlust von Neuronen im Gehirn, verwendet (Brandscheid et al., 2017; Eimer & Vassar, 2013; Oakley et al., 2006). Das am stärksten differentiell exprimierte Gen in allen Darmabschnitten, aber vor allem im Kolon, war Apolipoprotein A1 (ApoA1). Im Zusammenhang mit der AD gibt es kontroverse Publikationen in Bezug auf ApoA1. Zum einen konnten geringere Serum- und Plasmaspiegel von ApoA1 in AD-Patienten gefunden werden und in einer älteren Kohorte auch vor dem Auftreten der Erkrankung mit einer verringerten kognitiven Leistung in Zusammenhang gebracht werden (Kawano et al., 1995; Merched et al., 2000; Song et al., 2012). Eine hohe Konzentration an ApoA1 wurde mit einem geringeren Risiko für Demenzerkrankungen in Verbindung gebracht (Saczynski et al., 2007) Zum anderen korrelierten in einer anderen Studie die Plasmaspiegel von ApoA1 nicht mit dem Demenzrisiko und der kognitiven Funktion (Koch et al., 2020). In einer unserer vorherigen Untersuchungen konnten wir zeigen, dass die ApoA1-Konzentration im Liquor keine Unterscheidung zwischen AD-Patienten und Patienten mit anderen Demenz-Formen zuließ (Stoye et al., 2020). Im Kolon der ein und zwei Monate alten 5xFAD-Mäuse in dieser Untersuchung konnte eine geringere Expression von ApoA1, in acht Monate alten 5xFAD-Mäusen interessanterweise jedoch eine höhere Expression im Vergleich zu wildtypischen Geschwistertieren festgestellt werden. ApoA1 wirkt anti-inflammatorisch (Cimmino et al., 2009; Sirniö et al., 2017). Eine erhöhte Expression von ApoA1 in den älteren Tieren könnte durch inflammatorische Prozesse, hervorgerufen durch die AD, verursacht werden. Dies ist im Einklang mit der zuvor gefundenen erhöhten Expression von GFAP im Kolongewebe der älteren 5xFAD-Mäuse im Vergleich zu den Wildtypen. Da ApoA1 größtenteils im Darm und der Leber synthetisiert wird und ausgeschlossen werden sollte, dass die beobachteten Unterschiede durch einen veränderten Stoffwechsel in der Leber stammen, wurden Leberproben von einem Monat alten Mäusen auf die Expression von ApoA1 untersucht. Dabei konnten keine Unterschiede in der Expression von ApoA1 in der Leber zwischen 5xFAD- und wildtypischen Mäusen festgestellt werden.

Die Transkriptionsfaktoren Arp-1 (ApoA1-regulierendes Protein) und FoxA2 (Forkhead-Box-Protein A2) sind wichtige Regulatoren der ApoA1-Expression (Ladias & Karathanasis, 1991; Wruck et al., 2016). In den Kolon-Proben der ein und acht Monate alten Mäusen wurde aufgrund dessen die Genexpression von Arp-1 und FoxA2 untersucht. Zu beiden untersuchten Zeitpunkten konnte kein Unterschied in der Expression von Arp-1 gefunden werden, jedoch eine signifikante Reduktion der Expression von FoxA2 in einem Monat alten 5xFAD-Mäusen im Vergleich zu wildtypischen Mäusen und ein tendenzieller Anstieg der Expression von FoxA2 im Vergleich von acht Monate alten 5xFAD-Mäusen mit wildtypischen Wurfgeschwistern. Dies spiegelte das Expressionsniveau von ApoA1 wider. FOXA2 spielt eine wichtige Rolle in der Lipidhomöostase (Arciello et al., 2016; Friedman & Kaestner, 2006). Deshalb wurde die Ausscheidung von Lipiden in Fäzes von 5xFAD-Mäusen im Vergleich zu wildtypischen Mäusen untersucht. Die gleichen Tendenzen wie zuvor bei ApoA1 und FoxA2 konnten auch hier gezeigt werden: Die jungen, ein Monat alten 5xFAD-Mäuse schieden ca. 30% weniger Lipide über Fäzes aus als wildtypische Mäuse, ältere 5xFAD-Mäuse mit 40 Wochen schieden ca. 30% mehr Lipide aus als Wildtypen. Die Expression von FoxA2 ist auch in iPSC („induced pluripotent stem cells")-basierten AD-Modellen erhöht und vermittelt zudem im Mittelhirn dieser Mäuse die Entwicklung und Erhaltung dopaminerger Neuronen (Ferri et al., 2007; Stott et al., 2013; Wruck et al., 2016). Im Zusammenhang mit der AD wurde eine Reduktion von Dopaminrezeptoren und deren Neurotransmitter im ZNS gefunden (Pan et al., 2019). Dopaminerge Neurone können die GTT direkt beeinflussen. Die veränderte FoxA2-Expression könnte ein Hinweis sein, dass der dopaminerge Stoffwechsel im ENS ebenfalls gestört ist. Damit könnte z. B. die schnellere GTT der älteren 5xFAD-Mäuse im Vergleich zu den wildtypischen Wurfgeschwistern zu erklären sein. Eine erhöhte Ausscheidung der Lipide kann jedoch nicht nur mit einer gestiegenen GTT in Verbindung stehen, sondern auch mit einer verminderten Lipid-Resorption im Darm der acht Monate alten 5xFAD-Mäuse, durch die sich der Darmtransit beschleunigt. Daher können wir zum jetzigen Zeitpunkt nicht sicher sagen, dass die erhöhte GTT mit der gestiegenen FoxA2-Expression zu begründen ist. Mit der veränderten Lipidausscheidung einher ging ein signifikanter Anstieg des Gesamtcholesterins im Serum der jungen 5xFAD Mäuse und eine signifikante Reduktion in den Proben der älteren Mäuse im Vergleich zu wildtypischen Geschwistern. ApoA1 ist Hauptproteinbestandteil von HDL. Folglich sollte mit einer erhöhten Menge an ApoA1 ebenfalls ein Anstieg an HDL-Cholesterin und eine Abnahme des LDL/VLDL-Cholesterins zusammenhängen. Diese Vermutung konnte in den alten Tieren jedoch nicht mit signifikanten Ergebnissen bestätigt werden, obwohl Tendenzen in die erwarteten Richtungen gefunden wurden.

Im Zusammenhang mit der AD zeigten Untersuchungen an APP/PS1DeltaE9 AD-Modellmäusen, dass kognitive Defizite durch einen Mangel an ApoA1 über eine höhere A-beta Konzentration verstärkt werden, wohingegen eine Überexpression von ApoA1 für den Erhalt der kognitiven Fähigkeiten sorgten (Lefterov et al., 2010; Lewis et al., 2010). Zudem führte die Behandlung von AD-Modellmäusen mit ApoA1 zu einer Reduktion der zerebralen A-beta-Ablagerungen (Fernández-de Retana et al., 2017). Inwiefern ApoA1 mit A-beta interagiert und die Aggregation beeinflusst, sollte in einem Fluoreszenz-basierten Aggregationsassay über vier Tage untersucht werden. Wir konnten zeigen, dass ApoA1 zu einer erhöhten Aggregation von A-beta, abgeleitet von einem stärkeren ThT-Signal, führt. Paul-Lima und Kollegen zeigten, dass dieser Effekt abhängig von den stöchiometrischen Verhältnissen von ApoA1 zu A-beta ist: bei niedrigem molarem Verhältnis von 20:1 (A-beta: ApoA1) wurde das ThT-Signal reduziert, ein Verhältnis von 2:1 führte zu einer Erhöhung des ThT-Signals im Vergleich zur Kontrolle (Paula-Lima et al., 2009). In unserem Versuch haben wir ein molares Verhältnis von 10:1 verwendet, die Erhöhung des ThT-Signals blieb über die gesamte Dauer des Versuchs von vier Tagen stabil. ApoA1 in höher Konzentration scheint demnach die Aggregation des A-betas zu beschleunigen zu Gunsten der Pathogenese, da das neurotoxische Peptid dadurch eliminiert wird.

Zusammenfassend geben diese Ergebnisse erste Hinweise darauf, dass Veränderungen der AD eventuell nicht erst im Gehirn, sondern im peripheren Gewebe des Darms zu finden sind. Diese könnten im Zusammenhang mit den zuvor berichteten Dysbiosen der intestinalen Mikrobiota, aber auch mit Funktionsstörungen des Darms von AD-Patienten stehen. Die Veränderungen im Lipidstoffwechsel können zum weiteren Verständnis der AD als systemischer Erkrankung beitragen. Welche zusätzliche Rolle ApoA1 für die Pathogenese der AD im Menschen spielt und ob sich daraus neue Wege für die Diagnostik und Therapie ergeben, muss in weiteren Untersuchungen geklärt werden.

Nachdem festgestellt werden konnte, dass A-beta im Darm der 5xFAD-Mäuse exprimiert wird, die Darmphysiologie z. B. in Bezug auf die GTT beeinflusst wird und A-beta antimikrobielle Eigenschaften aufweist, sollte im Gegenzug der Einfluss eines modifizierten Mikrobioms auf die progressive Entwicklung der AD anhand von 5xFAD-Mäusen untersucht werden. Es konnte bereits gezeigt werden, dass eine keimfreie Aufzucht von APP/PS1-Mäusen zu einer geschwächten Pathologie führt (Harach et al., 2017). Zudem resultierte eine sechsmonatige Behandlung der AD-Modellmäuse mit einem Antibiotika-Cocktail zu reduzierten A-beta-Ablagerungen in der kortikalen und hippocampalen Region der männlichen Tiere im Vergleich zu Kontrollmäusen (Minter et al., 2016). Damit einhergehend konnte eine geringere alpha-Diversität der Darmmikrobiota, nicht jedoch eine verringerte Abundanz der Bakterien beobachtet werden. Zusätzlich wurde eine Erhöhung von Zytokinen wie CCL11 sowie ein Anstieg

der Mikroglia-Vernetzung um die senilen Plaques im Gehirn nachgewiesen (Minter et al., 2016). In einem Rattenmodell, in dem AD-ähnliche Symptome durch die Gabe von A-beta hervorgerufen wurden, konnte die Gedächtnisleistung durch die Gabe eines präbiotischen Oligosaccharids verbessert werden (D. Chen et al., 2017). Auch eine probiotische Mischung aus *Bifidobacterium lactis*, *Lactobacillus casei*, *Bifidobacterium bifidum* und *Lactobacillus acidophilus* führte zu einer Verbesserung der kognitiven Leistung von gealterten SAMP8-Mäusen, ein Mausmodell mit einem beschleunigten Alterungsprozess, und wirkte sich dabei zudem positiv auf die Darmbarriere und die Blut-Hirn-Schranke aus (Yang et al., 2020).

In unserer Studie wollten wir überprüfen, ob sich im aggressiven 5xFAD-Mausmodell ähnliche Effekte mit Antibiotika erzielen lassen. Da die ersten A-beta Ablagerungen bereits ab einem Alter von 1,5 Monaten nachgewiesen werden können, begann die Behandlung der Mäuse ab einem Alter von vier Wochen (Oakley et al., 2006). Das Mikrobiom sollte sowohl mit Antibiotika als auch Probiotika moduliert und die pathologischen Merkmale der AD in den behandelten 5xFAD-Mäusen verglichen werden. Die Mäuse wurden dazu für zwei Wochen mit einer hohen Dosis eines Antibiotika-Cocktails (bestehend aus Gentamicin, Vancomycin, Metronidazol, Neomycin, Ampicillin, Kanamycin, Colistin und Cefoperazon) und weitere 12 Wochen mit einer niedrigen Dosis (1/50) der Startkonzentration im Trinkwasser behandelt. Das Behandlungsschema wurde bereits an APP/PS1-Mäusen verwendet, jedoch für eine Zeitspanne von sechs Monaten und mittels einer täglichen Gabe über eine Schlundsonde (Minter et al., 2016). Die Probiotika-Gruppe erhielt für 14 Wochen einen Mix aus *Lactobacillus acidophilus* und *Lactobacillus rhamnosus* im Trinkwasser, als Kontrolle dienten Tiere, die lediglich Leitungswasser tranken. Eine langfristige Behandlung mit probiotischen Bakterien kann zu positiven Wirkungen führen, wie z.B. hemmenden Effekten gegenüber Pathogenen, einer Verhinderung von Darmschäden oder einer verbesserten Elektrolytabsorption (Borrelli et al., 2016; Borthakur et al., 2008; Candela et al., 2008; Roselli et al., 2009). Daher war unsere Hypothese, dass die verwendeten Probiotika einen positiven Effekt auf das Fortschreiten der AD haben sollten. Im APP/PS1 AD-Mausmodell wurde bereits gezeigt, dass *Lactobacillus plantarum* allein oder eine Kombination mit *Bifidobacterium bifidum* die krankheitsbedingten Symptome linderten (F. Wang et al., 2020; Q.-J. Wang et al., 2020).

Um die kognitive Leistung im Zusammenhang mit einem modulierten Mikrobiom zu untersuchen, wurde in der 14. Woche die Nestbaufähigkeit der Mäuse untersucht. Die Antibiotika-behandelten Tiere bauten signifikant bessere Nester im Vergleich zur Kontrollgruppe und verbauten das gesamte zur Verfügung gestellte Material in ihren Nestern, obgleich dies statistisch keine Signifikanz erreichte. Bei den Probiotika-Tieren konnte dagegen im Vergleich zur Kontrollgruppe kein Unterschied festgestellt werden. Das Ergebnis spiegelte

sich bei der Untersuchung der A-beta Ablagerungen im Gehirn der behandelten Mäuse wider. Im Bereich des Hippocampus der Antibiotika-behandelten 5xFAD-Mäuse konnten weniger Ablagerungen quantifiziert werden, was auch mikroskopisch bereits ersichtlich war. Die eingesetzten Probiotika, *L. acidophilus* und *L. rhamnosus* beeinflussten die A-beta Ablagerungen jedoch nicht. In einer humanen Studie mit AD-Patienten konnte eine *L. acidophilus*-haltige Probiotikamischung bereits positive Effekte auf die kognitive Leistung der Patienten zeigen (Akbari et al., 2016). Eine Metaanalyse zu drei Studien mit Probiotika-Interventionen bei AD-Patienten berichtete dagegen, dass keine kognitive Leistungsverbesserung im Zusammenhang mit den Probiotika gefunden werden konnte (in allen Mischungen wurde *L.acidophilus* verwendet) (Krüger et al., 2021). In einer Metaanalyse konnte gezeigt werden, dass Probiotikamischungen wesentlich bessere gesundheitsfördernde Eigenschaften bewiesen als einzelne Stämme (Chapman et al., 2011). In unserem Versuch könnte das gemeinsame Zusammenspiel mehrerer Probiotika fehlen, um einen Einfluss auf die Pathologie auszuüben. Um Probiotika als unterstützende Therapie mit gesundheitsfördernden Eigenschaften bei der AD einzusetzen, bedarf es jedoch noch weiterer Studien. Probiotika bieten jedoch eine nebenwirkungsarme Möglichkeit, um die Gesundheit des Patienten zu unterstützen.

Die orale Gabe von Antibiotika wirkt sich sowohl auf die Mikrobiota-Zusammensetzung als auch auf die Produktion der SCFAs aus (Zarrinpar et al., 2018). Ein gehemmter SCFA-Stoffwechsel führt zu einer veränderten Glukosehomöstase der Kolonozyten des Darms, was sich unter anderem auch auf den Blutzuckerspiegel auswirkt (Zarrinpar et al., 2018). Auch diesen Effekt konnten wir bei den Antibiotika-behandelten 5xFAD-Mäusen im Vergleich zu den Kontrollmäusen anhand eines gesenkten Blutzuckerspiegels um ca. 25% beobachten. Da die Mäuse am frühen Morgen seziert wurden, ist davon auszugehen, dass sie sich in einem nüchternen Zustand befanden. Wir konnten ausschließen, dass die Beobachtungen durch eine veränderte Futteraufnahme verursacht wurden, da das Fressverhalten über die 14-wöchige Behandlungsphase dokumentiert wurde und langfristig keine Unterschiede in der Futteraufnahme festgestellt werden konnten. Mit dem gesunkenen Blutzuckerspiegel einher gingen jedoch ein erhöhter Serum-Glukagonspiegel und ein tendenziell verringerter Insulinspiegel. Diese Ergebnisse deuten auf eine antidiabetische Wirkung der Antibiotika-Behandlung hin. Typ-2-Diabetes steht im direkten Zusammenhang mit dem AD-Risiko für den Menschen (Shieh et al., 2020). Außerdem sind mehrere Signalwege, die an der AD-Pathologie beteiligt sind, von Insulin abhängig(Pardeshi et al., 2017). Daraus resultiert, dass eine veränderte Mikrobiota-Zusammensetzung sich positiv auf den pro-Diabetischen Phänotyp im Zusammenhang mit der AD auswirken könnte.

Zusammenfassend unterstützen die Ergebnisse der Arbeit die Annahme, dass die AD nicht nur als Erkrankung des ZNS gesehen werden sollte und dass der Darm und dessen Mikrobiom als Schaltzentrale unseres Immunsystems unbedingt auch in zukünftigen Studien mit einbezogen werden sollte. Die Ergebnisse eröffnen den Blick auf neue Interventionsmöglichkeiten und therapeutische Maßnahmen. In den Versuchen konnte gezeigt werden, dass das Mikrobiom die Pathologie der AD beeinflusst bzw. vice versa verändert die AD die bakterielle Viabilität und Zusammensetzung im Darm. Wie das Mikrobiom so moduliert werden kann, dass es bei AD-Patienten gesundheitsfördernde Effekte hervorruft, konnte nicht geklärt werden und muss in zukünftigen Forschungen näher untersucht werden.

Möglichkeiten, die intestinalen Mikrobiota zu modulieren, gibt es dabei viele. Neben der Ernährung gehören dazu wie im hier vorliegenden Versuchsaufbau Probiotika und Antibiotika, aber auch Präbiotika wie resistente Stärke, die z. B. das Wachstum von Darmschleimhaut-ernährenden Bakterien fördert. Eine verbesserte Versorgung der Mukosa und eine damit einhergehende dichtere intestinale Barriere könnten zu einer Linderung der GIT-Beschwerden der Patienten führen. Dies konnte bereits für PD-Patienten gezeigt werden und sollte in einer Studie mit AD-Patienten untersucht werden (Becker et al., 2021). Weiterhin ist der fäkale Mikrobiomtransfer eine schon jetzt erfolgreich angewendete Methode bei Patienten mit CED. Wir konnten bereits in einer hier nicht vorgestellten Untersuchung zeigen, dass das Mikrobiom von jungen und alten wildtypischen Mäusen erfolgreich in 5xFAD-Mäuse transplantiert werden kann und die amyloiden Ablagerungen abhängig vom Spender beeinflusst werden (Valeri et al., 2021). Für eine Anwendung am Menschen fehlt nach wie vor die Grundlage, welche Bakterienstämme und -spezies einen gesunden Spender definieren. Hierzu benötigt es weitere, vor allem einheitliche diagnostische Untersuchungen der Mikrobiota und ihren funktionellen Eigenschaften. Ziel ist eine unterstützende Behandlung von AD-Patienten auf Basis des Mikrobioms, die sowohl zu einer Reduktion der A-beta Ablagerungen als auch einer damit einhergehenden kognitiven Leistungsverbesserung führen.

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6 Liste weiterer Publikationen

6.1 Übersichtsarbeiten

(1) The impact of the gut microbiome in Alzheimer's disease: cause or consequence?

Malena dos Santos Guilherme, Kristina Endres

Genetics, Neurology, Behavior, and Diet in Dementia.

ISBN 978-0-12-815868-5, doi: 10.1016/B978-0-12-815868-5.00049-9

6.2 Weitere Veröffentlichungen

1. Elevated Testosterone Level and Urine Scent Marking in Male 5xFAD Alzheimer Model Mice.

Gadomsky L, dos Santos Guilherme M, Winkler J, van der Kooij MA, Hartmann T, Grimm M, Endres K.

Curr Alzheimer Res. 2020;17(1):80-92. doi: 10.2174/1567205017666200217105537.

2. Identification of Patulin from Penicillium coprobium as a Toxin for Enteric Neurons.

Brand B, Stoye NM, dos Santos Guilherme M, Nguyen VTT, Baumgaertner JC, Schüffler A, Thines E, Endres K.

Molecules. 2019 Jul 30;24(15):2776. doi: 10.3390/molecules24152776.

3. Apolipoprotein A1 in Cerebrospinal Fluid Is Insufficient to Distinguish Alzheimer's Disease from Other Dementias in a Naturalistic, Clinical Setting.

Stoye NM, Jung P, dos Santos Guilherme M, Lotz J, Fellgiebel A, Endres K.

J Alzheimers Dis Rep. 2020 Feb 4;4(1):15-19. doi: 10.3233/ADR-190165.

4. The Synthetic Retinoid Acitretin Increases IL-6 in the Central Nervous System of Alzheimer Disease Model Mice and Human Patients.

dos Santos Guilherme M, Stoye NM, Rose-John S, Garbers C, Fellgiebel A, Endres K.

Front Aging Neurosci. 2019 Jul 23;11:182. doi: 10.3389/fnagi.2019.00182. eCollection 2019.

5. Dietary Wheat Amylase Trypsin Inhibitors Impact Alzheimer's Disease Pathology in 5xFAD Model Mice.

dos Santos Guilherme M, Zevallos VF, Pesi A, Stoye NM, Nguyen VTT, Radyushkin K, Schwiertz A, Schmitt U, Schuppan D, Endres K.

Int J Mol Sci. 2020 Aug 31;21(17):6288. doi: 10.3390/ijms21176288.

6. Shotgun lipidomics of liver and brain tissue of Alzheimer's disease model mice treated with acitretin.

Lauer AA, Janitschke D, dos Santos Guilherme M, Nguyen VTT, Bachmann CM, Qiao S, Schrul B, Boehm U, Grimm HS, Hartmann T, Endres K, Grimm MOW.

Sci Rep. 2021 Jul 27;11(1):15301. doi: 10.1038/s41598-021-94706-3.

7. Acitretin reverses early functional network degradation in a mouse model of familial Alzheimer's disease.

Rosales Jubal E, Schwalm M, dos Santos Guilherme M, Schuck F, Reinhardt S, Tose A, Barger Z, Roesler MK, Ruffini N, Wierczeiko A, Schmeisser MJ, Schmitt U, Endres K, Stroh A.

8. N6 -Methyladenosine Modification in Chronic Stress Response Due to Social Hierarchy Positioning of Mice.

dos Santos Guilherme M, Tsoutsouli T, Todorov H, Teifel S, Nguyen VTT, Gerber S, Endres K.

Front Cell Dev Biol. 2021 Aug 20;9:705986. doi: 10.3389/fcell.2021.705986. eCollection 2021.

9. Influence of Acetylcholine Esterase Inhibitors and Memantine, Clinically Approved for Alzheimer's Dementia Treatment, on Intestinal Properties of the Mouse.

Nguyen VTT, Sallbach J, dos Santos Guilherme M, Endres K.

Int J Mol Sci. 2021 Jan 20;22(3):1015. doi: 10.3390/ijms22031015.

10. Primary Cilia Structure Is Prolonged in Enteric Neurons of 5xFAD Alzheimer's Disease Model Mice.

Nguyen VTT, Brücker L, Volz AK, Baumgärtner JC, dos Santos Guilherme M, Valeri F, May-Simera H, Endres K.

Int J Mol Sci. 2021 Dec 17;22(24):13564. doi: 10.3390/ijms222413564.

11. Impact of the Age of Cecal Material Transfer Donors on Alzheimer's Disease Pathology in 5xFAD Mice.

Valeri F, dos Santos Guilherme M, He F, Stoye NM, Schwiertz A, Endres K.

Microorganisms. 2021 Dec 9;9(12):2548. doi: 10.3390/microorganisms9122548.

12. Selective targeting of chronic social stress-induced activated neurons identifies neurogenesis-related genes to be associated with resilience in female mice.

dos Santos Guilherme M, Tsoutsouli T, Chongtham MC, Winter J, Gerber S, Müller MB, Endres K.

Psychoneuroendocrinology. 2022 May;139:105700. doi: 10.1016/j.psyneuen.2022.105700. Epub 2022 Feb 19.

13. Resilience and Gut Microbiome: Insights from Chronically Socially Stressed Wild-Type Mice

Malena dos Santos Guilherme, Francesco Valeri, Jennifer Winter, Marianne B. Müller, Andreas Schwiertz, Kristina Endres

Microorganisms 2022, 10(6), 1077; doi: 10.3390/microorganisms10061077

14. Behavioral Phenotyping of Bbs6 and Bbs8 Knockout Mice Reveals Major Alterations in Communication and Anxiety

Rödig N, Sellmann K, Dos Santos Guilherme M, Nguyen VTT, Cleppien D, Stroh A, May-Simera HL, Endres K.

Int J Mol Sci. 2022 Nov 22;23(23):14506. doi: 10.3390/ijms232314506.

7 Anhang

7.1 Veröffentlichungen

(1) Alzheimer's disease in the gut-Major changes in the gut of 5xFAD model mice with ApoA1 as potential key player.

Stoye NM, dos Santos Guilherme M, Endres K.

FASEB J. 2020 Sep;34(9):11883-11899. doi: 10.1096/fj.201903128RR. Epub 2020 Jul 18.

(2) Impact of Acute and Chronic Amyloid-β Peptide Exposure on Gut Microbial Commensals in the Mouse.

dos Santos Guilherme M, Todorov H, Osterhof C, Möllerke A, Cub K, Hankeln T, Gerber S, Endres K.

Front Microbiol. 2020 May 20;11:1008. doi: 10.3389/fmicb.2020.01008. eCollection 2020.

(3) Impact of Gut Microbiome Manipulation in 5xFAD Mice on Alzheimer's Disease-Like Pathology

dos Santos Guilherme M, Nguyen VTT, Reinhardt C, Endres K.

Microorganisms. 2021 Apr 13;9(4):815. doi: 10.3390/microorganisms9040815.

7.2 Curriculum Vitae

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RESEARCH ARTICLE

THE FASEB JOURNAL

Alzheimer's disease in the gut—Major changes in the gut of 5xFAD model mice with ApoA1 as potential key player

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Abstract

Alzheimer's disease (AD) affects around 33 million people worldwide, which makes it the most prominent form of dementia. The main focus of AD research has been on the central nervous system (CNS) for long, but in recent years, the gut gained more attention. The intestinal tract is innervated by the enteric nervous system (ENS), built of numerous different types of neurons showing great similarity to neurons of the CNS. It already has been demonstrated that the amyloid precursor protein, which plays a major role in AD pathology, is also expressed in these cells. We analyzed gut tissue of AD model mice (5xFAD) and the respective wild-type littermates at different pathological stages: pre-pathological, early pathological and late pathological. Our results show significant difference in function of the intestine of 5xFAD mice as compared to wild-type mice. Using a pathway array detecting 84 AD-related gene products, we found ApoA1 expression significantly altered in colon tissue of 5xFAD mice. Furthermore, we unveil ApoA1's beneficial impact on cell viability and calcium homeostasis of cultured enteric neurons of 5xFAD animals. With this study, we demonstrate that the intestine is altered in AD-like pathology and that ApoA1 might be one key player within the gut.

KEYWORDS

A-beta, enteric nervous system, FoxA2, lipids

1 | INTRODUCTION

Over 50 million people currently suffer from dementia worldwide and estimates assume that this number will more than triple over the next 30 years.[1] Already in 2018 the costs for treatment were about one trillion USD.[1] About two thirds of people suffering from dementia are diagnosed with Alzheimer's disease (AD). However, there is still no cure for this fatal disease and only few drugs are available for treatment of AD symptoms.[2] Meanwhile, it is commonly accepted that the accumulation and deposition of amyloid-beta (A-beta) peptides in the brain presents one of the most prominent pathological hallmarks of the disease.[3-5] Changes in A-beta level and distribution are, inter alia, able to induce inflammatory processes, oxidative stress, disbalance in calcium homeostasis and finally can

Abbreviations: A-beta, amyloid-beta; AD, Alzheimer's disease; ApoA1, apolipoprotein A1; CdkII, cyclin-dependent kinase like 1; CNS, central nervous system; ENS, enteric nervous system; GTT, gut transit time; PD, Parkinson's disease; SCFAs, short chain fatty acids; TREM2, triggering receptor expressed on myeloid cells 2.

[Correction added on August 10, 2020, after first online publication: Projekt DEAL funding statement has been added.]

lead to neuronal dysfunction and cell death (reviewed in 4,6). So far, AD is seen mainly as a neurodegenerative disease of the brain, although certain comorbidities such as diabetes[7-10] and loss of weight in late life[11-13] are well known. Recent work shows increasing evidence that besides the brain, also the gut as a peripheral organ might contribute to CNS disorders (reviewed in 14,15). The intestine shows great complexity in its structure which is due to the diverse tasks needed for food digestion and protection against potential pathogens. For managing this variety of functions, the gastrointestinal tract is innervated by its own nervous system, the enteric nervous system (ENS), which is able to act partially autonomous.[16-19] The ENS consists of around 400-600 million neurons in humans, which are organized in mesh-like structures, called plexuses (Auerbach's and Meissner's plexus).[18] It has diverse functions and regulates, for example, motility of the gastrointestinal tract, secretion of gastric acid, blood flow, and nutrition absorbance (for an overview see 16-20). The ENS shows great homology to the CNS not only in its complex structure but also in neurotransmitters used for signal transmission (reviewed in 18). Therefore, it is not surprising that the ENS has already been connected to neurodegenerative diseases. In Parkinson's disease (PD) one of the pathological hallmarks are Lewy bodies, abnormal aggregates of protein with alpha-synuclein as their primary structural component. For PD, it has been shown that one of the drivers of pathology might be the expression of alpha-synuclein in the ENS, from where it migrates to the CNS,[21-23] finally resulting in death of neurons in the brain. Diseases, resulting in the loss of enteric neurons during development such as Hirschsprung's disease are often fatal when not treated (reviewed in 24). In AD, A-beta peptides typically found in the brain of patients, could be demonstrated already decades ago by Joachim and colleagues within selected gut specimen.[25] In experiments with AD model mice, it has been shown that overexpression of A-beta results in an increase of inflammatory markers (eg, COX-2) in the gut of transgenic mice, otherwise typically seen in AD brain samples.[26] Furthermore, a specific loss of nitrergic and cholinergic neurons could be observed in the transgenic mouse model APP/PS1.[27] For a deeper understanding of the gut with its nervous system, and its role in AD, we analyzed four representative gut segments of the 5xFAD mouse model[28] regarding differences in expression of 84 genes involved in AD pathogenesis. The most prominently affected gene product, ApoA1, was further investigated regarding its role in A-beta-driven changes in primary culture of enteric neurons. Furthermore, the modulation of ApoA1 expression by FoxA2, a central regulator of lipid metabolism, was investigated. Finally, we were able to show differences in viability and calcium influx of enteric neurons derived from model mice and the reversal of these impairments by ApoA1 administration.

2 | MATERIALS AND METHODS

2.1 | Animals

For maintenance, male B6SJL-Tg (APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax (5xFAD) mice (Jackson Lab, Bar Harbor, Maine, USA) were crossbred with female C57BL/6J mice.[28] The animals were group-housed (three to five animals) with a 12 hours day/night cycle. Food and water were available ad libitum. The non-transgenic offspring was used as control. All procedures were performed in accordance with the European Communities Council Directive regarding care and use of animals for experimental procedures and were approved by local authorities (Landesuntersuchungsamt Rheinland-Pfalz; approval number G17-1-035).

2.2 | Tissue sample collection

Male 5xFAD mice and their respective littermates were sacrificed by decapitation after isoflurane anesthesia. The gut was removed and 2 cm long pieces of duodenum, jejunum, caecum, and colon were prepared. These gut pieces were opened longitudinally and carefully rinsed two times with sterile, isotonic NaCl. Samples were collected from three different mice for each gut segment, time point, and genotype regarding the initial expression analysis. For preservation, tissue pieces were submerged in RNA later (Qiagen, Hilden, Germany) and stored at −80°C until RNA preparation was conducted.

For liver tissue, portal vein of sacrificed animals was isolated and flushed with high volumes of ice cold 10 mM Tris/HCl to remove the blood. The median lobe of the liver was utilized for analysis. Crude myenteric plexus preparations (from duodenum to colon) for Western blotting were conducted as described for the first steps of isolation of enteric neurons (see below). Six pieces per mouse were collected from wild-type and transgenic animals and combined per animal before further homogenization.

For serum samples, truncal blood was collected with sacrifice of the animals. After 45 minutes of clotting, serum was obtained by centrifugation and subsequently stored at −80°C until further use.

2.3 | Acetylcholinesterase activity measurement

A colorimetric assay (Ellman's method, 29) was conducted to assess the levels of Acetylcholinesterase. Therefore, small intestine was isolated as described before and whole brain was prepared from approx. 40-week-old male 5xFAD mice and their respective wild-type littermates. Tissue was homogenized with

100 mg/500 µL of potassium phosphate buffer (0.5 M) containing protease inhibitor cocktail (Roche, Basel, Switzerland) with the TissueLyzer LT (Qiagen) for 5 minutes at 50 Hz followed by sonication with 20% output and 10 pulses (UW 2070, Bandelin electronic). After sonication, homogenates were centrifuged at 4°C for 1 minute at 1000 *g*. Supernatant of the small intestine preparations was diluted 1:10 and supernatant of brain tissue preparation 1:100 with potassium phosphate buffer in clear 96-well plates. Acetylthiocholine iodid (Sigma-Aldrich, St. Louis, Missouri, USA) (1 mM) and 5,5′-Dithiobis-2-nitrobenzoic acid (Sigma-Aldrich) (0.25 mM) were added. After 5 minutes incubation time in the dark at RT samples were measured at 405 nm over a period of 125 minutes. Minimum of signal was subtracted from maximum and slope normalized to protein content. Protein content was measured using Roti-Nanoquant (Carl Roth, Karlsruhe, Germany) following the vendor's manual.

2.4 | Gut transition time measurement

For measurement of the gut transition time, wild-type and 5xFAD animals were fed with sky blue solution (PME; 0.58 µL/g body weight). After the feeding, mice were single caged and time till mice defecated blue feces was measured.

2.5 | RNA assay

For qPCR, the RNA concentration of samples obtained from three corresponding animals was adjusted to 75 ng/µL. Three microliters of each RNA-sample was pooled to give a better biological averaging.[30] Eight microliters of this RNA-pool sample was used for reverse transcription using the "RT^2 first strand kit" (Qiagen) following the manufacturer's instructions. The obtained cDNA was subjected to the RT^2 Profiler PCR Array (Qiagen) according to the manufacturer's instruction. Levels of RNA were normalized to at least three different housekeeping genes, selected out of five potentially applicable housekeeping genes on the array by the least variation within the respective tissue. Changes in mRNA level were designated as "hit", if effect size was greater than the mean variation of the corresponding housekeeping genes. The expression of transcription factors and Abca1 was analyzed using the QuantiTect SYBR Green RT-PCR Kit (Qiagen) following the manufacturer's instructions using 100 ng RNA per reaction and the respective QuantiTect Primer Assay (Qiagen).

2.6 | Western blotting

Gut, liver, and plexus preparation were carried out as described before. The rinsed tissue specimen was subjected to Tris-HCl buffer (10 mM, pH 8) with protease inhibitor cocktail (Roche) (200 µL/100 mg of tissue), snap frozen in liquid nitrogen, and stored at −80°C until further usage. Samples were homogenized and protein content was determined as described before. Protein concentration was adjusted to 30 µg per 15 µL in LDS NuPAGE buffer (1x, Life Technologies) containing DTT (1 M, 10% v/v). The solution was boiled for 5 minutes at 95°C. Proteins were separated on a 10%- or 14%- (for A-beta detection) SDS polyacrylamide gel and proteins were transferred to a nitrocellulose membrane (GE Healthcare). The membrane was blocked with I-block solution (0.2% in PBS) (Thermo Fisher Scientific) including 0.05% Tween 20 (AppliChem, Darmstadt, Hessen). Primary antibody incubation took place overnight at 4°C with antibody against ApoA1 (Thermo Fisher Scientific; for a specificity control of the antibody see Figure S1) or against GFAP (Cell Signaling) with a dilution of 1:1000 in blocking solution. Actin or GAPDH detection was used as loading control (antibodies from Sigma-Aldrich and Cell Signaling). For A-beta detection, antibody 6E10 (Covance) was used. The respective secondary antibody, coupled with horseradish peroxidase, was applied (Thermo Fisher Scientific) and light signals were detected after incubation with SuperSignal West Femto chemiluminescent substrate (Thermo Fisher Scientific) using a CCD-camera imaging system (Stella Camera, Raytest, Straubenhardt, Germany).

2.7 | A-beta/ ApoA1 interaction

To investigate the interaction of ApoA1 (in 10 mM NH_4HCO_3; Chemicon International, Temecula, USA) with A-beta (in 5 mM NH_4OH, PBS buffered; AnaSpec, Fremont California, USA) or BSA (in 5 mM NH_4OH, PBS buffered; Carl Roth), proteins were incubated with solvent or in the molar ratio of 1:10 as described before.[31] Formation of complex was detected using 4%-20% Mini-PROTEAN TGX Precast Gels (Bio-Rad, Hercules California, USA) under native conditions. PAA-Gels were silver-stained using the Roti-Black P kit (Carl Roth) following the vendor's recommendation.

2.8 | A-beta aggregation assay

For the A-beta aggregation assay, human AggreSure A-beta (1-42; 23.6 µM; AnaSpec) and Thioflavin T (250 µM; Sigma) were combined in 50 mM Tris/150 mM NaCl (pH 7.2). To investigate the influence of ApoA1 on A-beta aggregation, the mixture was incubated with either ApoA1 (2.4 µM) or morin (0.1 mM in ethanol; Sigma) or the respective solvent control on black 384-well plates (Greiner Bio-One, Kremsmünster, Austria). Fluorescence was measured at 37°C for a period of 40 minutes every minute, with shaking in between with the Fluostar Omega (BMG Labtech, Cary, NC, USA)

(Ex/Em = 440/484 nm). For long-term observation, single measurements were performed at the indicated time points. In between, samples were kept under constant shaking (300 rpm) at 37°C in the dark.

2.9 | Lipid extraction from mouse feces

Mice were moved to a fresh cage. After 24 hours, fecal pellets were collected in the morning. Lipid extraction was done according to Kraus et al, 2015[32] (modified Folch extraction). In brief, 5 mL of saline were added to 1 g of mouse feces and mixed with chloroform in methanol (2:1 by volume). The suspension was centrifuged at 1000 *g* for 10 minutes at 21°C and the lipid phase removed. After the liquid was evaporated, lipid mass was calculated in weight of lipids/ total feces weight.

2.10 | HDL/ LDL-VLDL assay

The kit was used as recommended by the manufacturer for the fluorometric procedure (Sigma-Aldrich). In brief, 10 µL of serum were subjected to precipitation; subsequently 3 µL from 10-fold dilutions were used for measurement of fraction-dependent cholesterol with the Fluostar Omega (BMG Labtech).

2.11 | Isolation of primary enteric neurons

Enteric neuron isolation was carried out with slight changes according to Smith and colleagues.[33] Briefly, 2- to 3-month-old mice were sacrificed as described before. The whole gut was dissected, the small and the large intestine were kept in carbogen-gassed Krebs buffer, and the caecum was discarded. Each segment was rinsed with 10 mL of Krebs buffer from each side and cut into segments of approximately 2 cm length. The longitudinal muscle layer was peeled off with a cotton swab and the tissue was washed by centrifugation in 1 mL of Krebs buffer three times at 350 *g* for 30 s at 4°C. After centrifugation, the tissue was digested in collagenase solution (1.3 mg/mL; Worthington, Lakewood, New York) for 1 hour at 37°C under gentle agitation while being carbogen-gassed. Afterwards, tissue homogenate was centrifuged at 400 *g* for 10 minutes at 4°C, and digested with a sterile 37°C warm, 0.05% trypsin (Gibco)/ PBS solution. Digestion was stopped by adding DMEM/F12 supplemented with 10% FCS, 1% Glutamine and 1x Antibiotic/Antimycotic (all Life Technologies GmbH). Cells were centrifuged at 400 *g* for 10 minutes at RT. After centrifugation, the cell pellet was resuspended in neuronal medium (Neurobasal A with B-27, 2 mM L-glutamine, 1% FCS, 10 ng/mL of GDNF, and 1x Antibiotic/Antimycotic; all Life Technologies) and filtered through a 100 µm cell strainer (Greiner bio-one). The filtered cells were incubated on a rotation mixer for 30 minutes at 4°C. After mixing, cells were washed with 3 mL of neuronal medium and then centrifuged at 400 *g* for 10 minutes at RT. Cells obtained from one gut were resuspended in 600 µL of neuronal medium; for a 24-well plate (Sarstedt AG & Co. KG, Nümbrecht, Germany) 100 µL of neuron-containing suspension were added to 750 µL of neuronal medium in one well, coated with Poly-L-Lysine hydrobromide (PLL, Sigma-Aldrich Chemie GmbH). After 4 days in culture experiments were conducted.

2.12 | Cell culture

Primary enteric neurons were cultivated in neurobasal A DMEM/F12 supplemented with 1% FCS, 1% Glutamine, 1x B-27, 1x Antibiotic/Antimycotic (all: Life Technologies), 10 ng/mL of Glial Derived Neurotrophic Factor (Acris, Herford, Germany) at 5% CO_2, 37°C in humidified air (95%).

2.13 | Toxicity assay

About 112.5 µL of medium were pipetted into PLL coated black glass-bottom 96-well plates and 15 µL of cell suspension (see above) were added. After an incubation period of 48 hours half of the medium was aspirated and 36.25 µL of fresh medium were added, supplemented with either 2.5 µM A-beta$_{42}$ (AnaSpec), or as control 2.5 µM scrambled A-beta$_{42}$ (AnaSpec), or 0.25 µM ApoA1 (Chemicon international) or the respective solvent control (for A-beta or scrambled A-beta 5 mM NH_4OH in 1x PBS, for ApoA1 10 mM NH_4HCO_3).

2.14 | Immunofluorescence

Enteric neurons were seeded on PLL coated coverslips in 24 well-plates. For this purpose, 100 µL of cell suspension (see above) were added to 750 µL of neuronal medium. After 3 days of incubation 50% of cell supernatant was changed. After 4 days of incubation, cells were washed with PBS and incubated for 20 minutes at RT in 4% PFA solution, followed by a second washing step. Cells were stored in 1xPBS at 4°C until being blocked in 1x PBS supplemented with 10% FCS for 1 hour. After blocking, cells were incubated with primary antibody for detection of either β III-tubulin or NeuN (both Cell Signaling Technology) in a 1:500 dilution in blocking solution over night at 4°C. After incubation, cells were washed with PBS and counterstained with DAPI (10 µg/mL; Carl Roth) for 5 minutes at RT followed by three washing

steps with PBS. The incubation with the secondary antibody (Life Technologies) was carried out for 1 hour followed by three PBS washing steps. After this, microscopy of coverslips was carried out using the AxioVert 200M microscope (Carl Zeiss, Oberkochen, Germany).

2.15 | Calcium influx measurement

Calcium influx was measured using the Calbryte 520-AM indicator (AAT Bioquest 20651, Sunnyvale, California, USA) as the vendor recommended. After 4 days of cultivation, 100 µL of 20 mM HEPES buffered HBSS solution containing 10 µM Calbryte 520-AM were added to 100 µL of cell supernatant. Cells were incubated for 45 minutes at 5% CO_2, 37°C, humidified air (95%) followed by a 15 minutes incubation at RT in the dark. After incubation, Calbryte 520-AM containing supernatant was exchanged with HEPES buffered HBSS solution and KCl was added (50 mM) resulting in calcium influx. Binding of calcium to Calbryte 520AM results in a strong fluorescence signal (485 nm excitation, 520 nm emission).

3 | RESULTS

3.1 | Alzheimer's disease-like pathological changes are not restricted to the brain of AD model mice

Changes in gut properties have occasionally been described for AD mouse models such as differences in enteric network density or amount of trypsin in the gut lumen.[34,35] One of the major changes in AD pathology is the activity of the enzyme acetylcholinesterase (AChE) within brain tissue (for a review see 36) which has not been investigated in gut so far to our knowledge. We, therefore, measured the activity of AChE simultaneously in brain and gut tissue of the 5xFAD mouse model for AD using the Ellmans assay.[29] AChE activity was reduced not only in brain homogenates of transgenic animals compared to wild-type littermates, but also in the small intestine of 5xFAD animals (Figure 1A). Cholinergic transmission is an important mediator of gut propulsion in rodents[37,38] and gut transit time is widely used as an indicator for a disturbed intestinal function.[39] Therefore, the gut transit time of 5xFAD and wild-type mice was investigated at three different stages: early pathological (8 weeks), advanced pathological (21 weeks), and late pathological (40 weeks) stage. No difference in gut transit time of 8-week-old mice was observed, but a decreased gut transit time in 21-week-old and 40-week-old 5xFAD animals as compared to their respective wild-type control (Figure 1B). Moreover, GFAP—one of the major indicators of inflammation—likewise showed a significant increase in the gut of aged transgenic animals as compared to same-age littermates (Figure 1C). In sum, this shows the state-dependent influence of the transgenes that have been used for establishing the rodent model, on gut in regard to different functions and physiological pathways. It has already been shown previously, that transgenic human amyloid precursor protein (APP) is expressed in the gut of murine AD models.[34,35] To confirm this for the different pathological stages of 5xFAD mice once more, we here analyzed crude myenteric plexus preparations, collected from small and large intestine, by Western blotting (Figure 1D): in transgenic mice of young as well as old age, a more intense protein band was obtained at 4 kDa which would match the monomeric peptide and some higher protein species only visible in the samples from transgenic animals, presumably representing oligomeric forms. However, also in the wild-type tissue specimen signals were observed that are not expected due to the species-selectivity of the antibody 6E10 (only staining human-derived A-beta containing material). This indicates that in gut tissue cross-reactivity might occur and special caution has to be exercised with interpreting findings. The higher protein intensity found in young transgenic animals as compared to older transgenic animals reflects what was found before in regard to mRNA levels.[35]

To investigate the potential time-dependent manifestation of AD hallmarks in tissue of the intestine, we analyzed the expression of 84 different genes (AD RT^2 Profiler PCR Array, Qiagen), linked to this disease. Investigation took place in four different gut segments at three different time points in the 5xFAD mouse model (Figure 1E). As a pre-pathological stage 1-month-old mice, which seem still not to be impaired by the transgene in the gut,[35] were investigated. Although 2-month-old mice showed no impairment in the gut transit time measurement, general pathological hallmarks such as soluble A-beta in brain are already detectable[28] and therefore, the respective time point was chosen as a stage for an early onset of pathology. At 6 months of age, neuronal loss is starting in the brain of 5xFAD mice[40]; therefore, 8 months were chosen to represent a manifest stage in AD pathology. Most genes within the array were expressed in gut tissue and over all, a minimum of 88% of the AD-linked genes fulfilled quality requirements to be included in further analyzes (Figure S2). Intuitively, a first glance was taken on the main genes associated with A-beta such as murine *App*, *Psen*, *Bace*, and *Mapt*.[41] Respective differences in gene expression could be detected at single time points and within single tissue segments but rather inconsistent and with mostly only minor effect size. Murine *App* itself and *Psen1* and *2* were not changed regarding expression over all time points and gut segments. *Bace1* showed only a minor change in expression in jejunum in 2-month-old 5xFAD animals as compared to controls (fold regulation: 1.58). *Bace2* expression

varied greatly over all gut segments and time points. The expression of *Mapt* was lower in caecum of 2-month-old 5xFAD animals as compared to controls (fold regulation: −1.89).The fold regulation of the main genes associated with A-beta is shown in Table S1.

3.2 | Gene expression in colon tissue is altered in AD model mice

It has already been shown that constipation, impaction of intestine, volvulus, and dilatation of the colon (megacolon) are correlated to AD in humans.[42] It is of note that the most differentially expressed gene over all age-groups was also found in colon samples of 5xFAD animals compared to wild-type mice: apolipoprotein A1 (*ApoA1*; Figure 1E). For this reason, colon was chosen for further analysis. *ApoA1* displayed lower expression in 1- and 2-month-old 5xFAD animals (Figure 2A) but interestingly higher expression in 8-month-old 5xFAD animals as compared to wild-type animals (Figure 2B). Cyclin-dependent kinase like 1 (*CdklI*), the second most strongly affected gene, for example, was higher expressed in 1- and 2-month-old animals as compared to wild-type, but showed no altered expression in 8-month-old animals (Figure 2B). As our first analysis was conducted using pooled RNA samples, not allowing for analysis of variation, we subsequently performed measurements regarding individual animals for the identified hits (*ApoA1*, *CdklI*). RNA analysis on samples obtained from single animals confirmed a higher expression of *CdklI* in 1-month-old 5xFAD animals, but not in 2-month-old animals (Figure 2C). No change in expression was observed in 8-month-old animals. For *ApoA1*, the results obtained from pooled RNA were verified for all time points. *ApoA1* was ~90% lower expressed in 1- and 2-month-old animals and 25 times stronger expressed in 8-month-old animals as compared to wild-type control (Figure 2C).

3.3 | Direct influence of A-beta on the expression of *ApoA1*

Amyloid precursor protein has been found to be present in the intestine of animals with the Thy1-driven transgenes APP and PS1[35] and A-beta could be identified in preparations of enteric plexus (Figure 1D). To investigate if A-beta alone is capable of evoking changes in gene expression of *CdklI* and *ApoA1*, primary cultures of murine enteric neurons from the myenteric plexus of wild-type animals were treated with synthetic A-beta$_{42}$ (for the typical mesh-like structure of these neurons formed in vitro see Figure 3A). The neuronal character of the cultured cells was verified via two neuronal markers, NeuN and β-III tubulin (Tuj1), using immunohistochemistry (Figure 3A) and Western blot (Figure S3). Enteric neurons were treated with either monomeric or oligomeric A-beta$_{42}$ or scrambled peptide as control (for demonstration of the inability of the scrambled, biological inactive peptide to aggregate see Figure S4). Neither monomeric nor oligomeric A-beta preparations influenced the expression of *ApoA1* or *CdklI* after 24 hours of incubation (Figure 3B). Since acute A-beta-treatment had no effect on mRNA levels, *CdklI* and *ApoA1* expression was compared between enteric neurons derived from 5xFAD animals and wild-type animals. Thereby, the analyzed enteric neurons were obtained from a situation with persistent exposure to the transgene. The expression of *CdklI* showed no change between groups. However, ApoA1 level was significantly increased in neurons from 5xFAD mice to ~187% of control (Figure 3C). Both results reflect the observations for gene expression in the 8-month-old animals.

To verify that the changes seen on *ApoA1* mRNA level are also present on protein level, Western blot experiments were conducted. ApoA1 protein level was analyzed in colon-homogenates in all three different age-groups. In 1-month-old 5xFAD mice, ApoA1 was reduced to ~40% of the protein level of wild-type animals. In 2-month-old 5xFAD animals protein level of ApoA1 was reduced to ~75% of wild-type animals while in 8-month-old animals protein level of ApoA1 was increased to ~142% (Figure 3D). This does not directly reflect effect sizes of mRNA levels, but direction of effect was consistent with our observations regarding mRNA. The main sources for ApoA1 production are intestine and liver. To ascertain that the observed elevation is not based on effects in liver and subsequent transport via blood stream, ApoA1 levels in liver of 1-month-old mice were assessed: no significant change could be observed when comparing transgenic and wild-type animals (furthest graph on the right and respective Western blot picture in Figure 3D).

The transcription factors FOXA2 and ARP-1 have been identified as majorly regulating ApoA1 expression.[43,44] We, therefore, assessed expression of both within colonic mRNA samples of 1-month-old and 8-month-old mice. While Arp-1 occurred unaltered when comparing wild-type and 5xFAD mice (Figure S5), FOXA2 was reduced to about 50% of wild-type level in young animals. Old 5xFAD animals revealed elevated mRNA level of about three times higher as their respective wild-type controls (Figure 3E); however, this did not reach statistical significance ($P = .09$). Since both, ApoA1 and FOXA2 are main regulators of lipid homeostasis[45,46] relevance of our findings for systemic lipid excretion was analyzed (Figure 3F). 5xFAD animals showed a decrease in fecal lipids of ~30% (4.35 mg/g feces) in the young cohort, while in the old cohort the lipids were increased by ~30% (5.33 mg/g feces) in comparison to wild-type controls (wild-type young: 6.13 mg/g feces and wild-type old: 4.23 mg/g feces). This was accompanied by an increase of total cholesterol in serum in the

young transgenic mice, while the older animals showed a decrease as compared to wild-type littermates (Figure 3G). An elevated amount of ApoA1 should in consequence also result in elevated amounts of HDL-cholesterol as ApoA1 is the main protein moiety of HDL: although the results did not reach statistical significance, a tendency of increased HDL-cholesterol and a decrease of LDL/VLDL-cholesterol could be observed in the old animals.

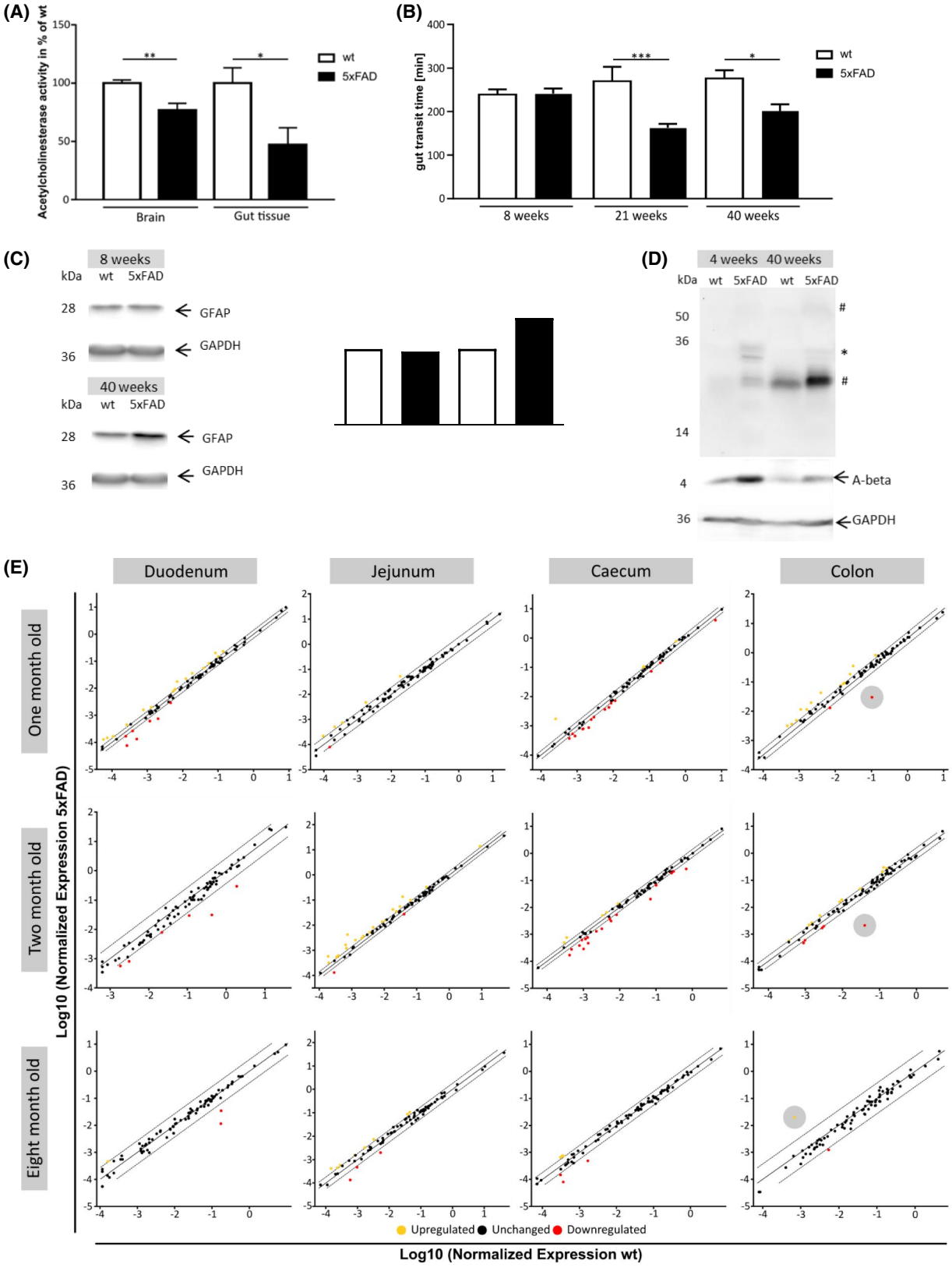

FIGURE 1 Neuronally expressed FAD transgenes (APP/PS1) induce major changes in the gut of Alzheimer's disease model mice. A, Activity of acetylcholinesterase was measured in tissue samples of whole brain and small intestine in 5xFAD and wild-type animals about 40 weeks of age. Values are presented as mean + SEM in % of values obtained from wild-type. Statistical analysis: Unpaired Student's *t* test (***P* < .01; **P* ≤ .05; n ≥ 5). B, Gut transit time (GTT) was monitored using an orally applied stain inert to resorption in animals at three different ages (8, 21, and 40 weeks; males and females evenly matched in every group). Values are presented in minutes (mean + SEM). Statistical analysis: One-Way ANOVA with Bonferroni's multiple comparisons test (***P* < .01; **P* ≤ .05; n ≥ 7). C, Analysis of GFAP expression in colonic tissue was conducted by Western blotting with homogenates from mice aged 8 or 40 weeks. Values have been normalized on GAPDH-dependent signal and are presented as mean + SEM. Statistical analysis: One-Way ANOVA with Tukey's multiple comparisons test (**P* ≤ .05; n = 7 for 8 weeks, n = 5 for 40 weeks). D, Detection of A-beta in crude myenteric plexus preparations was performed by Western blotting. An exemplary picture of A-beta- and GAPDH-signal from the respective samples is shown. In addition to specific signals (A-beta monomers and oligomeric forms, labeled by an asterisk) also unspecific signals were obtained (#), which might be based on the "mouse-on-mouse" incubation with antibody 6E10. E, Investigation of 84 Alzheimer's disease-relevant gene products in four different gut sections at three different ages was carried out with pooled RNA of 5xFAD or wild-type animals (three different male donors each). Dashed lines indicate borders set to identify upregulated (yellow), downregulated (red), and unchanged (black) gene expression. Borders were defined using the variation in expression of at least three housekeeping genes. The grey circle marks the most prominent differently expressed gene product, observed at all three stages of age (*ApoA1*)

In our initial screening (Figure 2), also Abca1 was identified as being differentially regulated in the transgenic animals at early stages in the gut as compared to wild-type controls. Abca1 acts as a receptor for ApoA1 and has also been shown to regulate its lipidation.[47,48] To proof, if the observed effects on lipid excretion solely are based on altered Apoa1 amount or if its receptor might contribute to it, we finally analyzed Abca1 expression in colon tissue as well. With 1 month of age Abca1 expression was not affected by the FAD transgenes; however, with 2 months of age a significant increase was observed (Figure 3H). In the oldest mice investigated, expression levels returned to wild-type levels.

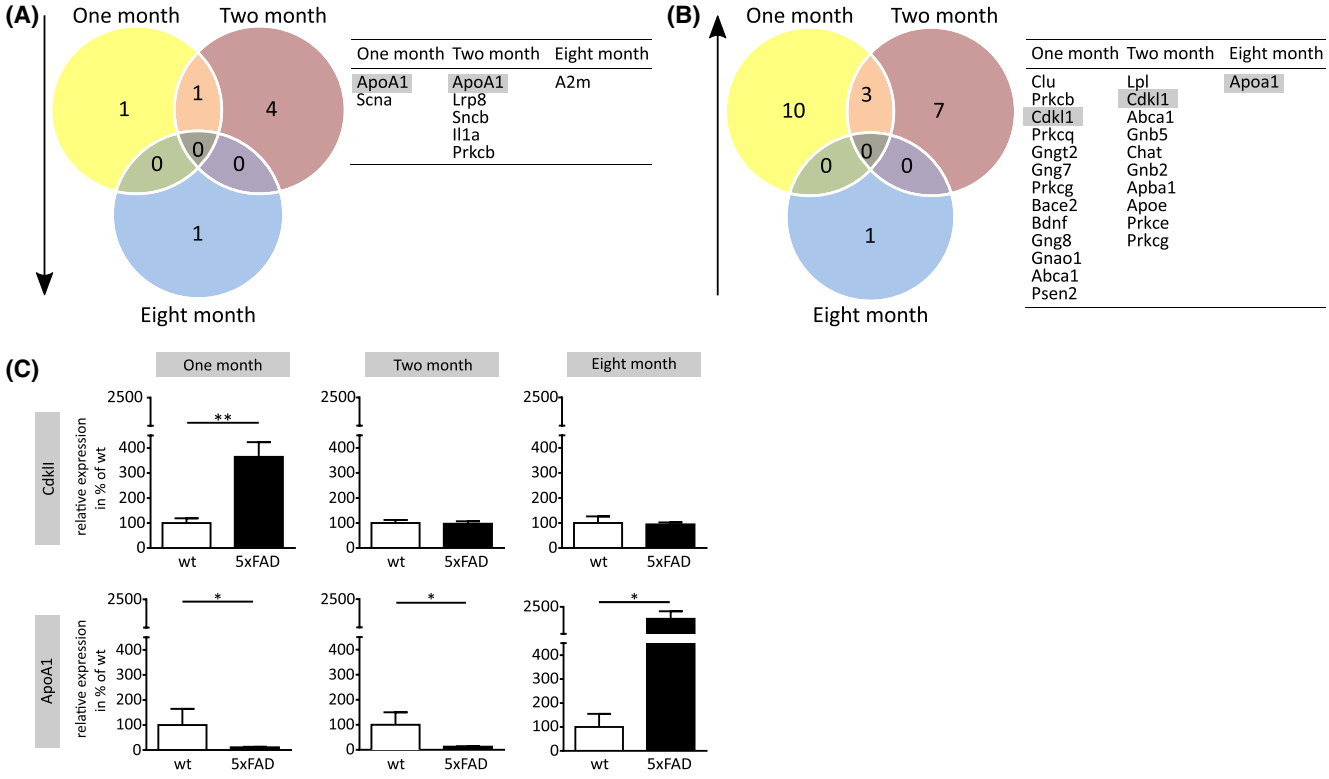

FIGURE 2 Colon shows an altered mRNA expression of AD-relevant genes. A and B, Up- and downregulated genes of animals were analyzed in three different age groups in colonic tissue. Analysis of downregulated genes is shown in A, upregulated genes in B. *ApoA1* showed the strongest downregulation in 1- and 2-month-old animals and the most upregulation in 8-month-old animals. *CdklI* showed the strongest upregulation in 1- and 2-month-old animals; both are marked in grey. C, For improvement of statistical validity of the experiments, mRNA of additional animals was used for single qRT-PCR reactions. Values are normalized to three different housekeeping genes and are shown as mean + SEM in % of values obtained from wild-type animals (statistical analysis: Mann-Whitney test; ***P* ≤ .01; **P* ≤ .05; n ≥ 6 animals for 1 and 2 months; n ≥ 3 animals for 8 months)

3.4 | ApoA1 interacts with A-beta

To further illuminate the role of ApoA1 in AD-like pathology, the potential effect of ApoA1 on typical pathological processes was investigated. A-beta monomers have been shown to be neurotoxic.[49] They are the predominant amyloid species synthesized by neurons[50] and since between monomeric and oligomeric A-beta there was no difference observed in modulating *ApoA1* expression, all further experiments were conducted with monomeric A-beta. To demonstrate that ApoA1

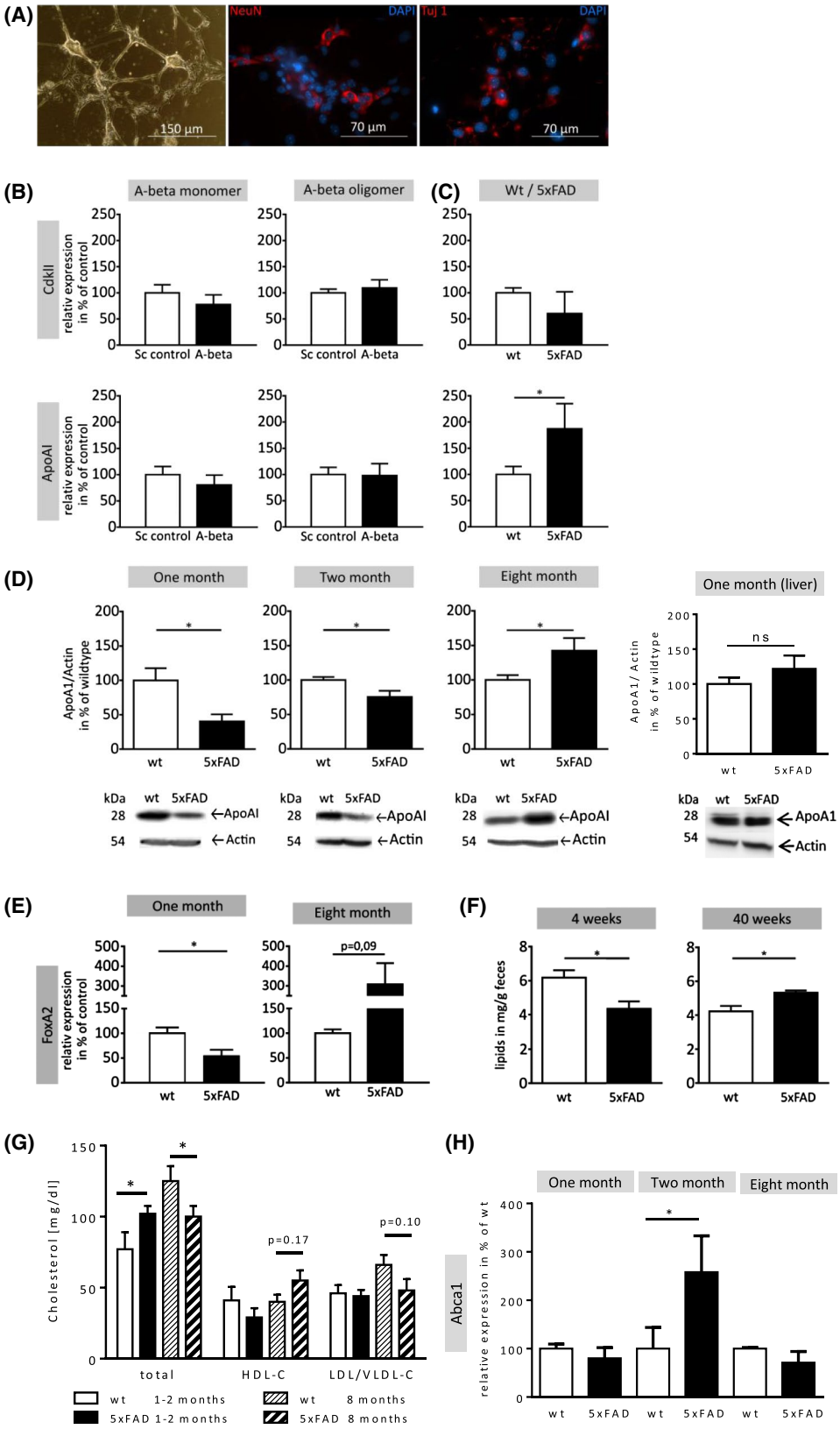

FIGURE 3 ApoA1 expression in enteric neurons is modulated due to chronic overexpression. A, Phase contrast image of cultured enteric neurons grown in typical mesh-like structure. Immunostaining of enteric neurons with neurospecificity markers NeuN and β-III tubulin (Tuj1) in red and DAPI as nuclei counterstain in blue, was carried out. B, Expression of *CdkII* and *ApoA1* was investigated in cultured enteric neurons of wild-type animals. Cells were treated with 2.5 μM monomeric or oligomeric A-beta or scrambled peptide (Sc control) for 24 hours and expression was compared between groups using qRT-PCR. C, In addition, gene expression was assessed by analyzing *CdkII* and *ApoA1* in enteric neurons of 5xFAD as compared to wild-type animals. Values are presented as mean + SD in % of values obtained from control. Statistical analysis: Unpaired Student's *t* test (*$P \leq .05$; n ≥ 4 different donor animals). D, ApoA1 protein amount was evaluated using densitometric analysis of Western blot images. Values obtained for ApoA1 were normalized to values obtained for Actin. Statistical analysis: Unpaired Student's *t* test (*$P \leq .05$; n ≥ 4 donor animals per group). E, Gene expression of *FoxA2* was investigated in colonic tissue of 5xFAD and wild-type mice. Values are normalized to the housekeeping gene *Actin* and are shown as mean + SEM in % of values obtained from wild-type animals (statistical analysis: Unpaired Student's *t* test; *$P \leq .05$; n = 4-8 for old and n = 5 for young animals per group). F, Lipid content in feces was measured for 5xFAD mice and their respective littermates and obtained values were normalized to total weight of feces. Statistical analysis: Unpaired Student's *t* test (*$P \leq .05$; n ≥ 7 for young and n = 4 for old animals per group). G, Total cholesterol, HDL- and LDL/VLDL-cholesterol were determined by a fluorometric assay using serum samples. Statistical analysis was performed by One-Way ANOVA with Fisher LSD post-test (*$P \leq .05$; n = 4-5 per group). H, *Abca1* mRNA level was determined in colon-derived RNA and normalized to *Actin* mRNA levels. Mean + SEM are shown in % of results for wild-type animals (statistical analysis: One-Way ANOVA with Sidak's multiple comparison test; *$P \leq .05$; n = 4-5 per group)

possesses the property to bind A-beta peptides and modulate A-beta aggregation, ApoA1 and A-beta were co-incubated and aggregation products analyzed on a native PAA-gel. At a molar ratio of 1:10, ApoA1 and A-beta formed high molecular weight aggregates, while incubation of A-beta or ApoA1 alone with the respective solvent only resulted in protein bands in the low molecular range (Figure 4A). Incubation with BSA did not result in formation of protein aggregates indicating specificity of the interaction between A-beta and ApoA1. This confirms earlier findings of Paula-Lima, et al.[31] who were able to show a formation of high protein aggregates if ApoA1 and A-beta were incubated at specific molecular ratio. To further investigate the influence of ApoA1 on A-beta aggregation, we conducted fluorescence-based aggregation assays by incubating A-beta and ApoA1 in the presence of Thioflavin T (Figure 4B). Incubation of A-beta with solvent control showed an increase of A-beta aggregation over 40 minutes as expected. Formation of A-beta aggregates could be inhibited by co-incubation with the aggregation inhibitor morin[51] while ApoA1 alone showed no increase of fluorescence signal over time. Incubation of ApoA1 and A-beta (molecular ratio of 1:10) resulted in an accelerated aggregation (Figure 4C) which also persisted up to 4 d after starting the co-incubation (Figure 4D).

3.5 | Enteric neurons derived from 5xFAD mice show changes in calcium homeostasis and cell viability, reversible by ApoA1

To investigate, if the effect of ApoA1 on aggregation influences the neurotoxic effect of A-beta, enteric neurons of wild-type mice were treated with either 2.5 μM A-beta, 0.25 μM ApoA1 or A-beta and ApoA1 together for 24 hours and cell viability was measured (Figure 5A). Treatment of enteric neurons with A-beta alone resulted in a decrease of cell viability of ~13%. Incubation of cells with ApoA1 showed no influence on cell viability, while co-incubation of enteric neurons with ApoA1 and A-beta lead to an elimination of A-beta-induced neurotoxic effects. To evaluate if the beneficial effect of ApoA1 was still existent in enteric neurons exposed chronically to the transgene, enteric neurons prepared from 5xFAD animals were treated with ApoA1. These neurons showed a ~19% higher viability than solvent-treated cells (Figure 5B), demonstrating that ApoA1's beneficial effect on cell viability is not restricted to acute A-beta exposure. Similar experiments conducted in the neuroblastoma cell line SH-SY5Y revealed that viability was comparably restored, when cells were incubated with A-beta in the presence of ApoA1 (A-beta/ApoA1-treated cells: 101% of control, for comparison of A-beta *vs* A-beta/ApoA1-treated cells: $P = .0002$, data not shown).

A-beta and Presenilin are known to modulate calcium-homeostasis and -signaling of neuronal cells.[3,52-54] For that reason, KCl-induced calcium influx was measured in enteric neurons of 5xFAD animals and their respective wild-type littermates (for an exemplary picture see Figure S6). Enteric neurons of transgenic animals showed a much stronger fluorescent signal increase than their wild-type littermates confirming hyper-activity of calcium signaling (Figure 5C). Incubation of these hyperactive enteric neurons with ApoA1 resulted in a ~28% decrease as compared to the solvent treated control (Figure 5D), which indicates that ApoA1 can at least partially restore calcium homeostasis.

4 | DISCUSSION

Although AD is mainly still seen as a neuronal disease of the brain, the gut gains more and more attention in this field of research. In this study we investigated the gut with its enteric nervous system regarding its role in AD. We were able to show a decrease in acetylcholinesterase activity in whole brain homogenates of 5xFAD mice, which is also observed

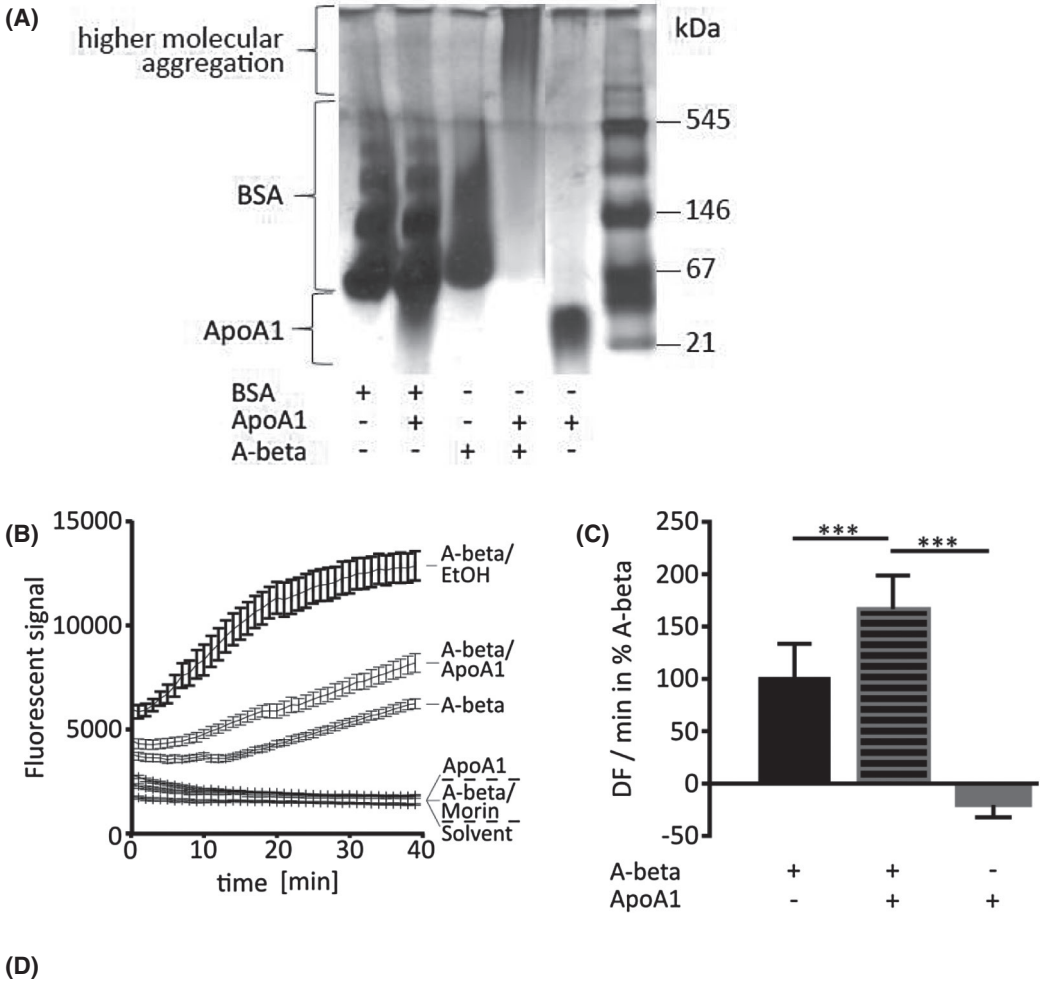

FIGURE 4 Interaction of ApoA1 and A-beta in vitro. A, ApoA1 was incubated with either BSA, human A-beta, or solvent for 24 hours at 37°C and loaded on a native PAA-gel. A couple of protein bands visualized at the same height as the BSA bands occurs in the lane where only A-beta has been subjected to. As these bands also are present in lanes where scrambled A-beta, which is not prone to aggregation, is run (data not shown), we assume that this is not aggregated peptide but material due to the purification process of the vendor (AnaSpec). B, To investigate a potential effect on A-beta aggregation, Thioflavin T was added to the indicated combination. Morin—an aggregation inhibitor—served as control. The respective slope of the fluorescent signal was calculated (C). Values are presented as mean ± SD (values obtained from A-beta without additives set to 100%). The experiment was conducted three times independently. Statistical analysis: One-Way ANOVA with Bonferroni's multiple comparisons test (***$P < .001$; $n \geq 5$). D, For long-term effect of ApoA1 in the co-incubation experiment, single measurements were conducted at $t = 0$ and $t = 40$ minutes as well as after 1 and 4 days. In between the measurements, samples were incubated at 37°C under constant shaking. Values are presented as mean ± SD (n = 4). Statistical analysis: Two-Way ANOVA Dunnett's multiple comparisons test (only comparisons between A-beta and A-beta/ApoA1 are shown; ***$P < .001$; **$P < .01$)

in brain homogenates of AD patients.[36,55,56] More interestingly, we could also demonstrate the decrease of AChE in the small intestine of 5xFAD mice, which confirmed the impact of AD on the enteric nervous system since this enzyme is mandatory for the acetylcholine-mediated signal transmission in the ENS.[57-60] This reduction might be of value for AD diagnosis since intestine biopsies are conducted on a regular basis for cancer screening in the elderly and might offer the possibility to study AChE levels of the enteric nervous system in people at risk.[61] To investigate if there is impairment in gut function and if this disease-driven change may already be recognizable at an early stage in disease progression, we analyzed the gut transit time (GTT). This motility test is the most basic tool used to assess disorders of the gut[39,62] and has already been linked to psychiatric disorders such as anxiety and depression.[63] GTT was changed at an age of 21 weeks in 5xFAD animals: at that time point mice display cortex-dependent learning memory stabilization impairments but no restriction in short-term memory (starting at 6 months of age).[64] Forty-week-old mice with severe AD-like pathology also displayed reduced gut transit time, indicating that the gut function is changed during the progression of the disease in 5xFAD animals. The microbiome is responsible for the digestion of dietary fibers to mainly short chain fatty acids (SCFAs).[65] These SCFA have multiple effects and are able to modulate enteric neuron properties (eg, butyrate interacts with the cholinergic system of the gut) which results, for example, in gut motility enhancement.[66,67] It has been shown, that the gut microbiome composition of 5xFAD mice differs from that of wild-type littermates,[35,68] and therefore might play an important role in modifying gut motility. It should be noted that an altered gut transit time is of major importance for further investigations of fecal microbiota in 5xFAD animals, since faster transit time itself has an impact on microbiota composition of the feces.[69]

The effect of an altered gut transit time and the reduced AChE activity in the gut underline the interplay of the intestine and AD-like pathology. In this study we investigated the gut with its ENS regarding expression of 84 AD-related gene products in the 5xFAD mouse model. We found *ApoA1* as the most differentially expressed gene product over all investigated gut segments of this animal model. ApoA1 is a 28 kDa lipoprotein synthesized mainly by the liver and the intestine.[46,70] Literature is not fully consistent regarding the role ApoA1 might have in AD: several reports show a decreased level in serum and plasma of patients with AD,[71,72] or even years before onset of AD.[73] However, there are also reports describing unaltered levels in brain parenchyma or even lowered levels in serum.[74,75] In addition, functional impact is not clear: the level of plasma ApoA1 was, for example, not found to correlate with cognitive function at baseline or with risk of dementia during follow-up in a case-cohort study nested within the Ginkgo Evaluation of Memory Study[76]

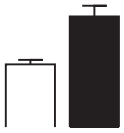

FIGURE 5 ApoA1 has an impact on cell viability of enteric neurons and plays a role in calcium signaling pathways. A, Enteric neurons of wild-type animals were treated with A-beta, ApoA1 or a combination of both. Scrambled A-beta served as a control. After 24 hours of incubation, cell viability was assessed. Values are shown as mean + SD in % values of Sc control. The experiment was conducted with five donor animals. Statistical analysis: One-Way ANOVA with Bonferroni's multiple comparisons test (***$P < .001$; **$P \leq .01$; $n \geq 12$). B, Enteric neurons prepared from 5xFAD mice were incubated with ApoA1 (0.25 μM, 24 hours) as described before and cell viability was assessed. Four independent experiments with four different donor mice were conducted. Statistical analysis: Unpaired Student's *t* test (***$P \leq .001$; $n \geq 19$). C, Calcium influx in wild-type and 5xFAD enteric neurons was measured by a calcium-dependent fluorescence dye and slope of the signal was calculated. Values are expressed as % values obtained for wild-type. The experiment was carried out five times with five donor animals. Statistical analysis: Unpaired Student's *t* test (***$P \leq .001$; $n \geq 21$). D, Enteric neurons of 5xFAD animals were treated with 0.25 μM ApoA1 for 24 hours. Calcium influx was measured and slope calculated. Values are shown as mean + SD in % of values of control obtained in three independent experiments. Statistical analysis: Unpaired Student's *t* test (*$P \leq .05$; $n \geq 16$)

while another earlier study concludes, patients with high concentration of ApoA1 in serum have a lower risk to suffer from AD later in life.[77] In addition, changes in ApoA1 CSF level might even report on general degenerative processes and not specifically on AD-related changes as this protein in CSF was—in our hands—not able to discriminate between a small panel of non-AD and AD-demented individuals.[78]

Here, we were able to demonstrate lower expression of *ApoA1* and reduced protein content in colon tissue of 5xFAD at a pre- and early pathological state while liver was not affected. Investigations in APP/PS1Δ9 mouse model for AD showed that a deficiency in *ApoA1* increases cognitive deficits due to higher A-beta levels in the vascular system,[79] whereas overexpression of *ApoA1* was able to preserve

cognitive function[80] in APP/PS1 mice. Additionally, it has been demonstrated in mouse models of AD that intravenous treatment with ApoA1 reduced cerebral deposition of A-beta.[81] Recently, an investigation of ApoA1 deficiency in APP/PS1 AD model mice revealed increased total and vascular A-beta deposition as well as astrocytosis in comparison to hemizygous controls.[82] This suggests that ApoA1-containing HDL can reduce amyloid-driven pathology and astrocyte reactivity. This effect might be due to ApoA1s ability to bind A-beta and accelerate the formation of higher molecular aggregates which are not neurotoxic.[83,84] While earlier reports described an inhibition of A-beta$_{40}$ aggregation by ApoA1,[85] Paula-Lima and colleagues showed that a different effect occurs in dependency from stoichiometric ratios[31]: at low molar ratio of ApoA1 (20:1 A-beta: ApoA1) ThT-evoked signal was reduced in an *in vitro* aggregation assay. However, at higher ratio (2:1 A-beta: ApoA1 molar ratio) shorter, curvilinear and thinner structures were built and ThT binding was increased. In our experiment, where we used a 10:1 ratio, ApoA1 likewise increased the ThT signal in comparison to samples only containing A-beta and this remained stable for 4 days. Consistent with this finding, ApoA1 used in the same molar ratio was able to neutralize A-beta–induced neurotoxic effects within enteric neuron primary cultures. Moreover, ApoA1 seems to reverse already existing neurotoxic effects induced in enteric neurons of 5xFAD animals.

Interestingly, we found the inverse effect of ApoA1 protein content and gene expression at the late pathological state of 5xFAD animals. The 5xFAD animal model is a rapid model with fast development of pathology. Eight-month-old animals display a severe pathological state with neuronal loss, cognitive impairment, and inflammation which are not observed in 2-month- or 1-month-old animals.[28,86] Therefore, levels of ApoA1 might be increased as part of an inflammatory response since ApoA1 possesses anti-inflammatory properties[87-92] which would also fit our observation of elevated GFAP in the colon of aged transgenic animals. Additionally, this elevation may contribute to neuronal regeneration which is continuously taking place in the enteric nervous system.[93] Like this late phase of AD, the secondary phase of traumatic CNS injury also is characterized by neuronal loss and inflammation and has even been associated with AD.[94-98] Sengupta et al[99] were able to show an increase of ApoA1 in serum of patients in the secondary phase of traumatic spinal cord injury. Moreover, a similar effect has been observed in scratch-wounded neuroblastoma cells: while ApoA1 decreased immediately after the injury, later-on it increased, which was interpreted as a self-protecting mechanism of the injured system.[99] While the neuronal cell line showed this behavior due to acute injury within hours, it is plausible that in the more complex mouse model such regulations occur in a slower time frame over months. An increased ApoA1 expression was also observed in cultured enteric neurons of 5xFAD animals when compared to wild-type animals. The fact that ApoA1 expression is not altered in enteric neurons exposed 24 hours to the recombinant peptide, but in neurons of transgenic animals, indicates that A-beta is not acting on transcription of ApoA1 directly. However, *FoxA2* was shown to mirror the expression level of *ApoA1* in 5xFAD mice: in young mice of 1 month of age it was decreased, while it increased in aged mice. FOXA2 is a major regulator of glucose and lipid metabolism.[100] Interestingly, it also has been described to be elevated in iPSC-based AD cellular models.[44] In midbrain, FOXA2 mediates important function in development and maintenance of murine dopaminergic neurons.[101,102] As dopaminergic neurons have been shown to strongly affect gut transit time,[103] disturbed FOXA2 homeostasis at later age in 5xFAD mice might also be the underlying mechanism for the here observed increased propulsion. For cholinergic transmission a direct link between FOXA2 and neuronal activity in the gut has not been described yet. However, FOXA2 has been identified as a regulator of α5 nicotinic acetylcholine receptor subunit in murine lung organogenesis[104] and this subunit has been shown to stabilize functional nicotinic receptors in at least non-neuronal tissues.[105] While α5 subunit was demonstrated in colonic epithelium of mice and its absence found to increase severity of experimental colitis in knock-out mice,[106] no data regarding enteric neurons are provided so far. Therefore, we cannot undoubtedly conclude that the elevated expression of *FoxA2* directly resulted in the observed faster gut transition via higher activity level of cholinergic neurons.

Nevertheless, faster transit time can directly explain a lower resorption and thereby higher fecal excretion of lipids.[107] This is exactly what we found in aged model mice and which might *vice versa* act on *FoxA2/ApoA1* expression. How the FAD transgenes start this vicious cycle was not resolved so far, however, it is interesting that decreased level of dopaminergic neurotransmitters and dopamine receptors in the CNS have been associated with AD progression.[108] If this is also the case for enteric neurons in AD model mice and patients has to be elucidated in the future. Besides *Apoa1*, also *Abca1* was found to be differentially regulated at 2 months of age in colonic tissue from the 5xFAD mouse model. Its pronounced increase might directly be the consequence of ApoA1 reduction observed at early age as ApoA1 deficiency leads to disturbed cholesterol efflux besides deterioration of other important cellular functions[109]: excess cholesterol accumulation has been shown to induce *Abca1* expression via LXR.[110,111]

How A-beta mediates neurotoxicity is still discussed: it is assumed that amyloid proteins form ion channel pores which disrupt the cellular ion homeostasis.[112-115] Especially calcium permeable channels are formed in membranes resulting in an increase in intracellular calcium concentration.[115-117] This increase in calcium uptake was

also observed in enteric neurons of 5xFAD animals, possibly explaining the neurotoxic effect mediated by A-beta. ApoA1 was significantly reducing calcium uptake of enteric neurons which might explain its neuroprotective effect. Additionally, ApoA1 has been found to be a ligand of the triggering receptor expressed on myeloid cells 2 (TREM2),[118] which loss leads to exacerbated functional decline of neurons in 5xFAD animals.[119]

In summary, we here widen the view on AD as a systemic disease where first molecular changes might not be found in brain but in peripheral tissue such as the gut. While dysfunction of the intestine in PD is a common comorbidity, only within the last years reports on AD mouse models but also patients describe changes in the tissue itself and also in the commensal microbial community. Why these occur is still enigmatic. With our finding that changing ApoA1 levels accompany disease development and thereby evoke changes in enteral lipid excretion, we might contribute to further understanding of this phenomenon. In human brain, evidence for significant ApoA1 mRNA levels is described not to be found and it is assumed that ApoA1 may enter CSF via the choroid plexus.[120-122] Therefore, intestinal ApoA1 production might directly affect the brain. Future investigations have to decipher if our findings from the mouse model also hold true for human patients and if this might open up new avenues for diagnostics.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the contents of this article.

AUTHOR CONTRIBUTIONS

K. Endres initiated the study and conducted the HDL/LDL assay. K. Endres and N.M. Stoye designed the research. N.M. Stoye performed qPCR for AD-related genes, cell culture, and animal experiments. M. dos Santos Guilherme conducted FoxA2 and Arp-1 qPCR, lipid extraction, animal feeding experiments, GFAP and A-beta Western blots and edited the manuscript. K. Endres and N.M. Stoye wrote the manuscript. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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Supplementary Information for

Alzheimer's disease in the gut – major changes in the gut of 5xFAD model mice with ApoA1 as potential key player

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Supplementary Information

Suppl. Fig. 1

Specificity control for ApoA1 detection in intestinal, murine tissue.

For specificity control of the ApoA1 antibody, recombinant human ApoA1 protein (30 ng) and 30 µg protein from tissue lysates were loaded as indicated on the PAA-gel. Western blot was carried out as described before. After blocking the membrane in I-block solution (0.2% in PBS), primary antibody incubation took place overnight at 4°C with antibody against ApoA1 (Thermo Fisher Scientific) and Actin (Sigma-Aldrich). Recombinant ApoAI, colon of wildtype animals and colon of 5xFAD animals showed a signal at 28 kDa specific for ApoA1; additionally a nonspecific signal was observed in colon tissue. Colon derived from both genotypes also showed a signal for Actin, which was not present in the recombinant protein sample.

Suppl. Fig. 2

Categorization of data quality.

To receive most reliable values, the data of the initial qPCR analyses were filtered for multiple criteria. Expression data were only included when showing Ct values below 35 cycles, and no multiple melting points in the product specific melting curve (multi Tm). At least 88% of all gene products in the four investigated gut segments fulfilled these criteria and could be included in further analysis (in total a minimum of 74 genes).

Suppl. Fig. 3

Neuronal character of enteric neuron culture.

To verify the neuronal character of the primary culture Western blotting was performed. Therefore, enteric neurons were seeded on a 6 well plate and cells scraped after 4 days in culture. Cells were resuspended in LDS NuPAGE buffer and Western blot was carried out as described before. After blocking with I-block solution (0.2% in PBS) (Thermo Fisher Scientific), primary antibody incubation took place overnight at 4°C with antibody against β-III Tubulin and anti GAPDH (both 1:1000; Cell Signaling). Whole brain homogenates

served as positive control. Cell culture lysate and whole brain showed both, a signal for the neuronal marker β-III Tubulin and GAPDH as loading control.

Suppl. Fig. 4

Aggregation of A-beta and scrambled A-beta peptides

A) To make sure that the scrambled, biological inactive control peptide, which was used for interaction with ApoA1 in the *in vitro* approach and in cell culture, is not able to deposit in aggregates such as formed by A-beta, we conducted a ThT aggregation assay as described in the methods section for the AggreSure peptide (conc. of the peptide: 125 µM). A time frame of 10 min of RFU measured for both peptides (n=4, mean + SD) is shown (normalized to min 0). B) Additionally, 5 µg of the peptides without further incubation or after 24 h incubation at 37°C were separated by SDS-PAGE and used for detection by Western blotting with the antibody 6E10. For A-beta$_{42}$ initially, the monomeric form (m) was detected and after 24 h a major proportion was converted to oligomers (o). The scrambled peptide cannot be detected with the antibody as expected; however, Coomassie staining identified a peptide of similar size as the A-beta peptide (lower part of the picture).

Suppl. Fig. 5

Expression of ApoA1 regulatory protein-1 (*Arp-1*) in colon of wildtype and 5xFAD mice

To investigate if *Arp-1* expression was altered in one- and eight-month-old male 5xFAD mice as compared to their respective littermates, animals were sacrificed and colonic tissue was collected and stored as previously described. The qRT-PCR experiment was conducted using the "QuantiTect® SYBR® Green RT-PCR Kit" (Qiagen) following the manufacturer's instructions using 100 ng RNA per reaction. Values were normalized to Actin as a housekeeping gene and are shown as mean + SEM in % of values obtained from wildtype animals (statistical analysis: Unpaired Student's t-test; n≥5 animals per group). No differences in expression of *Arp-1* in one- or eight-month-old animals were observed in 5xFAD animals as compared to their wildtype littermates (for one-month-old: p=0.91; for eight-month-old: p=0.64).

Suppl. Fig. 6

Calcium-induced fluorescence in enteric neurons

After four days of cultivation, enteric neurons were loaded with the fluorescence dye Calbryte™ 520-AM (AAT Bioquest 20651) as the vendor recommended. Briefly, 100 µl 20 mM HEPES buffered HBSS solution containing 10 µM reagent were added to the cell supernatant. Cells were incubated for 45 min at 37°C followed by 15 min incubation at RT in the dark. Subsequently, cell supernatant was exchanged by 100 µl buffer and KCl was added (50 mM), resulting in calcium influx. Calcium binds to Calbryte™ 520AM and thereby evokes a strong fluorescence signal. Exemplary pictures of enteric neurons are shown (taken by ZOE Fluorescent Cell Imager, BioRad) with the same patch of cells presented before and after adding KCl.

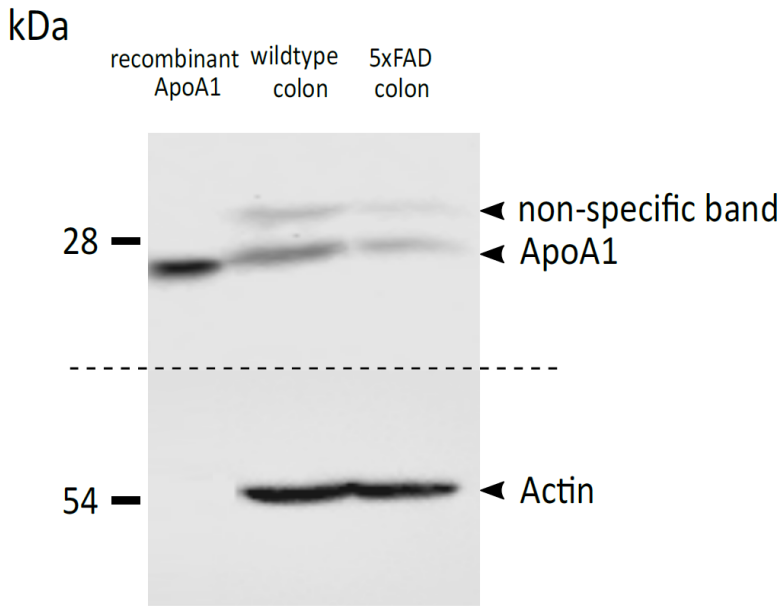
Suppl. Table 1: Initial analysis of the most common AD-linked genes in gut tissue

Values are shown as fold regulation in comparison to wildtype; grey marked values indicate potential hits. Their fold regulation effect is greater than the mean variation of the corresponding housekeeping genes and therefore these genes were included in further analysis. Grey marked positive values show a higher expression in the transgenic mice and negative values a lower expression in the transgenic mice.

Age	Gut segment	APP	Bace1	Bace2	Mapt	Psen1	Psen2
One month	Duodenum	-1.1987	-1.1511	1.8296	1.2277	-1.1198	1.1752
	Jejunum	1.1193	1.3100	1.6981	1.3482	1.0363	1.6075
	Caecum	1.0928	-1.0211	1.2023	1.0558	1.0949	1.0977
	Colon	1.0571	1.1790	1.9991	1.4015	-1.0328	1.6293
Two month	Duodenum	-1.1106	-1.0313	-13.7550	-2.0137	1.1462	-1.7354
	Jejunum	1.2179	1.5753	1.1274	1.2350	1.0586	1.0370
	Caecum	-1.0298	-1.2820	-3.5344	-1.8880	-1.1278	1.0436

	Colon	1.7655	1.4820	1.5374	-1.2171	1.4721	1.2736
Eight month	Duodenum	-1.0373	1.6764	1.0063	1.2417	1.1214	1.0724
	Jejunum	-1.1192	1.0683	1.7767	1.2034	1.1340	1.1632
	Caecum	1.0588	1.2213	-1.0428	-1.0475	1.0594	-1.1258
	Colon	1.0889	1.5775	-1.1539	-1.3170	1.1396	1.0498

Figure S1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recombinant ApoA1
wildtype colon
5xFAD colon
28
54
non-specific band
ApoA1
Actin

Figure S2

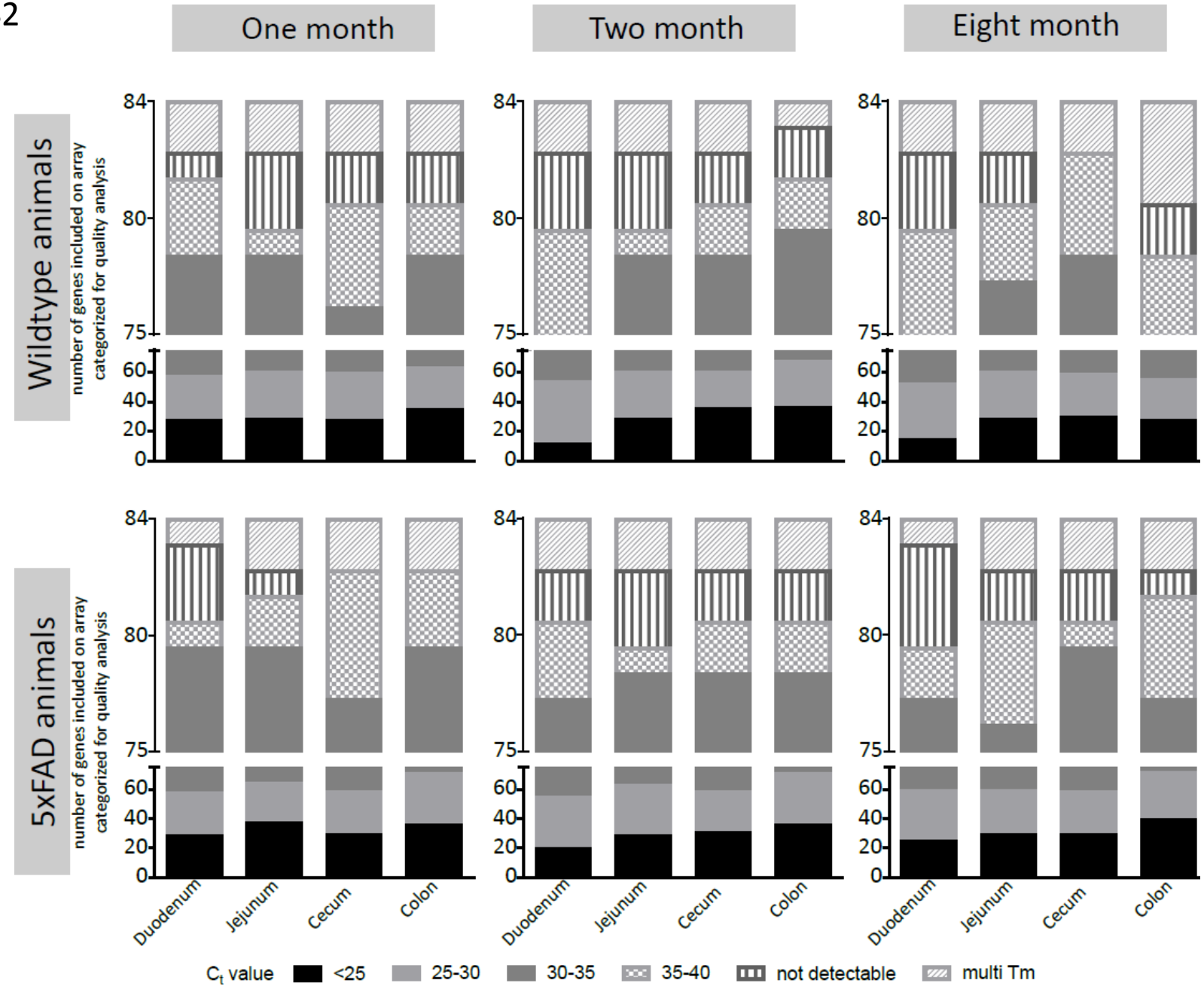

One month
Two month
Eight month
Wildtype animals
5xFAD animals
number of genes included on array categorized for quality analysis
84
80
75
60
40
20
0
Duodenum
Jejunum
Cecum
Colon
Ct value
<25
25-30
30-35
35-40
not detectable
multi Tm

Figure S3

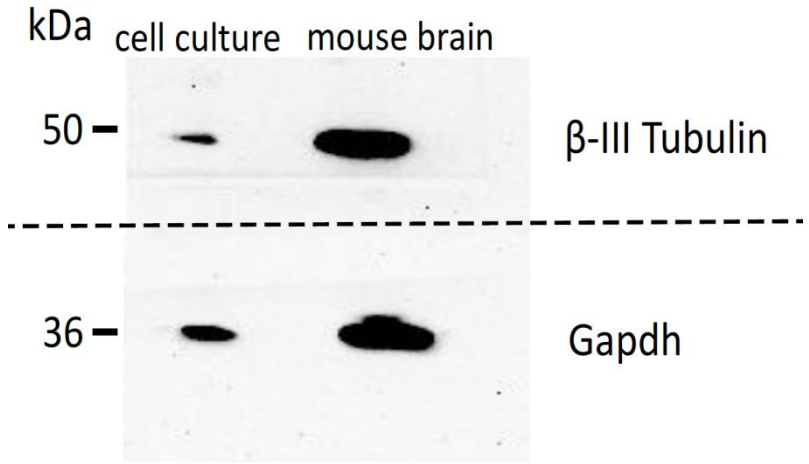
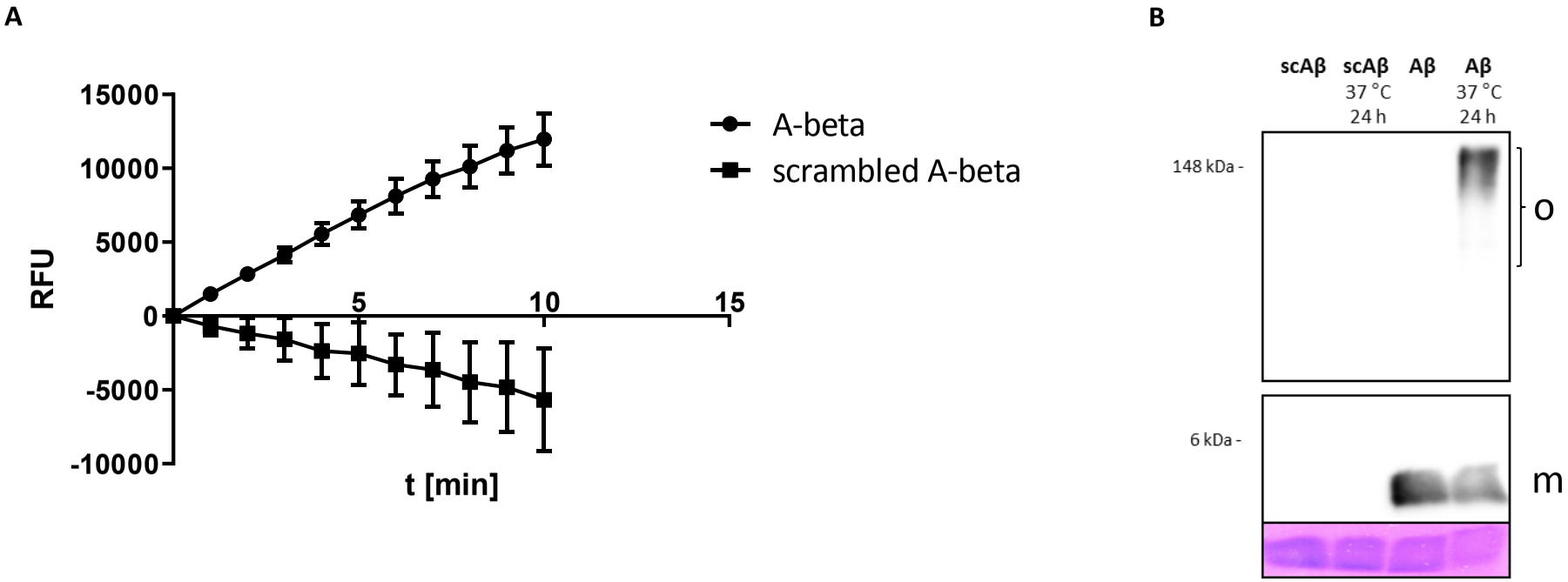


Figure S4


A
15000
10000
5000
0
-5000
-10000
RFU
5
10
15
t [min]
A-beta
scrambled A-beta
B
scAβ
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37 °C
24 h
Aβ
Aβ
37 °C
24 h
148 kDa -
6 kDa -
o
m

Figure S5

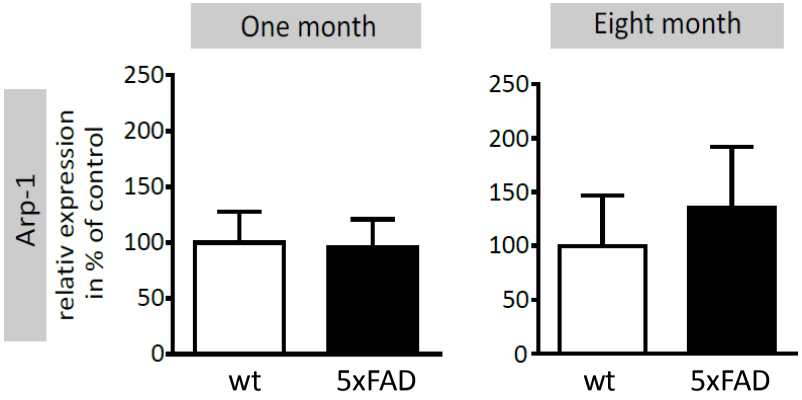
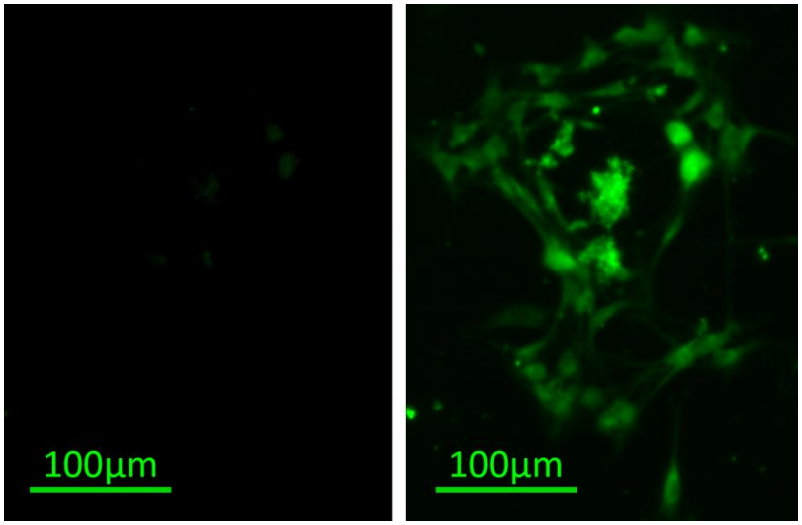

One month
Eight month
Arp-1
relativ expression
in % of control
250
200
150
100
50
0
wt
5xFAD
250
200
150
100
50
0
wt
5xFAD

Figure S6



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ORIGINAL RESEARCH
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Impact of Acute and Chronic Amyloid-β Peptide Exposure on Gut Microbial Commensals in the Mouse

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Alzheimer's disease (AD) is the most common form of dementia. Besides its cognitive phenotype, AD leads to crucial changes in gut microbiome composition in model mice and in patients, but the reported data are still highly inconsistent. Therefore, we investigated chronic effects of AD-characteristic neurotoxic amyloid-β (Aβ) peptides as provided by transgenic overexpression (5xFAD mouse model) and acute effects due to oral application of Aβ on gut microbes. Astonishingly, one-time feeding of wild type mice with $Aβ_{42}$ provoked immediate changes in gut microbiome composition (β diversity) as compared to controls. Such obvious changes were not observed when comparing 5xFAD mice with wild type littermates. However, acute as well as chronic exposure to Aβ significantly affected the abundance of numerous individual operational taxonomic units. This provides first evidence that acute *in vivo* exposure to Aβ results in a shift in the enteric microbiome. Furthermore, we suggest that chronic exposure to Aβ might trigger an adaptive response of gut microbiota which could thereby result in dysbiosis in model mice but also in human patients.

Keywords: Alzheimer's disease, microbiome, anti-microbial, Amyloid-β peptide, mouse model, 5xFAD

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INTRODUCTION

Improvements in our healthcare system have led to a global increase in the percentage of elderly people. Currently, nine percent of the population in the world is aged 65 or older; in 2050 this number will increase approximately to 16 percent and the older people will outnumber adolescents aged 15 to 24 years (United Nations Department of Economic and Social Affairs, 2019). Currently, 50 million people suffer from dementia (Alzheimer Disease International, 2019). The most common form of these divergent disorders is Alzheimer's disease (AD) and age is the major risk factor for developing AD (Alzheimer Disease International, 2019). One of the characteristic hallmarks in the brain of AD patients are amyloid plaques derived from amyloid-β (Aβ) peptides – a cleavage product of the amyloid precursor protein (APP). APP can be processed in two different ways: (1) cleavage by α-secretase gives rise to non-amyloidogenic APPs-α and (2) cleavage by

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; CFU, colony forming units; Sc, Scrambled; 5xFAD, Alzheimer's disease mouse model; OTU, operational taxonomic unit.

β-and γ-secretase generates neurotoxic Aβ peptides (for detailed reviews see Rossner et al., 2006; Endres and Deller, 2017). Imbalances of this processing or changes in Aβ degradation lead to oligomerization of neurotoxic Aβ peptides which are presumed to be the cause of sporadic AD (Hardy and Higgins, 1992; Yang et al., 2003; Roberts et al., 2017; Lane et al., 2018). Besides this, formation of neurofibrillary tangles of hyperphosphorylated tau protein and a loss of synapses as well as neurons are characteristics of the disease (Whitehouse et al., 1982; Kowall and Kosik, 1987; Shin et al., 1989). In sum, these pathological hallmarks are responsible for the observed decline in cognitive and general abilities in AD patients and finally lead to death. So far, there is no preventive or curative therapy. Treatment with, e.g., Rivastigmine, which acts as an acetylcholinesterase inhibitor, can help to ameliorate disease progression, but cannot stop it (Farlow et al., 2005). The etiology of sporadic AD is not yet fully understood; therefore a more systemic view – including other organs than CNS and also their microbiome – could provide new therapeutic targets.

Only recently, the interplay between brain and gut has gained more attention for researchers regarding neurodegenerative diseases (Quigley, 2017). However, investigations in the field of AD are still rare (for a detailed review see Endres, 2019). Research in the field of, e.g., Parkinson's disease (PD), a dementia with α-synuclein deposition in analogy to Aβ deposits in AD, is far more advanced and already gave insights into relevant gut-brain-connections. For example, differences in microbial composition of the gut, such as reduced Bacteroidetes and increased Enterobacteriaceae, were found when comparing fecal samples from PD patients with samples of healthy controls (Unger et al., 2016). Simultaneously, about 80% of PD patients suffer from gastrointestinal dysfunction (Poewe, 2008; Cersosimo and Benarroch, 2012). It has also been shown that gut microbiota can directly influence disease progression in PD model mice by regulating motor deficits and neuroinflammation (Sampson et al., 2016). Furthermore, α-synuclein, the main driver of the disease which was also found in gut tissue, is discussed as a biomarker for pre-motor PD by using colon biopsies (Shannon et al., 2012). These investigations give profound basis for the assumption that a neurodegenerative disease might derive from or be influenced by gastrointestinal properties. The avenues which are used for this are not fully understood so far.

Brain and gut are connected *via* the so-called gut-brain-axis which consists of the enteric nervous system (ENS), the *Nervus vagus*, immune cells and the microbiome (Mayer, 2011). The *Nervus vagus* allows bidirectional communication *via* efferent and afferent fibers (Quan and Banks, 2007). The connection between brain and gut enforces speculation if the microbiota and their metabolites may influence AD progression or vice versa if AD pathogenesis impacts the microbiome.

Joachim et al. (1989) already showed Aβ deposition in the gut of individual patients. Also, antimicrobial properties of Aβ on single microbial strains have been demonstrated (Soscia et al., 2010). Furthermore, different studies concluded, that transgenic AD model mice as well as AD patients present differences in microbiota composition in comparison to healthy controls (Brandscheid et al., 2017; Cattaneo et al., 2017; Harach et al., 2017; Shen et al., 2017; Vogt et al., 2017; Bauerl et al., 2018). The investigations of the role of microbiota in AD are still highly inconsistent. Harach et al. (2017), for example, showed an increase of Firmicutes, but a decrease of Bacteroidetes, in fecal samples of 1 month old AD model mice (APPPS1) as compared to wild type controls. The opposite result could be found in the same mouse strain at an age of 8 months. In a study using fecal samples of 9 week old 5xFAD mice, elevated amount of Firmicutes and less Bacteroidetes were detected as compared to wild type controls (Brandscheid et al., 2017). The investigated mouse strains represent genetic models, which can be used for observatory studies of chronic exposition of gut tissue with neurotoxic Aβ peptides especially because the peptide itself has also been detected in the gut (Brandscheid et al., 2017). However, acute effects have not been investigated so far *in vivo* and compared to the prolonged exposure.

The aim of our study was to investigate chronic as well as acute effects of Aβ exposition in the gut. 5xFAD mice were used as a model for AD, which express mutated human APP and PSEN1 transgenes with a total of five mutations and show Aβ deposition in the brain and gut tissue already at an age of 4 weeks (Oakley et al., 2006; Brandscheid et al., 2017). We analyzed gut bacteria of C57Bl/6J wild type mice in comparison to 5xFAD transgenic littermates by 16S-rDNA sequencing. Additionally, acute effects of Aβ were investigated *in vivo* after oral administration of Aβ peptides to wild type mice. As expected, differences in the gut microbiome could be observed by comparing 5xFAD mice with wild type controls but interestingly, also acute exposition of gut tissue for just 5 h with Aβ peptides (one complete gut passage) led to substantial differences in microbiota as compared to animals treated with a scrambled, non-active peptide.

MATERIALS AND METHODS

Peptides

For the incubation of feces bacteria and oral application to the mice, human $A\beta_{1-42}$ or scrambled, biologically inactive peptide (both AnaSpec, Seraing, Belgium) in 5 mM NH_4OH buffered in PBS was used. TAMRA-labeled peptide (AnaSpec, Seraing, Belgium) was used for detecting gut transition of Aβ.

Quantitation of Colony Forming Units and Bacterial Viability Assay

Anaerobic Cultivatable Community

Freshly collected fecal pellets were suspended in isotonic sodium chloride solution (0.9%, 100 μl/mg) using a hand-held electric stirrer (Xenox, Fähren, Germany). Aliquots of 50 μl were supplemented with 5 μl of peptide solution in 0.4 mM NH_4OH buffered in PBS. After 10 min incubation at room temperature under careful mixing for every 2 min, 20 μl of this suspension were added to 4 ml of thioglycollate bouillon (Becton Dickinson GmbH, Heidelberg, Germany) and kept for overnight at 4°C. The following day, 1 μl of the diluted fecal suspension was spread on Schaedler agar plates (Becton Dickinson GmbH, Heidelberg, Germany) and incubated for 48 h anaerobically (Anoxomat, Mart Microbiology B.V, Drachten, Netherlands). Lastly, colony

forming units (CFU) were counted and normalized to controls from the same donor mouse incubated with scrambled peptide.

Family-Specific Cultivation

For assessing toxic effects of Aβ on specific microbial families, freshly collected feces was suspended and incubated with 2 μM peptide solution as described above. Afterward, feces suspensions were diluted with 0.9% sodium chloride solution for plating 1 ml on 3M^{TM}-Petrifilm plates specific for Enterobacteriaceae and Lactobacillaceae (3M Deutschland GmbH, Neuss, Germany). Plates were incubated for 20 h at 37°C. CFU were counted and normalized to samples from the same donor mouse incubated with scrambled peptide.

Viability Assay

After incubation of diluted feces samples with Aβ peptide or scrambled control for plating (see above), additional 1:100 dilutions were prepared and viability measured by using the BacTiter Glo assay as indicated by the vendor (Promega, Mannheim, Germany). Relative luminescence was measured and normalized to controls from the same donor mouse incubated with scrambled peptide.

Animals

B6SJL-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax (5xFAD) mice (Jackson Lab, Bar Harbor, Maine, United States) were maintained by crossbreeding with C57BL/6J background as described in Reinhardt et al. (2018). Animals aged 9–10 weeks were used and non-transgenic offspring served as control and for acute treatment with peptides. All animals were group-housed with 3–5 animals per cage in a 12 h light/dark cycle with food (Ssniff Spezialdiäten GmbH, Soest, Germany) and water available *ad libitum*. At least one week before sampling of feces or chyme, animals were single-caged to avoid microbial transfer, e.g., by coprophagy. All procedures were performed in accordance with the European Communities Council Directive regarding care and use of animals for experimental procedures and were approved by local authorities (Landesuntersuchungsamt Rheinland-Pfalz; approval number G17-1-035).

Oral Peptide Application

Animals were lightly anesthetized with Isoflurane and 10 μg of peptide or solvent (10 mM NH_4OH) administered slowly into the oral cavity. Mice were kept in manual fixation up to ingestion of the fluid to prevent aspiration.

Collection of Chyme

Animals were deeply anesthetized with isoflurane and sacrificed by decapitation. For microbiome determination, feces pellets close to the rectum were taken. For assessing fluorescence after feeding of TAMRA-labeled Aβ, the whole gut was placed in ice cold isotonic sodium chloride to prevent further peristalsis movement. Segments of 2 cm length were then chopped starting from the stomach, the content collected by longitudinal opening and stored on ice until further use.

Western Blot and Fluorescence Detection

To measure the stability of TAMRA-labeled Aβ in the gastrointestinal milieu, chyme samples were collected as described before and diluted with 500 μl ice cold PBS. TAMRA-labeled Aβ (0.025 μM or 0.005 μM) was added to chyme or PBS as control and incubated for 2 h at 37°C with gently mixing every 20 min. Afterward, LDS NuPAGE buffer (1×, Life Technologies, Carlsbad, California, United States) and DTT (1 M, 10% v/v, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) were added and the solution was heated for 10 min at 70°C. Proteins were separated on a 12%-SDS polyacrylamide gel and subsequently transferred to a nitrocellulose membrane (GE Healthcare, Chalfont, Great Britain). The membrane was blocked with I-block solution (0.2% in PBS/T, Thermo Fisher Scientific) for 1 h before incubation with primary antibody in a dilution of 1:1,000 overnight at 4°C. On the next day, the membrane was washed with PBS/T and incubated with the secondary antibody coupled with horse reddish peroxidase (Thermo Scientific, Chalfont, Great Britain). Chemiluminescent signals were detected after application of SuperSignal West Femto chemiluminescent substrate (Thermo Scientific, Chalfont, Great Britain) by using a CCD-camera (Stella 3200 Camera, Raytest, Straubenhardt, Germany). Fluorescence signal was detected by using the Storm Scanner 860 (Molecular Dynamics, Caesarea, Israel).

Assessment of Gut Transition Time of Peptides

After oral application of TAMRA-labeled Aβ or NH_4OH as control and sacrifice of the animals 2, 4, or 24 h later, chyme samples were diluted with 500 μl ice cold PBS, incubated for 5 min and centrifuged. Fluorescence was measured by using 50 μl of the supernatant in duplicates (Exc 540 nm and Em 580 nm, FLUOstar Optima, BMG Labtech GmbH, Ortenberg, Germany) at intervals of 1 min over a period of 1 h at 37°C with shaking before every measurement. The mean of the values of the measurements between 15 and 45 min was used to calculate the difference between the TAMRA-labeled Aβ-treated animals and solvent-treated control animals.

DNA Extraction From Fecal Samples

Fecal samples were stored at −80°C before DNA extraction was done by using the QIAmp Fast DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) as recommended by the vendor.

Sequencing and Taxonomic Assignment of 16S rDNA Amplicons

Library preparation and Illumina sequencing was performed by StarSEQ GmbH (Mainz, Germany). For amplicon generation, the V3-V4 16S metagenomics sequencing system (Illumina) was used. Amplicons were paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform. De-multiplexed sequences were loaded into QIIME2 version 2018.6 (Caporaso et al., 2010) and de-noising was done using the DADA2 algorithm (Callahan et al., 2016). Due to a low quality of the reverse reads, neither read merging within QIIME nor external read merging with BBMerge

from the BBMap suite[1] retained a sufficient amount of reads, so we decided to exclude the reverse reads from downstream analysis. Forward reads were truncated to 255 bp after quality control. A naïve Bayes classifier was trained on Greengenes database 13_8 containing the V3–V4 region only with QIIME's internal implementation of scikit-learn. Taxonomic assignment was then done on 97% sequence identity. A multiple sequence alignment was done with MAFFT and a phylogenetic tree was calculated using the q2-phylogeny plugin.

Microbial Composition of Fecal Samples

We employed the *phyloseq* R package for downstream analysis of 16S data. Separate statistical comparisons were always performed between wild type vs 5xFAD and scrambled Aβ vs Aβ-fed animals, respectively. Alpha diversity was estimated using the Chao and Shannon index. In order to account for different library sizes, each sample was normalized to the smallest library size by random sampling with replacement. This process was repeated a 1,000 times and the average values from all runs were considered as final estimates for alpha diversity. Groups were compared statistically with an unpaired *t*-test. Differences in beta diversity were analyzed using canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003) as implemented in the *vegan* R package. Ordinations based on both the Bray–Curtis dissimilarity as well as the binary Jaccard distance were performed to investigate quantitative and qualitative differences in beta diversity. Community composition at the phylum and family level was compared based on relative abundance. Subsequently, we performed differential abundance analysis by estimating log2 fold changes (FC) of bacterial abundance with the DESeq2 package (Love et al., 2014). This method was chosen because DESeq2 was previously shown to offer increased sensitivity to detect differentially abundant operational taxonomic units (OTUs) while keeping false discovery rates below 0.05 with small sample sizes (Weiss et al., 2017). Briefly, absolute bacterial abundances were transformed to normalized counts using the median-of-ratios method implemented in DESeq2. FC of bacterial abundance were calculated using generalized linear models assuming a negative binomial distribution. The relationship between the mean and dispersion parameters was estimated using local regression. The significance of FC was evaluated with Wald *z* tests. Differences were considered statistically significant if the adjusted *p*-value of the respective FC was below 0.05. *p*-values are two-tailed and adjustment for multiple comparisons was performed by controlling the false discovery rate with the help of the Benjamini–Hochberg method.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 6 for Windows or R version 3.5. Data are graphically represented as mean + standard error of the mean (SEM) (**Figure 1**) or with boxplots and scatter plots showing individual values (**Figure 3**). Statistical analysis was performed with an unpaired two-tailed Student's *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$) if not stated otherwise.

[1]https://sourceforge.net/projects/bbmap/

RESULTS

Impact of Aβ on Viability of Murine Fecal Bacteria

An antimicrobial effect of $A\beta_{42}$ on single microbial species such as *Enterococcus faecalis* or *Candida albicans* has been shown before (Soscia et al., 2010). In order to ensure the toxic properties of Aβ peptides in complex microbiota mixtures, fecal samples from 5xFAD mice and wild type littermates were incubated with $A\beta_{42}$ or scrambled peptide as control (incubation with solvent only did not statistically deviate from scrambled control, data not shown). Ten minutes of incubation sufficed to significantly reduce viability of fecal microorganisms from wild type mice in comparison to scrambled peptide treated samples (**Figure 1A**). Interestingly, no such toxic effect of the active peptide could be shown within samples derived from transgenic AD model mice. Furthermore, samples derived from wild type mice treated with 2 or 10 μM $A\beta_{42}$ were plated on Schaedler agar plates to analyze the effect on all anaerobically cultivatable bacteria. Two different concentrations were selected to address a wider range of bacteria. CFU were counted after 48 h of incubation. At both concentrations, a significant reduction of about 30–40% of bacterial growth was observed (**Figure 1B**). To assess if differences in vulnerability due to Aβ exist in different bacterial families, diluted bacterial suspension from transgenic and wild type animals were subsequently plated on agar plates selective for Lactobacillaceae (**Figure 1C**) or Enterobacteriaceae (**Figure 1D**). Both families were significantly reduced after $A\beta_{42}$ treatment in comparison to scrambled peptide control when fecal material was derived from wild type mice. However, no significant effects could be observed for Lactobacillaceae from transgenic 5xFAD animals, and only a non-significant reduction of Enterobacteriaceae occurred ($p = 0.09$).

Gastro-Intestinal Passage of Orally Administered Aβ Peptides

Before oral treatment of the mice, the stability of the Aβ peptide in the gastro-intestinal milieu had to be proven. Therefore, TAMRA-labeled $A\beta_{42}$ was incubated with PBS-diluted content of the stomach of wild type mice or PBS as control for 2 h at 37°C (exponential decay constant of stomach emptying in mice: 74 min; Schwarz et al., 2002). Detection of Aβ-dependent signals by Western blotting using the antibody 6E10 showed similar patterns of Aβ monomer and oligomers incubated with gastric content or PBS, independent of Aβ concentration (**Figure 2A**, upper panel). Fluorescence detection supported a clear assignment of the TAMRA-labeled peptide as the same pattern could be obtained as with the antibody (**Figure 2A**, lower panel). In sum, this indicated that the peptide would at least be stable within a 2 h period of digestive passage. After the stability of the Aβ peptide in the gastrointestinal milieu

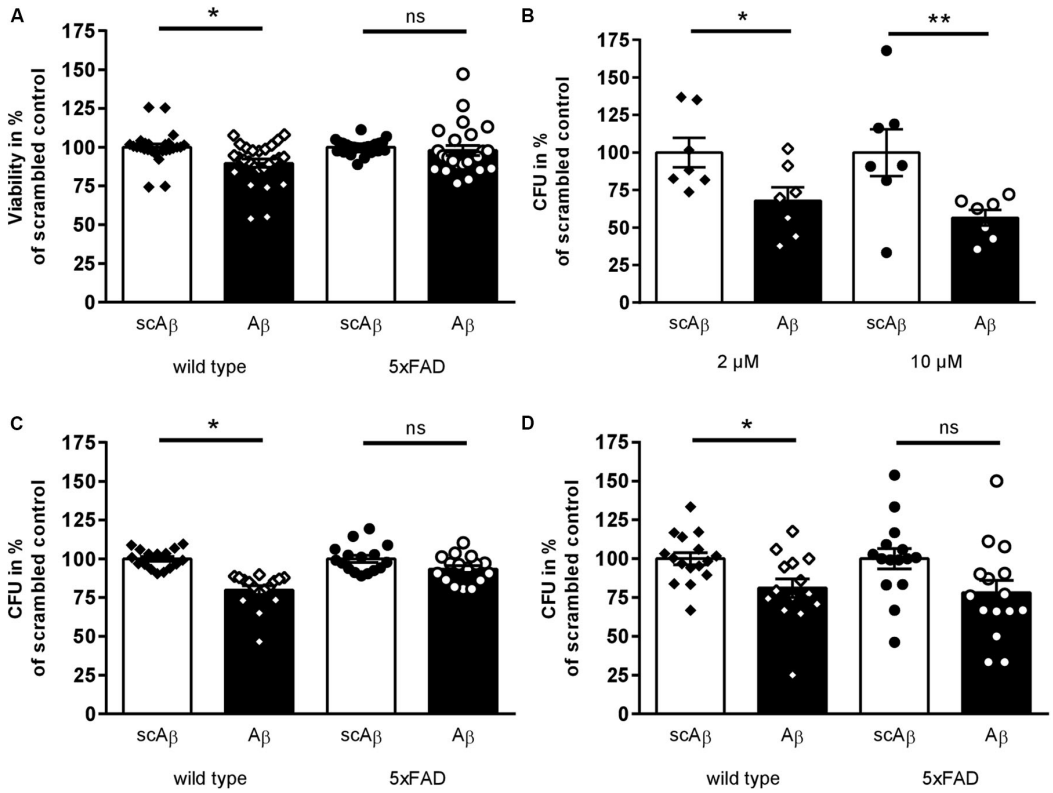

FIGURE 1 | Toxic effects of synthetic $A\beta_{42}$ on murine fecal microbial organisms. **(A)** Fecal samples of transgenic 5xFAD mice and wild type littermates were diluted and incubated with $A\beta_{42}$ peptide or scrambled control (2 µM) for 10 min. Viability was measured and calculated in percent of scrambled peptide treated samples (n = 13 animals per group, ♂ = 6–7, ♀ = 6–7). **(B)** Fecal material suspension from wild type mice were incubated with 2 or 10 µM $A\beta_{42}$ or the respective amount of scrambled peptide. Subsequently, aliquots of further diluted bacteria suspension were plated on Schaedler agar and incubated anaerobically for 48 h at 37°C. Number of colony forming units (CFU) of samples treated with scrambled peptide control were set to 100% (n = 7 animals per group; ♂ = 3, ♀ = 4). **(C, D)** After incubation of fecal samples from transgenic or wild type mice with 2 µM $A\beta_{42}$ or scrambled peptide, suspensions were spread on plates selective for Lactobacillaceae **(C)** and Enterobacteriaceae **(D)** (n = 10–11 per group, ♂ = 5–6, ♀ = 5). Datapoints for individual normalized measurements are shown in each graph (*$p < 0.05$; **$p < 0.01$).

could be ensured, mice were orally treated with the TAMRA-labeled peptide and sacrificed after 2, 4, and 24 h. To measure the transit time of the peptide, the gut was divided into 2 cm segments and fluorescence was measured within diluted chyme. Two hours after treatment, the TAMRA-labeled peptide could be detected in the 10th segment of the gut of the wild type animal (according to cecum) and in the 11th segment of the transgenic one (**Figure 2B**). Two hours later, in both animals the signal was measured in the 11th segment (cecum ampulla). No signal could be detected in any sample 24 h after the treatment. For further experiments, a complete gut passage was suspected after 5 h in mice of respective age and strain which fits to earlier reports on C57Bl/6 mice (Schwarz et al., 2002).

Diversity of the Gut Microbiome Under Chronic and Acute Exposure to Aβ Peptides

We employed high-throughput sequencing of the bacterial 16S rRNA gene in order to investigate the impact of acute or chronic exposure to the toxic Aβ peptide on the murine gut microbiome *in vivo*. Therefore, feces from 5xFAD mice and wild type littermates as well as feces from wild type mice treated with Aβ or scrambled Aβ were collected. Subsequently, DNA was purified and subjected to 16S rDNA sequencing. All animals used in these experiments were female.

In the first step of the analysis we compared the alpha and beta diversity of the negative control sample to all experimental groups as means of quality control. Estimates of species richness as well as evenness were considerably lower for the negative control sample as indicated by the Chao and Shannon index, respectively (**Supplementary Table S1**). Furthermore, unconstrained analysis of principal coordinates based both, the Bray-Curtis dissimilarity and Jaccard index, revealed that the negative control was distinctly separated from all experimental groups in reduced space (**Supplementary Figure S1**). This accounted for a large amount of the dispersion in sample scores, therefore the negative control was removed from the subsequent downstream analysis in order to better represent differences between experimental groups.

We did not observe differences in the alpha diversity estimates between wild type and 5xFAD animals in terms of species evenness (**Figure 3A**) and richness (**Figure 3B**). The comparison of mice fed with scrambled Aβ or the active form of the

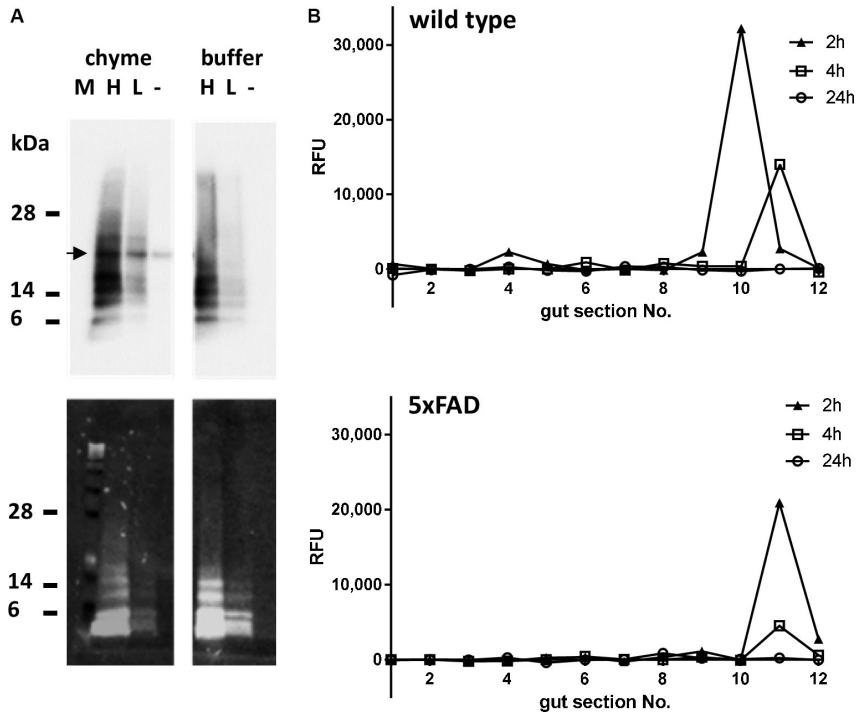

FIGURE 2 | Stability and time-dependent localization of *per os* administered Aβ peptides in the murine gastrointestinal tract. **(A)** TAMRA-labeled Aβ peptide was incubated in two different concentrations [high (H): 0.025 μM; low (L): 0.005 μM] with PBS-diluted gastric content of wild type mice for 2 h at 37°C. Solvent of the peptide was used as a negative control (–). Additionally, samples with PBS instead of chyme were incubated as described (buffer). Proteins were separated on PAA gel and transferred to nitrocellulose. Detection of Aβ-dependent signal was conducted by antibody 6E10 (upper panel) or by fluorescence (lower panel). The arrow indicates a cross reactivity only occurring in chyme. **(B)** To assess the passage of orally administered Aβ peptides, wild type or 5xFAD mice were fed with 10 μg of TAMRA-labeled Aβ peptides and sacrificed at indicated time points. TAMRA-dependent fluorescence was determined in the chyme of 2 cm gut sections numbered from 1 (oral end = stomach) to 12 (rectal section). Values are presented as measured difference between the TAMRA-Aβ-treated animals and solvent-treated control animals (one animal per time point, technical duplicates).

peptide yielded similar results with a very slight non-significant trend of reduced species richness in the Aβ group (mean Chao index = 423.4 vs 387.9 in scrambled Aβ compared to Aβ-fed animals, $p = 0.24$, **Figures 3C,D**).

Comparison of beta diversity of 5xFAD vs wild type mice using CAP resulted in better separation of experimental groups along the constrained dimension based on the Jaccard index (**Figures 3E,F**). Nevertheless, experimental group assignment only explained a small amount of the dispersion between samples and the grouping effect was not significant. In contrast, beta diversity significantly differed between Aβ- and scrambled Aβ-fed animals in the Jaccard distance based ordination. This was indicated by non-overlapping 95% confidence ellipses (**Figure 3H**). The difference was not significant when beta diversity was measured with the Bray–Curtis dissimilarity, implying that the shift in microbiota composition was qualitative in nature (**Figure 3G**).

Community Composition of Gut Microbiome

Community composition of the gut microbiome at the phylum level was highly similar in 5xFAD mice as compared to wild type littermates (**Figure 4A**). The most dominant phylum was Bacteroidetes with an average relative abundance around 60%, followed by Firmicutes with an average relative abundance of approximately 30% in both groups. Proteobacteria and Verrucomicrobia were the only remaining classified phyla with a relative abundance of above 1%. The phylum composition of Aβ- compared to scrambled Aβ-fed animals revealed similar trends (**Figure 4B**). The average relative abundance of Bacteroidetes was slightly lower in the Aβ group (53.9% compared to 59.5% in the scrambled Aβ group, $p = 0.463$). In contrast, Firmicutes average relative abundance of 25.4% in Aβ-fed animals was marginally higher than the scrambled peptide group (20.2%, $p = 0.102$). Nevertheless, differences in relative abundance at the phylum level were not statistically significant. The community composition at the family level is shown in **Supplementary Figure S2** for wild type and 5xFAD animals and **Supplementary Figure S3** for animals fed with Aβ or the scrambled peptide.

Differential Abundance Analysis

We performed a differential abundance analysis on the OTU level by calculating log2 FC of bacterial abundance using the DESeq2 package. Diagnostic plots from this analysis are shown in **Supplementary Figure S4**. This analysis revealed 109 phylotypes with a significantly different abundance in 5xFAD animals

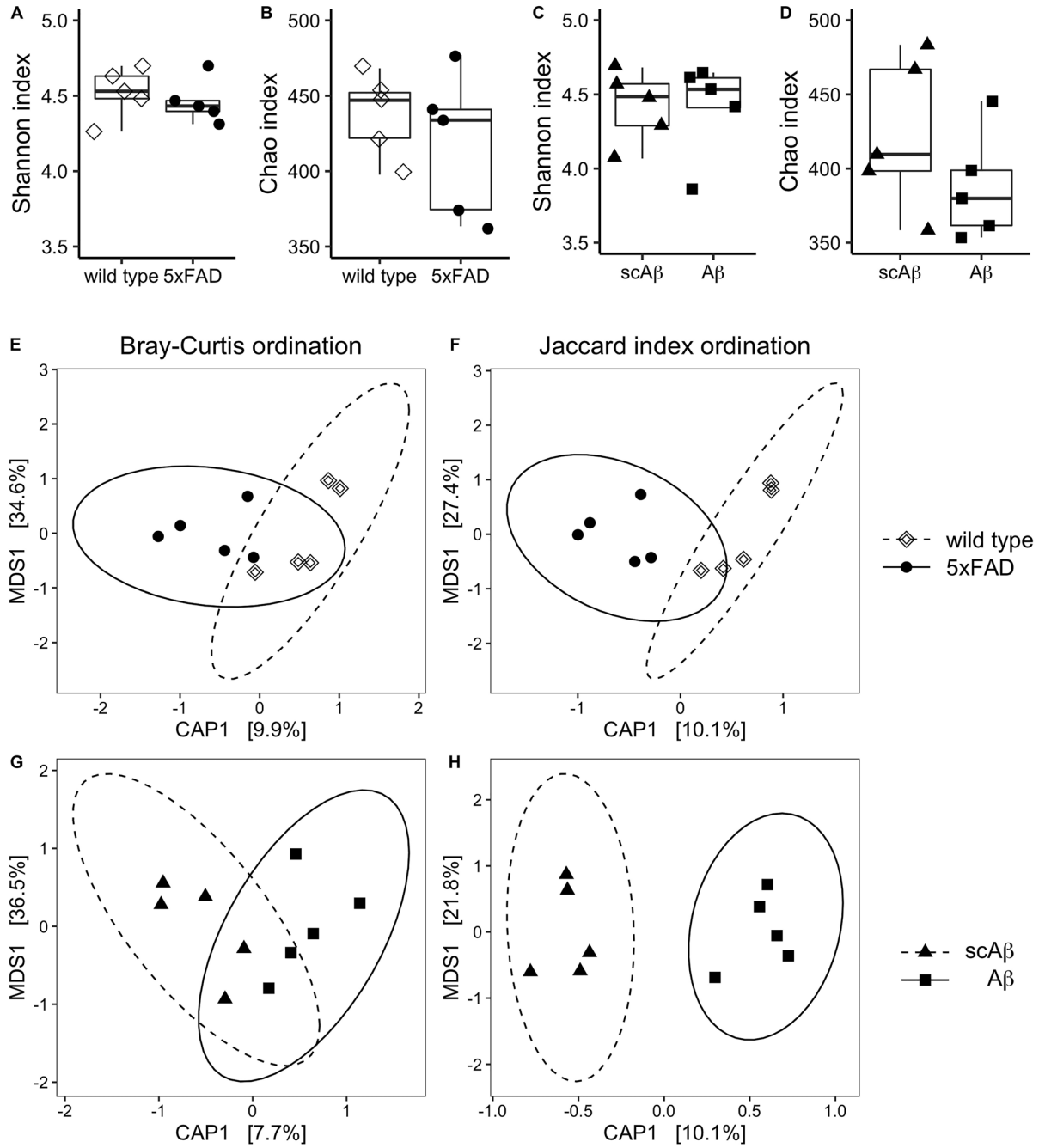

FIGURE 3 | Diversity measures of gut microbiota following chronic or acute exposure to Aβ. Species richness and evenness of each experimental group were evaluated by estimating the Chao index and Shannon index, respectively. Panels **(A)** and **(B)** show results for wild type compared to 5xFAD mice. Differences in alpha diversity between wild type animals receiving scrambled Aβ (scAβ) or Aβ are depicted in panels **(C)** and **(D)**. Data are shown as boxplots and individual values. Beta diversity was evaluated by calculating Bray–Curtis dissimilarity **(E, G)** and binary Jaccard distance **(F, H)**. Results were visualized using canonical analysis of principal coordinates (CAP) for each dissimilarity measure separately. Since there are two groups in each ordination analysis, only one constrained dimension is calculated (CAP1), which is shown on the *x*-axis. The *y*-axis corresponds to the first unconstrained dimension (MDS1). Confidence ellipses around the respective group centroid were drawn at the 95% confidence level. The percentage of total inertia captured by each axis is shown in brackets. MDS: Multi-dimensional scaling.

relative to the wild type group (**Figures 5A,B**). The majority of these OTUs were associated with the Clostridiales order within the Firmicutes phylum. A large amount of the differentially abundant OTUs could not be classified to a reference taxonomy beyond the order and family level. These taxonomic units included OTUs with either significantly increased or decreased abundance in 5xFAD animals which implies different influence of chronic Aβ exposure on the growth of bacteria at lower

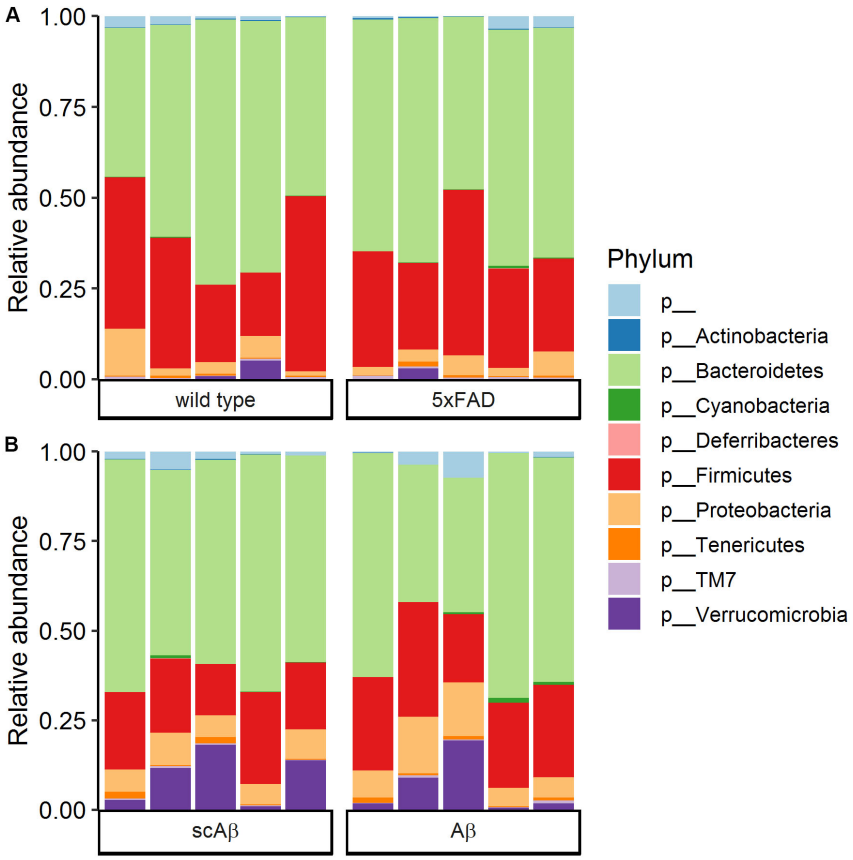

FIGURE 4 | Community composition at the phylum level. Bars show the relative abundance of bacterial phyla in **(A)** wild type compared to 5xFAD mice and **(B)** wild type animals fed with scrambled Aβ (scAβ) versus active Aβ. Phyla with a relative abundance below 0.05% were filtered out from the data set. The label "p__" corresponds to operational taxonomic units that could not be classified to a reference phylum.

taxonomic levels such as genera, species, or strains. At the genus level, we detected individual OTUs associated with *Allobaculum*, *Prevotella*, *Anaeroplasma*, *Bacteroides*, *SMB53*, and *Turicibacter*, respectively, all of which demonstrated significantly increased abundance in 5xFAD animals as compared to wild type littermates. At the species level, we observed an enhanced growth of individual OTUs belonging to *Bacteroides acidifaciens*, *Lactobacillus reuteri*, and *Ruminococcus gnavus* in the 5xFAD group. In contrast, abundance of *Helicobacter hepaticus* was significantly decreased relative to wild type animals whereas individual OTUs associated with *Alistipes massiliensis* demonstrated both, increased and decreased abundance in 5xFAD animals (for single fold changes and adjusted *p*-values see **Supplementary Table S2**).

Our comparison of the abundance of individual OTUs between Aβ- and scrambled Aβ-fed animals resulted in 55 differentially abundant OTUs (**Figures 5C,D**) most of which belonged to the Clostridiales order. Similarly to the previous analysis, multiple OTUs could not be classified beyond the order and family level. At the genus level, one OTU assigned to *Anaeroplasma* showed significantly decreased abundance in the Aβ group. In contrast, OTUs classified to *Oscillospira* and *Ruminococcus* were associated with instances of both, increased and decreased abundance, relative to the samples derived from scrambled Aβ-fed animals. At the species level, we identified one OTU associated with *Lactobacillus reuteri* and two OTUs belonging to *Ruminococcus gnavus* which demonstrated significantly reduced growth following feeding with Aβ (fold changes and adjusted *p*-values are given in **Supplementary Table S3**).

DISCUSSION

The link between AD and alterations in the gut microbiome composition has already been established in several experimental animal models (Brandscheid et al., 2017; Harach et al., 2017; Shen et al., 2017; Zhang et al., 2017; Bauerl et al., 2018; Peng et al., 2018; Zhan et al., 2018) and clinical studies (Cattaneo et al., 2017; Vogt et al., 2017; Zhuang et al., 2018). However, the exact causal mechanism is still not understood. On one hand, perturbations in gut bacterial homeostasis might lead to increased intestinal permeability, low grade inflammation or insulin resistance and obesity (Turnbaugh et al., 2006; Cani et al., 2008, 2009), the last two of which are risk factors for developing AD (e.g., Kivipelto et al., 2005; Fitzpatrick et al., 2009; Luchsinger et al., 2012;

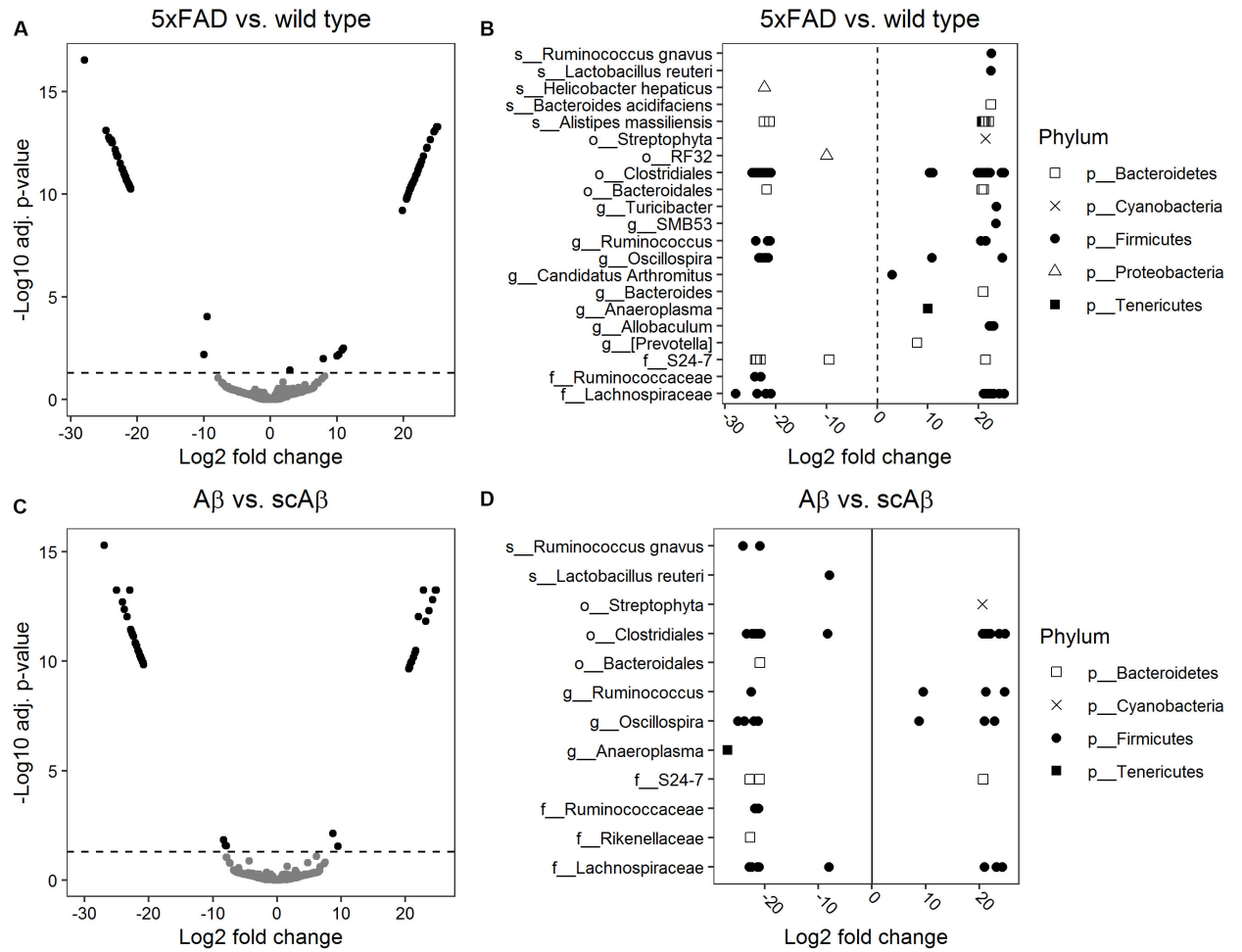

FIGURE 5 | Differential abundance analysis of bacterial operational taxonomic units (OTUs). Log2 fold changes of bacterial abundance were calculated using DESeq2. Volcano plots show the log2 fold changes versus –log10 adjusted *p*-values for 5xFAD relative to wild type mice **(A)** and animals fed with Aβ relative to mice receiving the scrambled peptide **(C)**. OTUs were considered to be differentially abundant if the adjusted *p*-value (Benjamini–Hochberg method) was below 0.05 (indicated by a dashed horizontal line). Significant log2 fold changes appear as black dots in panels **(A)** and **(C)**. The lowest level of taxonomy which could be assigned to the differentially abundant OTUs is depicted in panel **(B)** for 5xFAD mice relative to wild type littermates and in panel **(D)** for mice fed with Aβ relative to the scrambled peptide control group. Log2 fold changes greater than 0 correspond to OTUs significantly enriched in 5xFAD or Aβ-fed mice relative to the respective control group.

Ferreira et al., 2018). On the other hand, it is plausible that shifts in gut bacteria in AD patients might occur as a result of life style and dietary changes after onset of the disease. For instance, in a cross-sectional study, AD patients had an exacerbated nutritional status compared to age-matched controls (Saragat et al., 2012). An alternative mechanism of how the host affects microbiota in the context of AD has been hypothesized by us in a recent *in silico* study (Hewel et al., 2019). In this previous work, we demonstrated that host miRNAs differentially expressed in patients suffering from AD have the potential to bind on key regulatory sequences in commensal microbiota, thereby possibly regulating transcription in bacterial pathways.

Despite representing a major hallmark of AD pathology, the exact physiological function of the Aβ peptide is not known. However, both *in vitro* (Soscia et al., 2010; Kumar et al., 2016; Spitzer et al., 2016) and *in vivo* (Kumar et al., 2016) studies have demonstrated that Aβ exerts anti-microbial properties. Furthermore, oligomerization and fibrillization of the peptide, which are involved in the process of plaque deposition, are also characteristics shared with known anti-microbial peptides (Soscia et al., 2010; Kagan et al., 2012). This has led to the proposal of a new paradigm for the role of Aβ in AD pathogenesis referred to as the antimicrobial protection hypothesis of AD (reviewed in Moir et al., 2018). Considering the fact that Aβ depositions are also found in the intestine, we investigated if acute or chronic exposure to the toxic peptide modulates mouse gut microbiota composition *in vitro* and *in vivo*. Previous *in vitro* experiments on the anti-microbial properties of Aβ were performed under aerobic conditions (Soscia et al., 2010; Spitzer et al., 2016), which is not representative for the predominantly anaerobic environment of the intestine. Therefore, we incubated fecal samples from wild type and 5xFAD mice anaerobically in the

current study. Strikingly, Aβ inhibited bacterial growth in fecal samples originating from wild type animals. Only a tendency of bacterial growth reduction of Enterobacteriaceae of 5xFAD mice could be observed. This might suggest that prolonged exposure to the peptide in 5xFAD animals triggers an adaptive response in some gut microbiota, but individual species could still be affected. This assumption is corroborated by *in vitro* experiments showing that Aβ inhibited *E. faecalis* growth at early time points, whereas bacterial growth resumed at later time points (Soscia et al., 2010).

After observing the growth-inhibiting effects of Aβ on fecal bacteria *in vitro*, we also investigated how this translates to the *in vivo* situation. We did not detect any difference in alpha diversity measures between wild type and 5xFAD animals or between wild type mice fed with Aβ or scrambled peptide which is in line with previous reports in mice of similar age (Harach et al., 2017; Shen et al., 2017; Zhang et al., 2017). In contrast, conflicting results have been described regarding longitudinal changes of alpha diversity in mouse models of AD. For instance, Zhang et al. (2017) and Bauerl et al. (2018) observed a significant increase in alpha diversity in APP/PS1 transgenic mice over time whereas Shen et al. (2017) reported a significant decrease in older APP/PS1 mice. Differences were not gender-driven since the two studies showing an increase used male and female mice, respectively. Furthermore, APP/PS1 mice had significantly higher alpha-diversity compared to control animals at 8 months of age in a study by Harach et al. (2017). In the senescence accelerated mouse prone 8 (SAMP8) model of AD, alpha diversity was significantly lower in 7-month old SAMP8 mice as compared to age-matched controls (Zhan et al., 2018). While differences in the mouse models of AD and experimental designs might be partly responsible for these conflicting results, the true association between alpha diversity of the gut microbiome and AD still needs to be elucidated.

5xFAD animals did not significantly differ from wild type littermates in terms of beta diversity which is in agreement with existing studies in mice of comparable age (Harach et al., 2017; Zhang et al., 2017; Bauerl et al., 2018). Remarkably, wild type mice fed with Aβ were clearly separated from animals receiving the scrambled peptide on the ordination plot when beta diversity was measured with the Jaccard distance. This might again be an indication that intestinal bacteria in wild type mice are more susceptible to the acute effects of Aβ, whereas prolonged exposure in 5xFAD mice already led to compositional adaptation. However, longitudinal investigations or studies in older mice consistently reported a difference in beta diversity between mouse models of AD and controls at later times points such as 6, 7, 8, and 24 months of age (Harach et al., 2017; Bauerl et al., 2018; Peng et al., 2018; Zhan et al., 2018). Therefore, we hypothesize that increased Aβ burden might be an early driver of changes in the gut microbiome followed by an adaptive response to the direct anti-microbial effects of the peptide. This is also reflected by the lack of *in vitro* toxic effects of Aβ peptides administered to fecal samples derived from 5xFAD mice. However, the initial perturbations of gut homeostasis might trigger a larger cascade of subsequent events contributing to AD development and long-term alterations in microbiome composition. For instance, we previously described a significantly reduced body weight in male 5xFAD mice compared to wild type controls as early as 6 weeks of age (Brandscheid et al., 2017). Altered gut microbiome is well known in the context of obesity (Turnbaugh et al., 2006; Cani et al., 2008), therefore it is plausible to assume that body weight reduction might also be associated with shifts in fecal bacteria.

With regard to community composition of intestinal bacteria in female mice aged 10 weeks, we did not detect any significant changes at the phylum level. In an earlier study, we described a significantly increased relative abundance of Firmicutes accompanied by a significant decrease in Bacteroidetes in male 5xFAD mice compared to wild type littermates at the age of 9 weeks (Brandscheid et al., 2017). It is important to mention that we previously employed a targeted approach to only quantify a specific subset of phyla and species using qPCR. Here, we performed a more comprehensive microbiome characterization by high-throughput amplicon sequencing of the 16S rRNA gene. Therefore, methodological differences might in part account for the discrepancy in results. However, gender itself is also associated with differences in intestinal microbiota composition (Yurkovetskiy et al., 2013; Fransen et al., 2017; Elderman et al., 2018). Importantly, female mice were reported to have a higher diversity and richness which might be an indicator of a more robust microbiome (Yurkovetskiy et al., 2013; Elderman et al., 2018). Furthermore, male mice exhibited an altered gut bacterial composition at puberty and this was not observed in female animals (Yurkovetskiy et al., 2013). Therefore, shifts at the phylum level in the context of AD might take longer to develop in female mice as compared to males which might explain why we did not detect any changes in the current study. In line with this, Bauerl et al. (2018) described a significantly increased abundance of the phylum Proteobacteria in 6-month-old APP/PS1 female mice compared to controls. The difference was not present at 3 months of age.

Even though the composition of gut microbiota was not altered after acute or chronic exposure to Aβ at higher taxonomic levels, we detected numerous differentially abundant OTUs pointing to early perturbations in gut homeostasis at the species and strain level. In line with the *in vitro* growth inhibiting effect of Aβ on Lactobacillaceae family, we observed a significantly lower abundance of an OTU associated with *Lactobacillus reuteri* in animals fed with Aβ compared to scrambled peptide. Interestingly, abundance of *L. reuteri* was drastically decreased in the offspring of mice kept on a high fat diet compared to mothers on a regular diet in an autism mouse model (Buffington et al., 2016). Moreover, supplementation with this bacterium significantly improved deficits in social behavior in the offspring of the high-fat diet mice. However, we observed an increased abundance of this bacterium in 5xFAD mice compared to wild type littermates, which in theory may again point to a compensatory reaction after a prolonged exposure to the toxic Aβ peptide. However, the possible role of *L. reuteri* in AD needs to be further validated. Another potential target of interest could be *Ruminococcus gnavus* which belongs to the Lachnospiraceae family and has been linked to inflammatory bowel disease (Joossens et al., 2011; Hall et al., 2017; Henke et al., 2019). We identified two OTUs corresponding to this species with significantly lower abundance in mice fed with Aβ, however,

one OTU demonstrated an increased abundance in 5xFAD mice relative to wild type controls. Interestingly, OTUs associated with *R. gnavus* were identified to have an increased abundance in patients with clinically diagnosed AD dementia compared to age-matched individuals with normal cognitive function (Zhuang et al., 2018). In the same study, an OTU associated with *Prevotella* was more abundant in the AD group which is in line with our observation of enhanced growth of a *Prevotella* OTU in 5xFAD mice compared to controls. In contrast, a decreased abundance of *Prevotella* was described in AD mice as compared to controls (Shen et al., 2017; Peng et al., 2018), so results remain controversial. An OTU belonging to the *Bacteroides* genus demonstrated an enhanced growth in 5xFAD mice in our study. Increased abundance of this bacterial genus was also described in patients with AD by Vogt et al. (2017). Furthermore, *Bacteroides* abundance in the AD group was positively correlated with $A\beta_{42}/A\beta_{40}$ and p-tau/$A\beta_{42}$ in cerebrospinal fluid, both of which are biomarkers for exacerbated AD pathology.

Overall, the abundance of multiple other OTUs, which belong to the same higher taxonomic unit, was regulated in an opposite direction after acute or chronic exposure to Aβ. This demonstrates that species and strains react differently possibly even within the same genus. However, the majority of OTUs could not be classified to these lower taxonomic levels. This highlights the need for optimization of reference databases for taxonomic assignment especially if pre- or probiotic treatments should emerge as an option for ameliorating AD pathology. Namely, such therapies would have to target specific bacterial species or strains which would first have to be identified with high certainty in 16S marker-gene survey studies.

In conclusion, to the best of our knowledge, we provide first evidence that acute *in vivo* administration of Aβ is associated with a shift in gut microbiota composition. Our study does not answer the question if this is the factor that leads to long-term intestinal bacteria alterations in the context of AD. However, we suggest that increased exposure to the Aβ peptide might be an early trigger of a process that ends in a vicious circle of exacerbated AD pathology promoting impaired gut homeostasis and vice versa.

DATA AVAILABILITY STATEMENT

The raw sequencing data generated in this study have been deposited in the NCBI SRA data base under the accession number PRJNA627235.

ETHICS STATEMENT

The animal study was reviewed and approved by LUA Rhineland-Palatinate, Germany.

AUTHOR CONTRIBUTIONS

KE conceived the project and designed the experiments. MS, AM, and KC performed the experiments. HT and CO conducted bioinformatics data analysis. MS and HT wrote the manuscript. KE, TH, SG, MS, and HT edited the manuscript. KE and SG supervised the study. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.01008/full#supplementary-material

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Conflict of Interest: HT was employed by Fresenius Kabi Deutschland GmbH at the time the research was conducted.

The remining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

Table S1. Alpha diversity measures for the negative control sample

Shannon index	Chao index
3.38	113.87

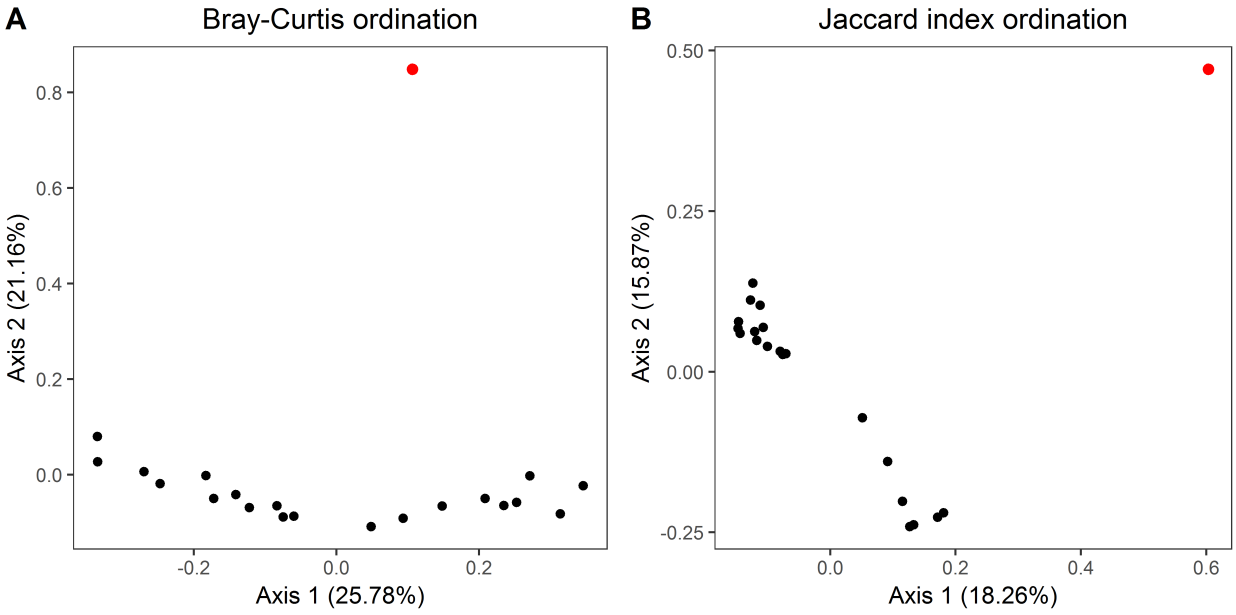

Figure S1. Unconstrained ordination analysis of all experimental groups including negative control. Ordination analysis was performed based on the **(A)** Bray-Cutis dissimilarity or **(B)** binary Jaccard distance. The negative control sample appears as a red dot on the plots. The amount of dispersion in sample scores (inertia) in percent captured by each multivariate dimension is given in brackets on the respective axis.

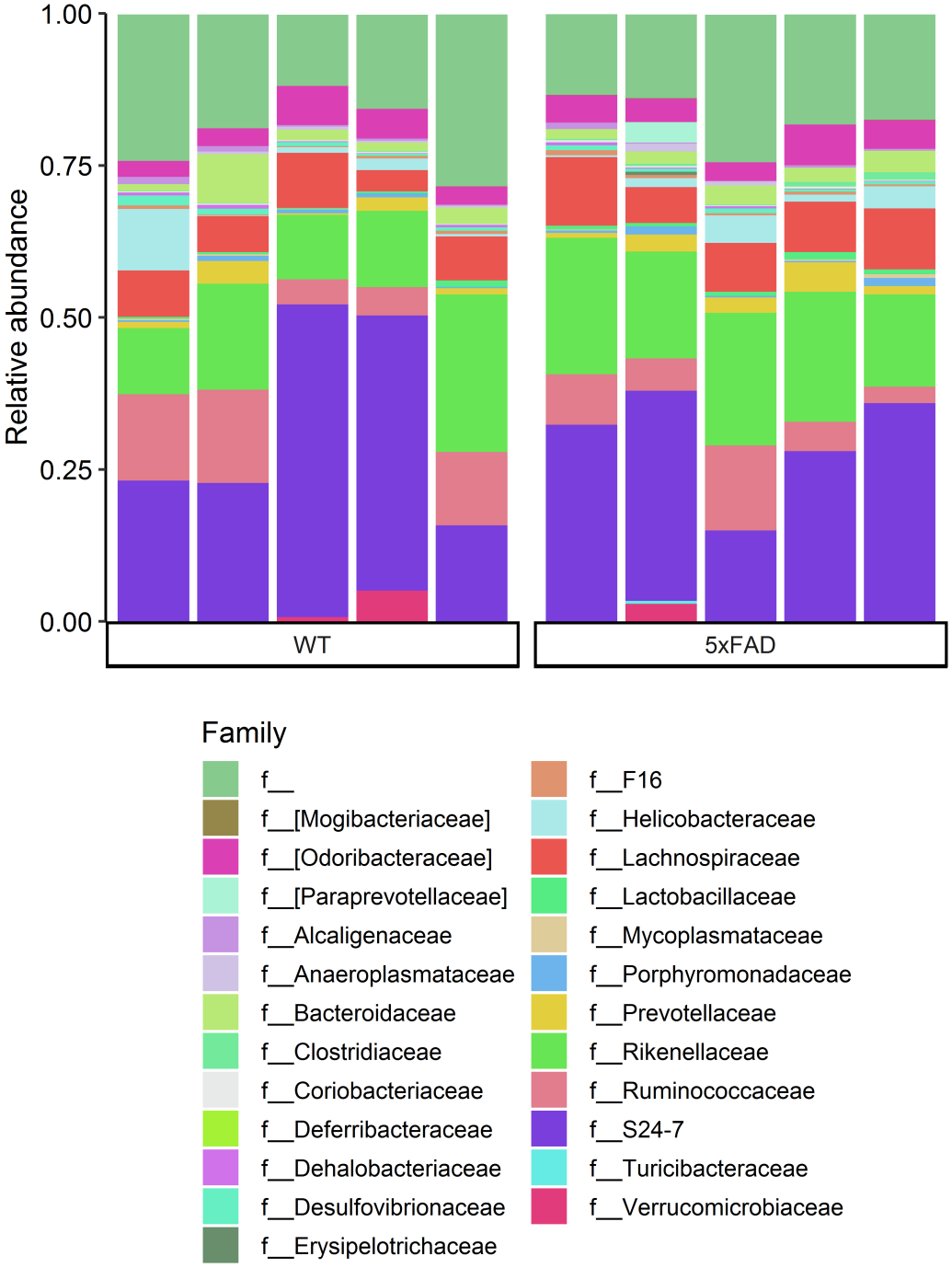

Figure S2. Community composition of wildtype and 5xFAD animals at the family level. Each bar shows the relative abundance of bacterial families for individual animals.

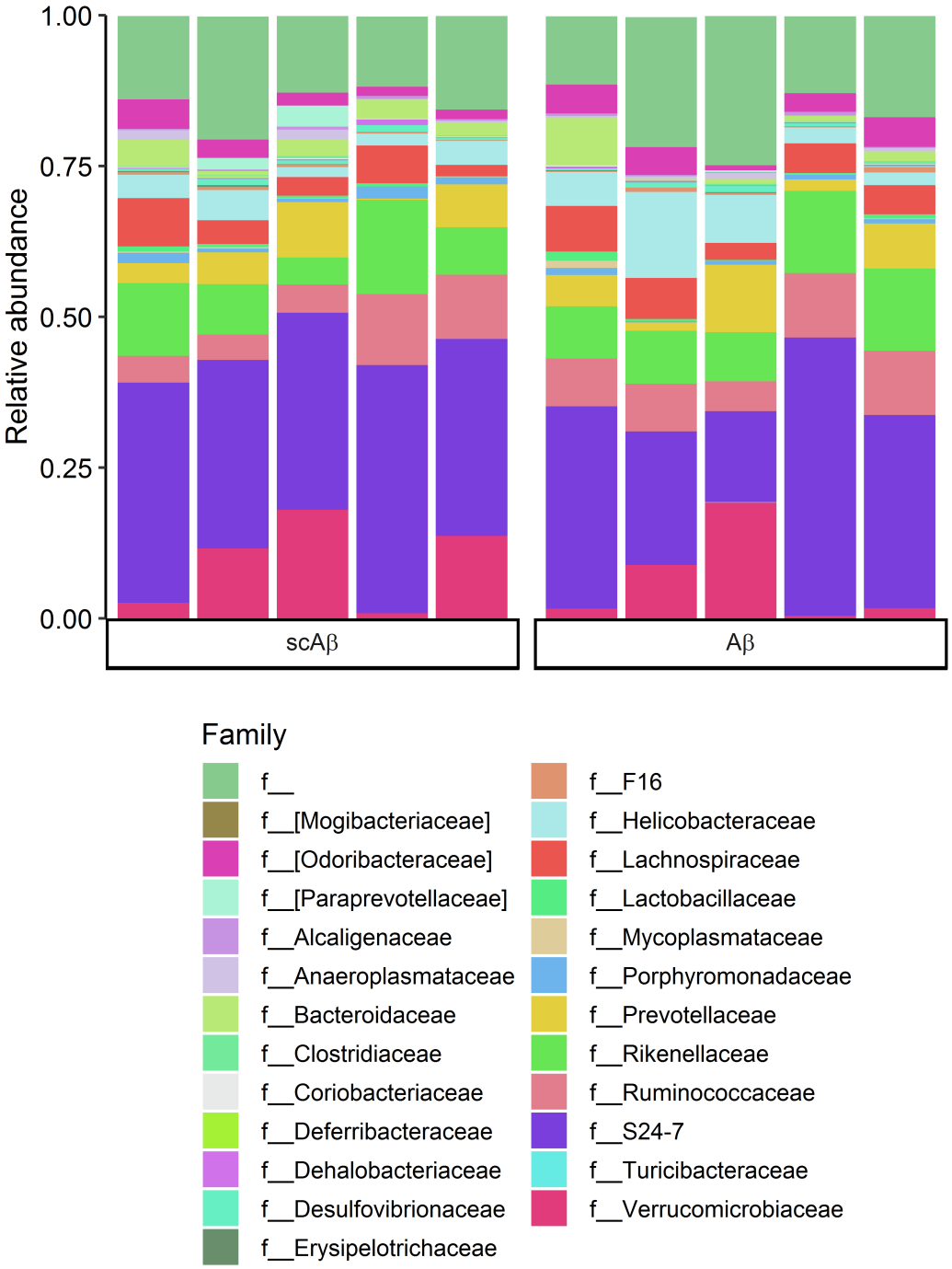

Figure S3. Community composition of scrambled Aβ or Aβ-fed animals at the family level. Each bar shows the relative abundance of bacterial families for individual animals.

Table S2 Differentially abundant operational taxonomic units (OTU) in 5xFAD animals relative to wildtype controls. The taxonomy classification for each OTU, the fold change (FC) together with the corresponding adjusted p-value (Benjamini-Hochberg method) are reported. FC values below 0 indicate OTUs with significantly lower abundance in 5xFAD animals whereas positive FC values correspond to enriched OTUs in the 5xFAD group.

Taxonomy	Log2 fold change	Adj. p-value
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-27.93	2.96E-17
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	25.01	5.15E-14
p__Firmicutes; c__Clostridia; o__Clostridiales	25.09	5.15E-14
p__Firmicutes; c__Clostridia; o__Clostridiales	-24.67	7.96E-14
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	24.73	7.96E-14
p__Firmicutes; c__Clostridia; o__Clostridiales	24.54	9.32E-14
p__Firmicutes; c__Clostridia; o__Clostridiales	-24.26	1.73E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	-24.13	2.15E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	-24	2.24E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	24.04	2.24E-13
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	-24.02	2.24E-13
p__Firmicutes; c__Clostridia; o__Clostridiales	-23.91	2.38E-13
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	-23.77	3.16E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-23.74	3.23E-13
p__Firmicutes; c__Bacilli; o__Turicibacterales; f__Turicibacteraceae; g__Turicibacter	23.52	5.40E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53	23.46	5.95E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-23.36	6.97E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; o__Clostridiales; o__Clostridiales	-23.36	6.97E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-23.19	1.01E-12
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	-23	1.38E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-23.03	1.38E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	-23.01	1.38E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	23.02	1.38E-12
p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Allobaculum	22.96	1.41E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.98	1.41E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.92	1.50E-12
p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Allobaculum	22.71	2.46E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	22.59	3.15E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.59	3.15E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__Ruminococcus gnavus	22.49	3.89E-12
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__Bacteroides acidifaciens	22.46	4.08E-12
p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__Lactobacillus reuteri	22.41	4.50E-12

p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	-22.29	5.87E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.26	5.88E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	22.27	5.88E-12
p__Proteobacteria; c__Epsilonproteobacteria; o__Campylobacterales; f__Helicobacteraceae; g__Helicobacter; s__Helicobacter hepaticus	-22.26	5.88E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-22.23	6.05E-12
p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Allobaculum	22.17	6.90E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	22.14	7.22E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.06	8.80E-12
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	22	9.66E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-21.99	9.82E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-21.97	1.03E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.95	1.04E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	21.93	1.06E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.88	1.18E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.83	1.28E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.82	1.32E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales	-21.81	1.33E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.75	1.50E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.67	1.77E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	-21.65	1.84E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.59	2.08E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.57	2.17E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.55	2.18E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-21.52	2.32E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.52	2.32E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.42	2.71E-11
p__Cyanobacteria; c__Chloroplast; o__Streptophyta	21.41	2.71E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.43	2.71E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	21.42	2.71E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	21.42	2.71E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	21.38	2.91E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.37	2.92E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.37	2.96E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.35	3.01E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.35	3.02E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	21.32	3.10E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.28	3.33E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.28	3.41E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.24	3.63E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	21.23	3.63E-11

p__Firmicutes; c__Clostridia; o__Clostridiales	21.22	3.63E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	21.22	3.63E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	-21.22	3.67E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.19	3.77E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	-21.19	3.78E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	21.17	3.91E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	21.14	4.14E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales	21.05	4.97E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.06	4.97E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.04	5.03E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.04	5.09E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.02	5.17E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.02	5.17E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides	20.98	5.48E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	20.97	5.48E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-20.97	5.49E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-20.98	5.49E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	20.95	5.59E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	20.9	6.18E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	20.79	8.12E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__Alistipes massiliensis	20.76	8.54E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales	20.66	1.07E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.58	1.27E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.52	1.36E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.5	1.50E-10
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	20.45	1.63E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.45	1.63E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.43	1.70E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	19.82	6.26E-10
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	-9.49	9.01E-05
p__Firmicutes; c__Clostridia; o__Clostridiales	10.94	0.003137
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira;	10.77	0.003848
p__Proteobacteria; c__Alphaproteobacteria; o__RF32;;;	-10.01	0.00635
p__Firmicutes; c__Clostridia; o__Clostridiales;;;	10.31	0.00664
p__Tenericutes; c__Mollicutes; o__Anaeroplasmatales; f__Anaeroplasmataceae; g__Anaeroplasma	9.99	0.007711
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]	7.91	0.010456
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Candidatus Arthromitus	2.91	0.03776

Table S3. Differentially abundant operational taxonomic units (OTU) in amyloid-β (Aβ) relative to scrambled amyloid beta (scAβ)-fed animals. The taxonomy classification for each OTU, the fold change (FC) together with the corresponding adjusted p-value (Benjamini-Hochberg method) are reported. FC below 0 indicate OTUs with significantly lower abundance in Aβ animals whereas positive FC values correspond to enriched OTUs in the Aβ group.

Taxonomy	Log2 fold change	Adj. p-value
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae;	24.24	1.72E-16
p__Tenericutes; c__Mollicutes; o__Anaeroplasmatales; f__Anaeroplasmataceae; g__Anaeroplasma	-26.96	1.84E-16
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-24.99	3.50E-14
p__Firmicutes; c__Clostridia; o__Clostridiales	24.8	4.45E-14
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	24.68	4.98E-14
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__Ruminococcus gnavus	-24.11	5.89E-14
p__Firmicutes; c__Clostridia; o__Clostridiales	23.75	7.10E-14
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-23.78	7.10E-14
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	23.94	7.10E-14
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-22.93	2.22E-13
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	22.81	2.89E-13
p__Firmicutes; c__Clostridia; o__Clostridiales	-23.38	6.71E-13
p__Firmicutes; c__Clostridia; o__Clostridiales	22.02	1.22E-12
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	-22.79	2.60E-12
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae	-22.71	2.99E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-22.58	3.83E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	-22.55	3.94E-12
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-22.48	4.45E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.38	5.50E-12
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.09	1.07E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-22.06	1.10E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-22.01	1.17E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	-21.89	1.51E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.87	1.52E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.63	2.42E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.64	2.42E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.59	2.56E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-21.53	2.93E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	21.46	3.27E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-21.36	4.14E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	-21.34	4.16E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	-21.32	4.27E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	21.23	4.90E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	-21.24	4.90E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	-21.2	5.19E-11

p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-21.1	6.11E-11
p__Firmicutes; c__Clostridia; o__Clostridiales;	-21.12	6.11E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-21.08	6.42E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae;	20.97	7.70E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	-20.97	7.88E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	20.95	7.92E-11
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__Ruminococcus gnavus	-20.94	7.96E-11
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales	-20.89	8.55E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	20.89	8.55E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	20.85	9.09E-11
p__Firmicutes; c__Clostridia; o__Clostridiales	-20.77	1.07E-10
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7	20.71	1.21E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.66	1.33E-10
p__Firmicutes; c__Clostridia; o__Clostridiales	20.58	1.49E-10
p__Cyanobacteria; c__Chloroplast; o__Streptophyta	20.55	1.67E-10
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus	9.5	0.003558
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira	8.73	0.009744
p__Firmicutes; c__Clostridia; o__Clostridiales	-8.32	0.01723
p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__Lactobacillus reuteri	-7.92	0.02619
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	-8.06	0.02619

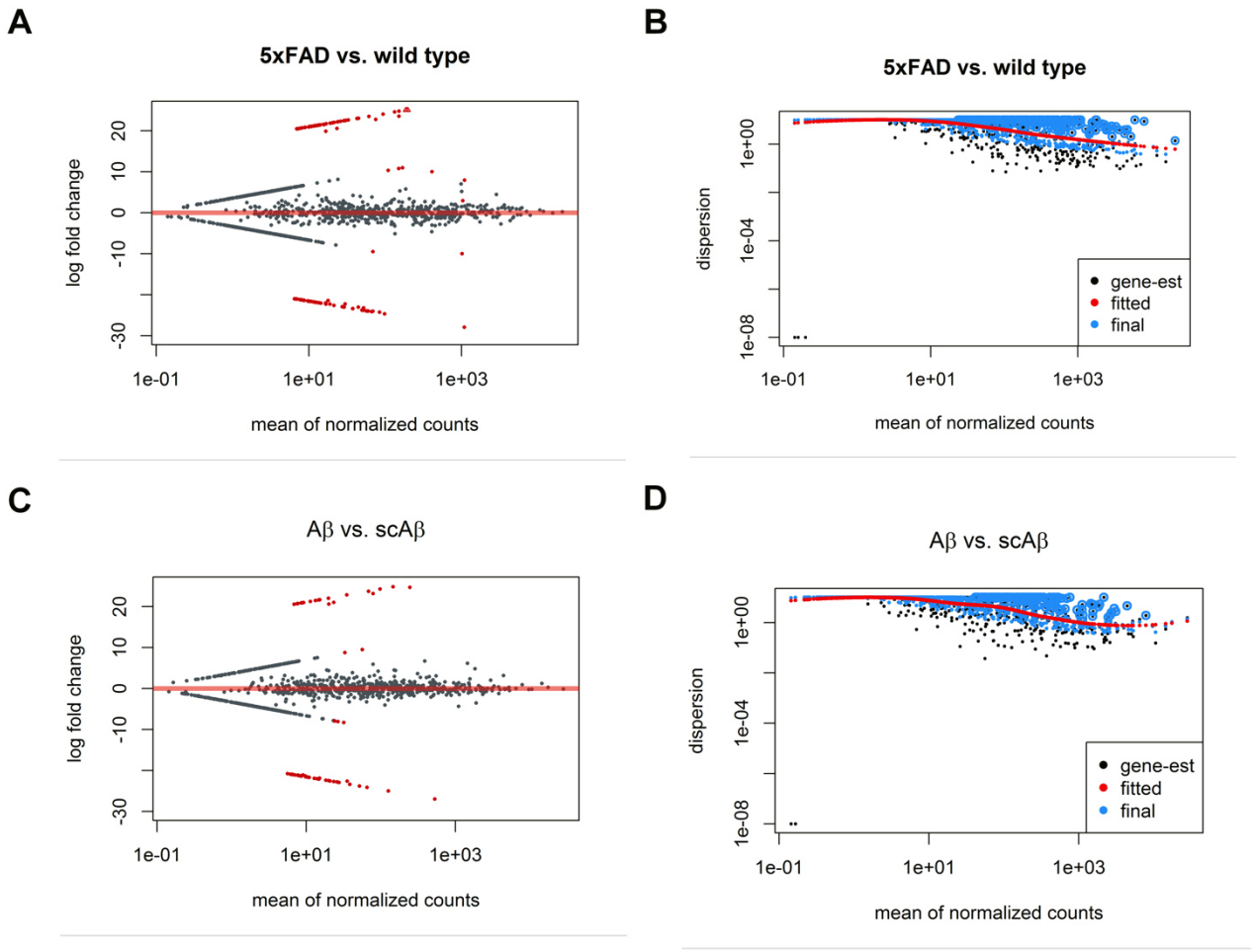

Figure S4. Diagnostic plots for the differential abundance analysis performed with DESeq2. The relationship between the mean normalized bacterial abundance and log2 fold change is shown in panel A for 5xFAD mice relative to wild type mice and panel C for Aβ-fed wild type mice relative to animals receiving scrambled Aβ (scAβ). Fold changes with an adjusted p-value less than 0.05 are colored red. The relationship between the dispersion and mean parameters of the negative binomial distributions used to estimate log2 fold changes is shown in panels B and D. Dispersions estimates directly obtained from the data appear as black dots, the red line corresponds to the fitted values using local regression. The blue dots correspond to the final estimates of dispersion after shrinkage.

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Impact of Gut Microbiome Manipulation in 5xFAD Mice on Alzheimer's Disease-Like Pathology

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Abstract: The gut brain axis seems to modulate various psychiatric and neurological disorders such as Alzheimer's disease (AD). Growing evidence has led to the assumption that the gut microbiome might contribute to or even present the nucleus of origin for these diseases. In this regard, modifiers of the microbial composition might provide attractive new therapeutics. Aim of our study was to elucidate the effect of a rigorously changed gut microbiome on pathological hallmarks of AD. 5xFAD model mice were treated by antibiotics or probiotics (*L. acidophilus* and *L. rhamnosus*) for 14 weeks. Pathogenesis was measured by nest building capability and plaque deposition. The gut microbiome was affected as expected: antibiotics significantly reduced viable commensals, while probiotics transiently increased *Lactobacillaceae*. Nesting score, however, was only improved in antibiotics-treated mice. These animals additionally displayed reduced plaque load in the hippocampus. While various physiological parameters were not affected, blood sugar was reduced and serum glucagon level significantly elevated in the antibiotics-treated animals together with a reduction in the receptor for advanced glycation end products RAGE—the inward transporter of Aβ peptides of the brain. Assumedly, the beneficial effect of the antibiotics was based on their anti-diabetic potential.

Keywords: microbiota; antibiotics; probiotics; intestines; neurodegenerative diseases; diabetes mellitus; receptor for advanced glycation end products; glucagon

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1. Introduction

The eliciting factors for sporadic Alzheimer's disease (AD) are still to be identified. Several genetic and environmental risk factors have been identified such as the ApoE4 allele or diabetes (for example [1,2]) that contribute in different proportion to the overall risk load of the individual. Within the last decade, a new approach was introduced by considering the human microbiome as one of the disease modifiers if not even a trigger [3]. Differences in the gut microbiome as well as other microbiomes such as the salivary one have been studied in AD patients and aging cohorts [4–7]. It is tempting to assume that, for example, the gut–brain axis might translate such changes within distinct microbial communities towards the brain of the host and thereby initiate, ameliorate or aggravate pathological mechanisms within the central nervous system [8]. For example, neurotrophic factors such as BDNF or dendritic spine formation within the hippocampus have been shown to be associated with presence of a gut microbiome or its composition (for a recent review see [9]).

Interestingly, the relation between the microbiome and this devastating disease might be double-edged. One of the major hallmarks of AD are the neurotoxic Aβ peptides derived by proteolytic processing of the amyloid precursor protein (AβPP). These peptides

have been suggested to act in an anti-microbial manner, and in vitro as well as in vivo effects on selected bacteria as well as viral species and microbial communities have been reported [10–13]. Recently, the occurrence of Aβ has been demonstrated in the saliva of AD model mice and comparably in samples derived from human patients [14]. Additionally, App NL-G-F but not APP/PS1 mice displayed an altered oral microbiome. Another study revealed that in C57BL/6J wild type mice a single oral administration of Aβ altered the gut microbiome after inoculation for the time of a single gut passage only [15].

This might hamper the analysis of the microbiome and might explain why a conclusive picture about the typical "AD-microbiome" still is lacking. Some bacterial commensals seem to at least be repeatedly identified such as *Alistipes*, *Blautia*, *Odoribacter*, *Ruminococcus*, and *S24-7* [16].

An approach to unravel the effect of the microbiome on pathogenesis is the manipulation of the microbiome and here, most work has been focused on the gut commensal community. Under germ-free conditions, for example, APP/PS1 mice showed an attenuated pathology [17]. A six-month treatment regime with an antibiotics cocktail did not reduce microbial abundance in general (measured by 16SrRNA copy number, [18]) but affected the community as α-diversity was lowered. This was accompanied by a more than two-fold decrease in combined cortical and hippocampal Aβ plaque burden in male antibiotics-treated mice as compared to vehicle-receiving animals. As a potential explanation for this beneficial effect, the authors demonstrated elevations of circulating cytokines such as CCL11, and more importantly increased ramification of microglia surrounding the senile plaques within the brain.

Furthermore, pre- and probiotics have been tested with regard to an anti-AD potential. In a rat model, where AD-like symptoms were evoked by treatment with D-galactose and Aβ, oligosaccharide extracted from *Morinda officinalis* (OMO) was administered orally for four weeks [19]. This resulted in attenuation of learning and memory deficits of the AD model as shown by Morris Water Maze test, recovering of control cytokine levels in blood and decreased levels of Aβ1-42 and Tau proteins. By administration in an inflammatory bowel disease model, OMO proved its prebiotic character. Probiotic *Lactobacillus* strains rescued the rough eye phenotype that is found in AD-induced *Drosophila* [20]. ProBiotic-4, a probiotics mixture of *Bifidobacterium lactis*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus* was capable of attenuating disruption of the intestinal barrier and blood–brain barrier and improved memory deficits in aged SAMP8 mice [21]. This inbred strain shows an accelerated aging phenotype and resembles several pathological hallmarks of AD (for example [22]).

Aim of our study was to compare the probable efficacy of either antibiotics treatment or probiotics administration on AD-like pathology within the 5xFAD mouse model in a direct, parallel comparison approach. The 5xFAD mouse model is a rather aggressive and fast developing model with the occurrence of first plaques at the age of 1.5 months [23]. Therefore, we chose to start the treatment paradigm at an age of four weeks and to proceed up to the age of 18 weeks where first behavioral deficits can be observed.

2. Materials and Methods

2.1. Animals

B6SJL-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax (5xFAD) mice (Jackson Lab, Bar Harbor, Maine, USA) were stably crossbred on a C57BL/6J background. The animals were group-housed (three to five animals) until the start of the experiment, then they were single-caged to prevent coprophagy. Animals were kept with a 12 h day/night cycle; food and water were available *ad libitum*. All procedures were performed in accordance with the European Communities Council Directive regarding care and use of animals for experimental procedures and were approved by local authorities (Landesuntersuchungsamt Rheinland-Pfalz; approval number G17-1-035, approval date June 2017). Germ-free C57BL/6J that were used for assessing viability of probiotic bacteria after stomach passage were maintained as colonies in sterile flexible film mouse isolator systems at

the Translational Animal Research Center (TARC) [24,25]. The germ-free status of the mice was verified every second week by 16S rDNA PCR and by bacterial culture testing.

2.2. Treatment of Mice via Drinking Water

All animals received food (Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water *ad libitum*. At an age of 4 weeks, animals received an antibiotics mixture as described previously [18]. We assumed a maximal gavage volume of 500 µL and adopted for a mean drinking volume of 4 mL the following concentrations: gentamicin (0.1251 mg/mL), vancomycin (0.0635 mg/mL), metronidazole (0.25 mg/mL), neomycin (0.0635 mg/mL), ampicillin (0.1251 mg/mL), kanamycin (0.3753 mg/mL), colistin (7,506,000 U/mL), and cefoperazone (0.1251 mg/mL). Metronidazol, vancomycin, ampicillin, cefoperazone, and kanamycin were obtained from Cayman (Ann Arbor, Michigan, MI, USA), gentamicin and neomycin from Sigma; colistin from J&K Scientific (Beijing, China). The animals were kept for two weeks on the high dosage, then dosage was reduced to 1/50 and animals maintained on the treatment for a further 12 weeks. Control animals received tap water only, probiotics-treated animals received 10^9 CFU/mL (OptiBac for those on antibiotics, OptiBac Probiotics HQ Wren Laboratories Ltd., Hampshire, UK, contains *L. acidophilus* and *L. rhamnosus*). The water was exchanged two-times a week, animals were treated for 14 weeks in total. Consumption of drinking water was indistinguishable between the three groups (controls: 4.6 $\pm$ 1.2 mL, antibiotics group: 4.8 $\pm$ 1.2 mL, probiotics group: 4.2 $\pm$ 0.7 mL).

2.3. Viability of Lactobacillaceae in Stomach Content

From germ-free mice, 10 mg of stomach content were collected during sacrifice, dissolved in 750 µL PBS, and incubated for 10 min at 600 rpm at room temperature. OptiBac powder, which served as the probiotic treatment, was dissolved in PBS to reach 400 CFU/mL. Equal volumes of bacterial suspension were diluted 1:1 either with PBS (positive control) or with stomach content. Stomach content diluted 1:1 with PBS served as a negative control. All samples were incubated for 70 min (estimated stomach passage of mice, [26]) at 500 rpm and 37 °C.

2.4. Bacteria Plating and CFU Assessment

Samples were diluted appropriately with sodium chloride and plated on 3M™ Petrifilm plates (3M Deutschland GmbH, Neuss, Germany) for *Lactobacillaceae* and *Enterobacteriaceae*. Colony forming units were counted after 20 h incubation at 37 °C.

2.5. Nest Building Test

The nest building test was performed as described previously [27]. In brief, animals were habituated to a special nesting material and received 10 g of nesting material for overnight nest building. The next morning before 9:00 a.m., nest quality was scored and material not integrated into the nest was weighed.

2.6. Sacrifice and Tissue Preparation

Animals were weighed, anesthetized with isoflurane (Piramal, Mumbai, India), and sacrificed by decapitation. Truncal blood was collected and blood sugar immediately measured from one droplet by a blood glucose guide meter (Accu-Chek, Roche, Basel, Switzerland). The residual blood was used for serum preparation by two consecutive centrifugation steps (first time at 680× *g*, second time at 15,680× *g*, both for 10 min at 10 °C). Thymus, adrenal glands, liver, spleen, epididymal fat and colon were dissected and weighed or measured (colon length). The brain tissue was collected as hemispheres without olfactory bulbs and the left hemisphere drop-fixed in 4% formaldehyde for 24 h.

2.7. Immunohistochemistry and Densitometric Analysis

IHC sections were stained with anti-APP antibody 6E10 (Covance) as described previously [28]. For the densitometric analysis, two sections per mouse were used (total magnification of 40×). Five areas were determined to be measured. All areas were corrected for the value of the background area. For cortical tissue, two distinct areas were analyzed and mean value of both measures was used. Experimenters were blinded for the treatment of the mice during the analysis. Microscopic pictures of the IHCs were acquired by an EVOS XL microscope (Life Technologies, Darmstadt, Germany). AIDA image analyzer 4.26 software (Raytest, Straubenhardt, Germany) was used for quantitative analysis.

2.8. Measurement of Serum Insulin and Glucagon

Serum was prepared from truncal blood by two consecutive centrifugation steps after a minimum clotting time of 45 min. Samples were stored at −80 °C until further usage. 10 (Insulin ELISA) and 25 (Glucagon ELISA) µL were subjected to analysis following the vendor's recommendations (Mercodia, Uppsala, Sweden).

2.9. Western Blotting

Right brain hemispheres were homogenized in Tris HCl buffer supplemented with proteinase inhibitor cocktail (Roche complete mini) by using the Tissue Lyzer (Qiagen, Hilden, Germany). Proteins (40 µg per lane, determined by Roti Nanoquant reagent) were separated on 10% SDS PAA gel and blotted onto nitrocellulose. Non-specific binding was blocked with 0.2% I-Block (Thermo Fisher Scientific, Waltham, MA, USA) solution including 0.05% Tween20. Primary antibodies were as follows: anti-RAGE (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA) and XBP-1 (1:1000, Abcam, Cambridge, UK). As a loading control, GAPDH was detected (14C10, Cell Signaling, Danvers, MA, USA). Blots were incubated with respective secondary antibody coupled with horseradish peroxidase (Thermo Scientific, Karlsruhe, Germany) and signals obtained by administration of Super-Signal West Femto chemiluminescent substrate (Thermo Scientific, Karlsruhe, Germany). Chemiluminescence signals were captured using a CCD-camera imaging system (Raytest, Straubenhardt, Germany) and densitometric analysis performed by using AIDA image analyzer 4.26 software (Raytest, Straubenhardt, Germany).

2.10. Statistical Analysis

For comparisons one-way analysis of variance (ANOVA) was performed followed by a post hoc test as indicated. p-values < 0.05 were considered statistically significant and results were presented as mean + SEM. Data analyses were performed using GraphPad Prism 8 (Graph Pad Software, La Jolla, CA, USA).

3. Results

3.1. Manipulation of the Gut Microbiome of 5xFAD Mice

The reduction of the gut microbiome within the APP/PS1 AD mouse model has been shown to exert a beneficial effect on hallmarks of the disease-like state within the animals [18]. All transgenic mouse models of AD display unique characteristics e.g., concerning the timeline of symptoms or severity of symptoms. We therefore wanted to analyze if the comparably aggressive 5xFAD mouse model might also benefit from such treatment. We modified the treatment paradigm by administering two weeks a high dosage and then providing a low maintenance dosage for another 12 weeks. Start point of the treatment were male mice aged four weeks (schematic of the experiment provided in Figure 1A). The relatively early age was chosen as first plaque depositions can already be observed at about 1.5 months of age in this mouse line [23]. To assess the influence of bacteria assumed to exert a probiotic function, 5xFAD mice were also treated with a mixture of *L. acidophilus* and *L. rhamnosus* (animals designated as probiotics group). As all treatments were conducted via drinking water and with this by oral passage, firstly the survival rate of *Lactobacilli* was tested using the stomach contents of germ-free mice

(Figure 1B). Bacteria were added to stomach contents or PBS and plated after 70 min incubation at 37 °C, which simulated time to enter the intestine in mice [26]. CFU obtained for bacteria incubated with stomach content was indistinguishable from numbers of control (PBS)-treated bacteria ($p = 0.97$). Therefore, these probiotic bacteria were sufficiently viable to enter and colonize the intestine after ingestion.

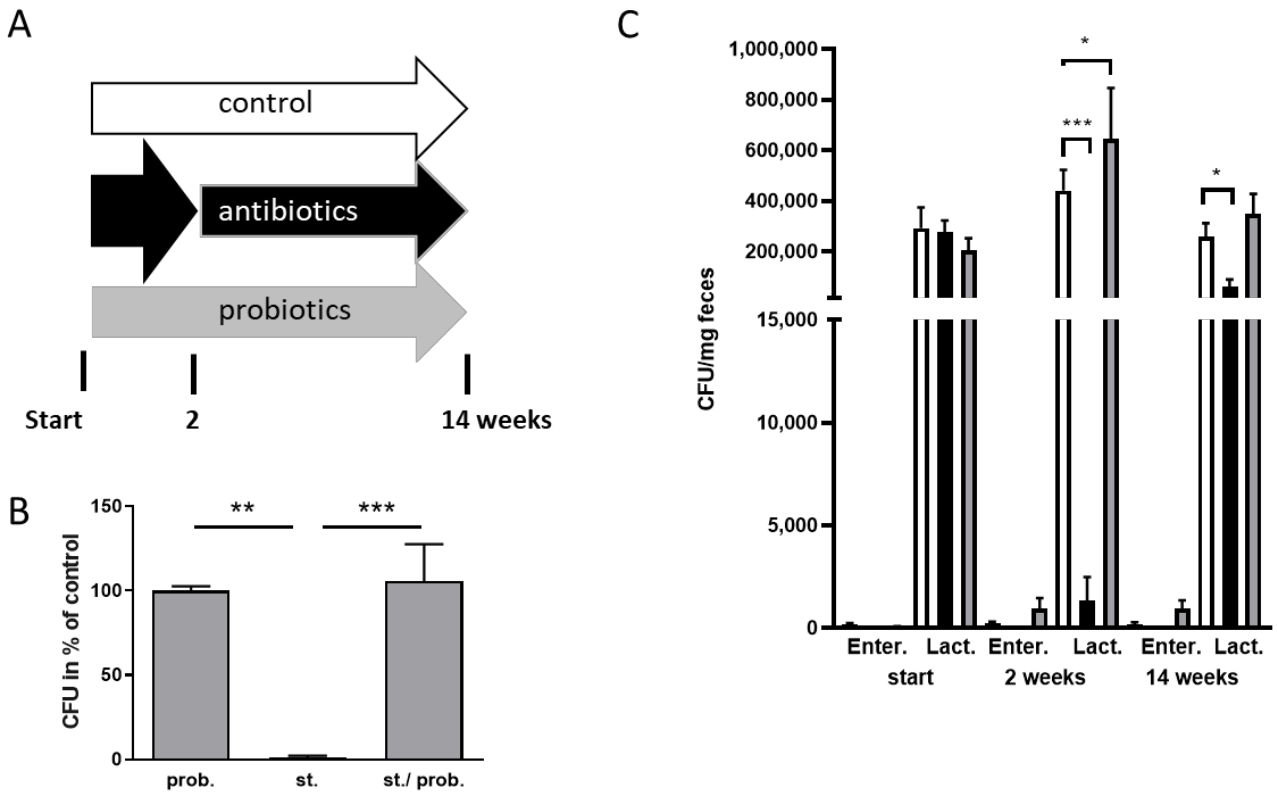

Figure 1. Manipulation of gut microbiota by application of antibiotics and probiotics with drinking water in 5xFAD mice. Male mice aged 4 weeks were subjected to three treatment groups: control, antibiotics or probiotics ($n = 8$ for control, $n = 7$ for pro- and antibiotics each). Within the antibiotics group two weeks of high dosage and 12 weeks of low maintenance dosage were applied via the drinking water (**A**, scheme). (**B**) To control for general viability of the orally administered probiotics within the intestinal passage, stomach contents from germ-free, gnotobiotic mice ($n = 18$) were spiked with a defined amount of the probiotic bacteria, incubated for 70 min and plated on *Lactobacillaceae*-specific plates. As a positive control, probiotic bacteria in solvent were used, (prob., $n = 8$); stomach contents from the animals diluted with solvent served as a negative control (st.). Colonies observed after 20 h were counted (colony forming units, CFU) and normalized to the positive control. (**C**) Feces from the animals of the three treatment groups were collected at the indicated time points, diluted with sodium chloride and plated on *Enterobacteriaceae*- and *Lactobacillaceae*-specific plates. CFU were related to the used fecal material amount. Data are presented as mean + SEM. Statistical analysis was performed by one-way ANOVA with the appropriate post-test (B: Tukey's, C: Fisher's LSD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

To monitor effect of the treatment by anti- and probiotics, fecal samples were analyzed after two weeks and at the end of the experiment (Figure 1C). As representatives, cultivatable *Enterobacteriaceae* and *Lactobacillaceae* were quantified by using specific plates. The latter family also served as the proof of probiotics colonization. Before start of the treatment, colonies from all three treatment groups were comparable in regard to both bacterial families. After two weeks, the high dosage of antibiotics resulted in a reduction of both, reaching significance for the *Lactobacillaceae*. In the probiotics-treated groups, *Lactobacillaceae* were significantly elevated, demonstrating a successful colonization by exogenously added members of this family. At the end of the treatment period, the antibiotics effect was still present even if the low dosage allowed growth of more bacteria than after the high dosage application. Within the probiotics-treated mouse group, an increase in

Lactobacillaceae was still measured; however, this did not reach significance (control: 259,286 CFU; probiotics group: 350,333 CFU; $p = 0.298$).

3.2. Effect of Gut Microbiome Alteration on Pathological Hallmarks of 5xFAD Mice

Assessing the quality of the nest built by the mouse, reports on well-being of the animal and on integrity of the hippocampus as has been demonstrated i.e., by hippocampal lesion experiments [29]. When scoring the quality of the nests shortly before the end of the experiment (14th week), better nests were obtained for antibiotics-treated 5xFAD mice and the integration of material was also optimized—even if the latter did not reach statistical significance (Figure 2A). An analysis of Aβ deposition in the hippocampal area confirmed the finding of ameliorated pathology (Figure 2B,C), while in cortical areas only subtle, non-significant decrease of deposited material was observed, the dentate gyrus and subiculum both showed reduced staining intensities. Treatment with probiotics elicited no change in any of the investigated regions.

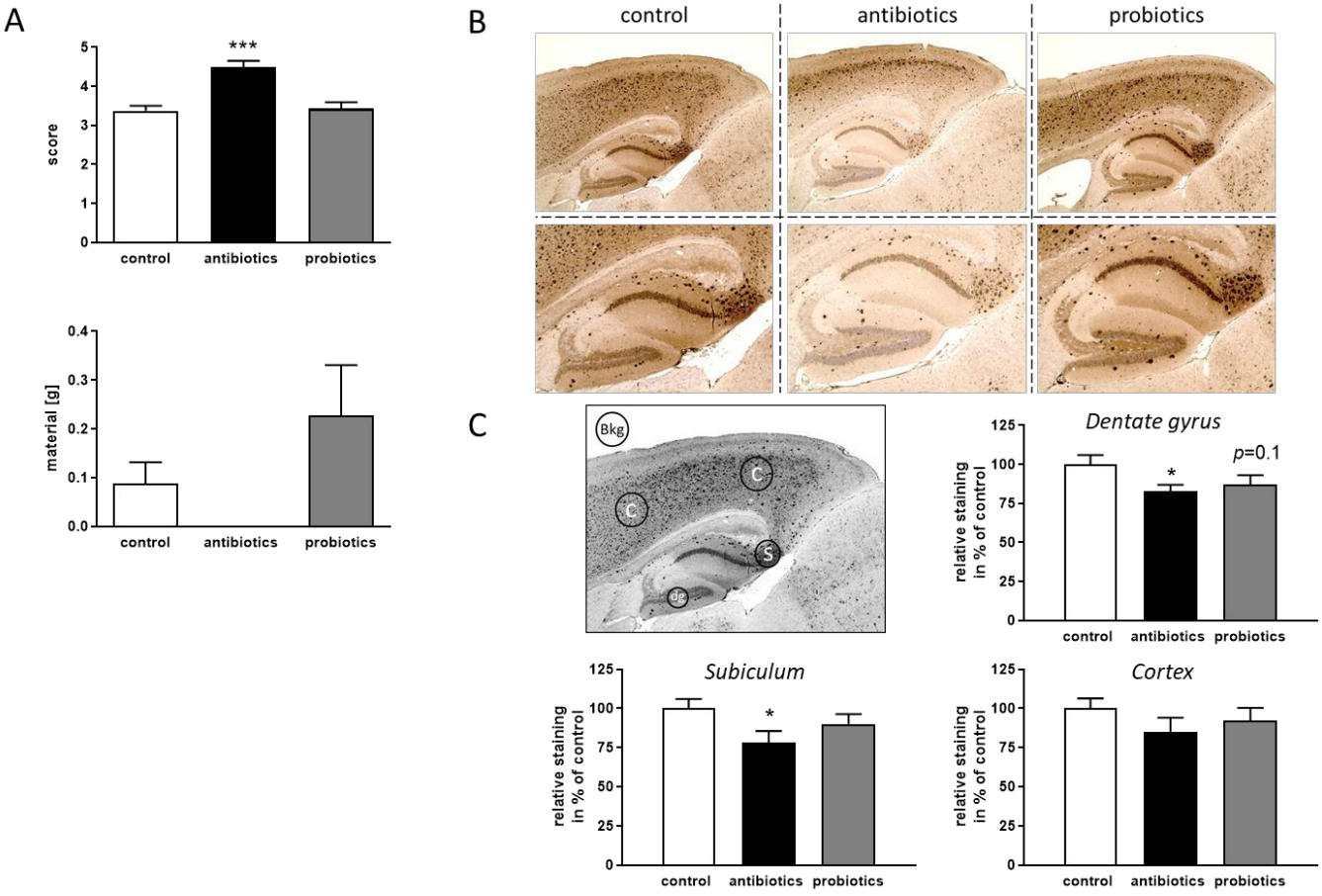

Figure 2. Influence of gut microbiota manipulation on pathological hallmarks of 5xFAD mice. (**A**) Within the 14th week of treatment, nest-building ability as a proxy for hippocampal function was assessed: quality of the nests (score) as well as the amount of not integrated material were measured. Statistics were performed with a one-way ANOVA and Tukey's post-test (***, $p < 0.001$). (**B**) After 14 weeks of treatment, animals were sacrificed and sagittal sections of the brain were stained for Aβ-depositions. The area of the hippocampus is shown magnified within the exemplary pictures. (**C**) Aβ-depositions were quantified densitometrically in the indicated brain regions of two independent slices per animal and corrected for background staining intensity (Bkg.). Pixel measures were normalized to the mean of control-treated animals and are depicted as percentage. Data are presented as mean + SEM. (Multiple t-test; *, $p < 0.05$; one sample from the antibiotics-treated animals was not analyzed due to technical reasons).

3.3. Antibiotics-Treatment of 5xFAD Mice Reveals Anti-Diabetic Properties

Both, depletion of the microbiome as well as the administration of probiotics, might reveal non-brain effects—e.g., on tissues relevant to immunity or hormone signaling. Therefore, additional parameters were assessed with sacrifice, such as thymus and spleen weight, adrenal gland weight, weight of abdominal fat pads (Supplementary Materials Figure S1) or body weight (Figure 3A). None of these were affected, either by antibiotics nor by probiotics when measures were compared to control animals. However, blood sugar levels were specifically decreased in the antibiotic-receiving mice (Figure 3B). As sacrifice of the mice was performed around 10 a.m., it must be assumed that the mice were at a fasting state (lights on in the animal facility was at 6 a.m.).

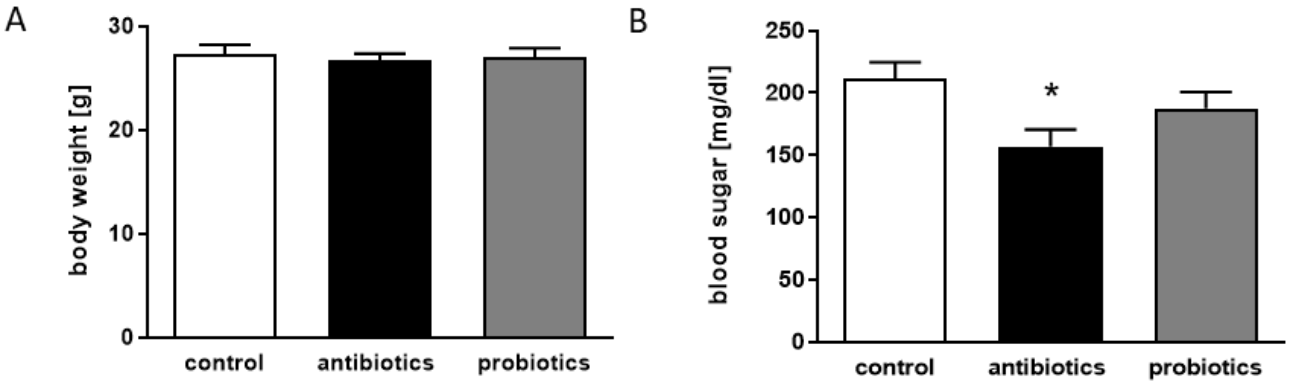

Figure 3. Antibiotics reduce blood sugar level of 5xFAD mice. (**A**) Body weight of the mice was measured after 14 weeks of the respective treatment. (**B**) Samples from truncal blood collected during sacrifice were used for quantification of blood sugar levels. Data are given as mean + SEM. Statistical analysis was performed by one-way ANOVA with Tukey's post-test (*, $p < 0.05$).

To ascertain that the observation was not related to altered feeding behavior, food consumption within the first two weeks and within the last 12 weeks of treatment was compared between control animals and treated groups (Figure 4A). Probiotics had no effect on food consumption overall. Antibiotics reduced food intake during the period of high dosage treatment by 0.5 g per day. However, within the subsequent 12 weeks on low dosage, this reduced level returned to control levels. Therefore, general hypophagia can be excluded as a cause for the observed reduction in blood glucose level.

For lean mice, a drop in blood glucose together with rise in glucagon and decrease in insulin levels has to be expected [30], while in obese or insulin resistant mice, for example, the fasting-induced decrease in serum glucose is blunted. When measuring insulin in both, samples from antibiotics- and probiotics-treated mice, a decrease in insulin was assessed that did not reach statistical significance ($p = 0.426$ and 0.209, Figure 4B). For glucagon, a significantly higher mean value was obtained in antibiotics-receiving animals as compared to control 5xFAD mice, while probiotics showed no effect. We therefore interpreted the observed effect as an anti-diabetic shift in blood glucose control as due to the 14 weeks administration of antibiotics.

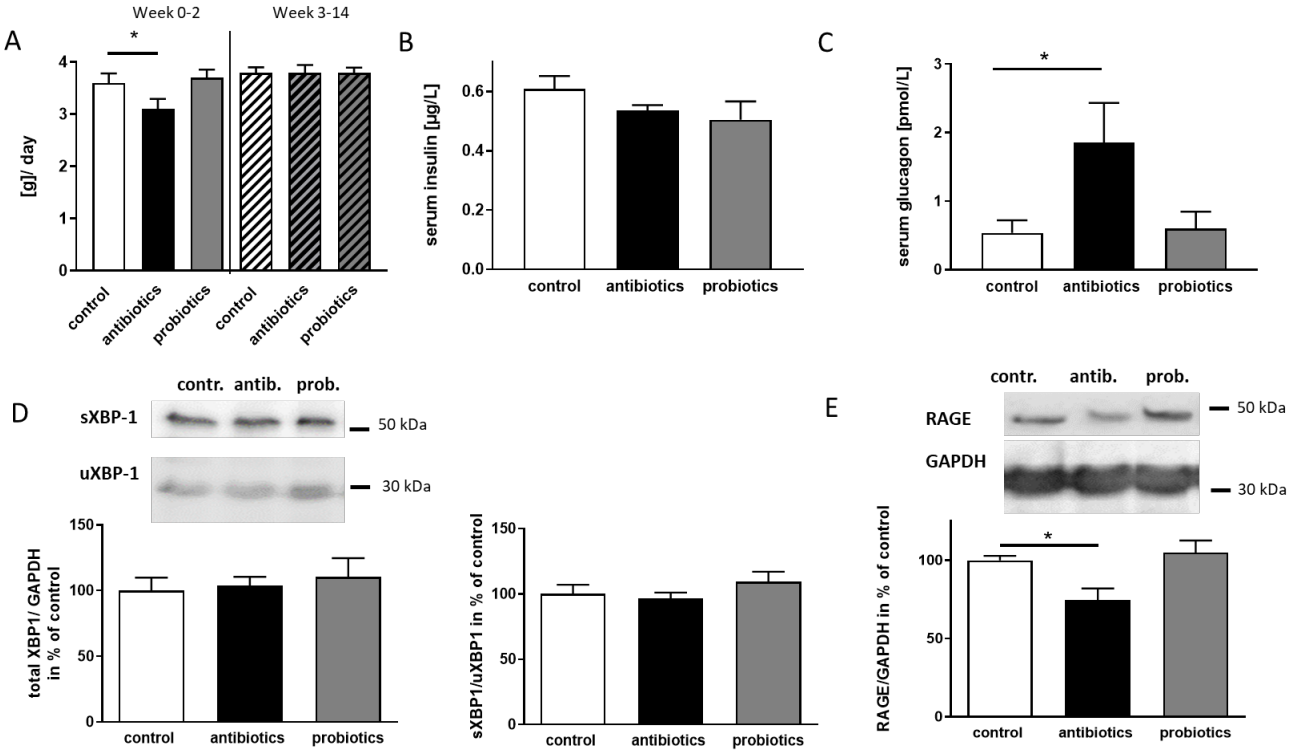

Figure 4. Effect of antibiotics on blood glucose homeostasis and insulin-dependent pathways. (**A**) Food consumption of the mice was measured twice a week. Columns without pattern show the food intake within the first two weeks, patterned columns show the food consumption within the weeks 3 to 14. (**B**) Serum insulin was measured by ELISA using 10 µL sample in duplicate. For glucagon (**C**) 25 µL were used in technical duplicates. (**D**) Splice variants of XBP-1 (S: spliced; u: unspliced) were quantified by Western blotting and ECL-based signal development due to appropriate HRP-labelled secondary antibody. (**E**) RAGE and GAPDH, which served as a loading control, were visualized as described ($n \geq 5$ per group for Western blots and ELISAs). Mean + SEM are presented. Statistics were performed with one-way ANOVA and Dunnett's post-test (*, $p < 0.05$).

Several pathways involved in AD pathology have been considered to depend on insulin [31], and type II diabetes is one of the risk factors for this disease in humans [32]. An altered microbiome might have favorable effects on each of the conditions, but also on the cross-talk between AD and a diabetic phenotype (reviewed in [33]). To understand if any of the known molecular mechanisms might be involved in the here observed pathology-ameliorating effects, we exemplary investigated XBP-1 and RAGE. The IRE1 α/XBP-1 pathway is activated by ER stress as, for example, evoked by unfolded proteins and results in a spliced mRNA. The resulting protein (sXBP-1) is double the size of the one encoded by the unspliced mRNA (uXBP-1, 30 kDa, [34]). Components of this pathway have been shown to function at an increased basal level in the presence of insulin resistance and hyperinsulinemia [35]. Moreover, sXBP-1 has been shown to act as a transcriptional activator of the α-secretase ADAM10 [36]. Quantitation of both, s- and u-XBP-1 revealed no elevated protein amounts—neither by anti- nor by probiotics treatment (Figure 4D). Moreover, no alteration in the ratio between both could be observed.

The formation and accumulation of advanced glycation end products (AGEs) is part of the normal aging process but occurs accelerated under diabetic conditions [37]. AGEs bind to RAGE, the receptor for AGEs and thereby lead to sustained NFκB activation and inflammation (for a review see [38]). Moreover, RAGE is also responsible for brain-directed transport of Aβ peptides from the periphery as a counterpart to LRP1 [39]. In antibiotics-treated 5xFAD mice, a reduction of RAGE by an amount of about 30% was assessed, while probiotics did not affect the expression of the receptor (Figure 4E).

4. Discussion

Within the 5xFAD mouse model, treatment with antibiotics over a period of 14 weeks, starting at an early pathological stage, resulted in an ameliorated pathogenesis while probiotics administration remained without effect. In addition, the antibiotics led to an anti-diabetic shift that also was accompanied by decreased expression of RAGE, which might have contributed to the beneficial property of the intervention.

For the probiotics treatment, we obtained a statistically significant increase in *Lactobacillaceae* in the early phase of the treatment. The selective plates used in the experiments allowed growth of *Lactobacillaceae*, the family that also includes the administered bacterial species *L. acidophilus Rosell-52* and *L. rhamnosus Rosell-11*. It can therefore be assumed that colonialization with the exogenously applied probiotics was successful, even if their amount was not specifically addressed. After 14 weeks of continuous administration, no significantly elevated *Lactobacillaceae* amount was noted anymore. However, it has to be stated that administration of probiotics not only affects the amount of the added species but also has far-reaching consequences for the whole community. This probiotics-driven effect on the gut microbiome has been demonstrated for several species and bacterial strains before [40–42]. Especially, beneficial effects against enteropathogens can be assumed by the potential to supersede these from the surface of host cells (demonstrated on Caco-2 cells, [43]). Moreover, additional positive effects of probiotic strains directly exerted on the hosts' intestinal cells have been reported, such as electrolyte absorption, prevention of intestinal damage by TNBS, and downregulation of Glut2 using rodent or cellular models [44–46]. Therefore, we in principle hypothesized a disease-ameliorating effect in 5xFAD mice by the administered probiotics, as has been shown for other models before. In senescence accelerated mouse models and in several attempts using Aβ-injection or other pharmacologically-derived rat models of AD, such probiotics improved memory deficits and ameliorated neuroinflammation [21,47–52]. In the murine model strain APP/PS1, recent publications also reported that *L. plantarum* administration (alone or in combination with *B. bifidum*, [53,54]) alleviated pathological symptoms. In one of the studies, however, the effect of *L. plantarum* was mainly noted to be an augmentation when administered together with memantine by inhibiting synthesis of the gut microbial metabolite trimethylamine-N-oxide, and decreasing neuroinflammation [54]. Within another study, the beneficial effect regarding cognitive performance was mainly seen in the approach where two strains (*L. plantarum* and *B. bifidum*) were combined [53].

SLAB51, a formulation made of nine live bacterial strains (containing *L. acidophilus*), was also able to positively affect several disease-associated pathways in 3xTg AD model mice [55]: for example, SIRT1 activity was increased in brain homogenates, as was the activity of antioxidant enzymes such as GST. However, cognitive improvement has not been shown in the respective investigation. In a meta-analysis, it was shown for 12 out of 16 studies, in which a direct comparison between single strain administration and treatment with probiotic mixtures was reported, that mixtures were more efficient in providing health-promoting effects [56]. In our study, we treated the 5xFAD mice with a mixture of two strains, *L. acidophilus Rosell-52* and *L. rhamnosus Rosell-11*, and at least *L. acidophilus* has been used successfully as a component of such mixtures before. Nevertheless, no significant effect on behavior, plaque deposition or metabolism was obtained in 5xFAD mice. Potentially, the concerted interplay of another probiotic here was missing that could not be compensated sufficiently by *L. rhamnosus*. Usage of probiotics in AD patients still is only a future perspective and data from human studies up to now have not been convincing: a small study in 30 patients per group described a positive effect of a *L. acidophilus*-containing probiotic mix on cognitive function and some metabolic parameters [57]. A recent meta-analysis, however, concluded from three randomized clinical trials, suiting the filter criteria and involving 161 individuals with AD, no benefit for cognitive function (all studies used *Lactobacillus* and *Bifidobacterium* strains, *L. acidophilus* contained in all, [58]). Interestingly, treatment resulted in improved plasma triglycerides and insulin resistance.

For antibiotics-based depletion of the microbiome of 5xFAD mice, a treatment regimen was applied that has been reported before [18] with slight deviations, namely a shorter treatment period (14 weeks instead of 6 months) and delivery via drinking water instead of gavage from the very beginning. After two weeks of high dose antibiotics, a strong reduction in viable *Lactobacilli* occurred, which flattened out with the end of the experiment. This coincides with the data obtained for APP/PS1 mice at the end of the treatment reported by Minter and colleagues [18]. No general reduction of prokaryotic fecal abundance as measured by PCR copy number was observed, but rather a shift in the composition. Moreover, a reduction in relative abundance in OTU reads for *Lactobacillaceae* was also found but it was not reported if this difference was statistically significant. A limitation of our study might be seen within the lack of a detailed microbiome analysis via sequencing which does not allow a direct comparison with the study initially describing, for example, the antibiotics treatment. However, our aim was more to investigate the outcomes concerning pathology in the model mice.

There are controversial opinions on the usage of antibiotics cocktails in comparison to germ-free raised animals. Both methods have their pros and cons as e.g., germ-free conditions might impact gut development (for example [25,59,60]) but antibiotics might not deplete bacterial commensals completely and have side effects [61]. By comparing both attempts in 5xFAD mice, both strategies revealed lowered plaque burden in the hippocampus in mice and ameliorated behavioral deficits, however, microglial activation was selectively found in germ-free raised animals [62]. The antibiotics treatment regimen differed from the here used one (containing 1 mg/mL vancomycin, 1 mg/mL cefoxitin, 1 mg/mL gentamicin and 1 mg/mL metronidazol for two months). As an investigation in APP/PS1 mice demonstrated, however, a single administered antibiotic drug is not effective, but rather a mixture is [63]. This study also reported that only minor amounts of the administered drugs could be found in brain parenchyma. In particular, only 3% of metronidazole was found by LC-MS, while all other antibiotics were below detection range. This indicates that the drugs cannot exert a direct effect on pathomechanisms localized in the brain. Interestingly, Zarrinpar and colleagues described that oral gavage of C57BL/6 mice with antibiotics (ampicillin 100 mg/kg, vancomycin 50 mg/kg, metronidazole 100 mg/kg, neomycin 100 mg/kg, and amphotericin B 1 mg/kg, every 12 h for maximally 30 days, [64]) not only altered the microbiome indicated by a decrease in Firmicutes and Bacteroidetes. It consequently also affected bacterial metabolites, namely decreasing luminal short-chain fatty acids (SCFA) such as butyrate, and the secondary bile acid pool. This enforced the colonocytes of the hosts gut to shift from metabolizing SCFA towards glucose consumption and by this altered glucose homeostasis. For example, blood glucose levels were reduced both after short and extended fasting in the mice. The same observation was made in our study, where a reduction of blood glucose level of about 25% was observed in animals that were sacrificed 3 h after entering the period of rest where no food intake was expected. The elevated glucagon and the decreased insulin level furthermore underlined the anti-diabetic effect, even if the latter did not get statistically significant. A strong correlation of reduction of SCFA-producing bacteria was found in adult wild type mice receiving fecal transplantation from old donor mice in a recent publication [65]. This went along with, for example, an ageing-like phenotype of microglia in the CNS of the animals and could potentially hint at a deleterious effect of the observed age-related change in the microbiome. However, our study is based on a disease model, which comprises a completely different baseline condition.

To understand what aspect of the anti-diabetic effect of the antibiotics cocktail might be involved in ameliorating behavioral impairment of the 5xFAD mice, we investigated two exemplary pathways that are closely linked to glucose metabolism/insulin signaling: the XBP-1-linked ER-stress response and expression of RAGE. XBP-1 splicing and thereby activation of the transcription factor probability was considered as it has previously been identified as an alpha-secretase gene expression enhancer [36]. Stimulation of alpha-secretase ADAM10 expression and by this elevation of enzymatic activity could have

explained the reduced Aβ deposition and in consequence improved the cognitive function of the mice (e.g., as shown by the pharmacological inducer acitretin, [27]). However, neither the amount of XBP-1 derived from the unspliced mRNA nor the protein derived from the spliced mRNA was affected in the brains of antibiotics-treated 5xFAD mice. In contrast, the RAGE protein amount was decreased. This receptor is able to transport Aβ peptides over the blood–brain barrier from the periphery, and thereby leads to an increase in cerebral amyloid load. Its blockade therefore has been assumed to be of therapeutical value [66,67]. This result corresponds with a study using type two diabetic male db/db mice [68]: chronic administration of antidiabetic drugs such as metformin or glibenclamide decreased Aβ influx across the blood–brain barrier by decreasing RAGE abundance.

In sum, we here provide evidence that antibiotics might elicit a beneficial effect on AD pathology by their anti-diabetic potential and the subsequent drop in the influx of Aβ. This once more demonstrates the complexity of studies on the involvement of the microbiota in non-gut disorders. The presence and composition of the commensal community has a multitude of systemically relevant effects on the host that in second line may affect molecular mechanisms at the border to or within the brain.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/microorganisms9040815/s1, Figure S1: Organ weights and colon length of 5xFAD mice treated with antibiotics or probiotics.

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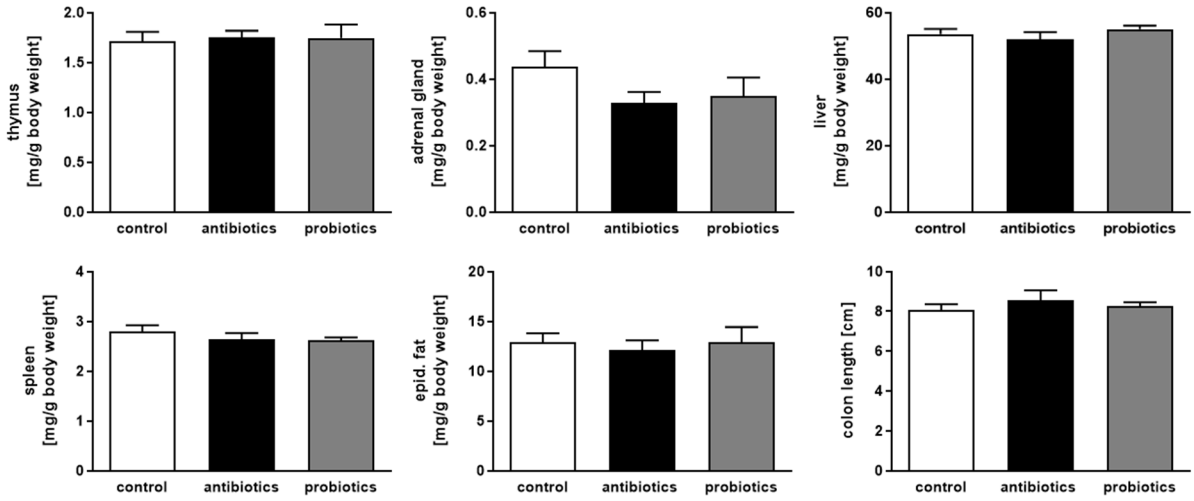

Suppl. Figure 1: Organ weights and colon length of 5xFAD mice treated with antibiotics or probiotics

Male mice (n=8 for control, n=7 for anti- or probiotics) were sacrificed after 14 weeks of treatment via drinking water at an age of 18 weeks. The respective organs were dissected, weighed and normalized for body weight. Colon was dissected and length from caecum ampulla to the rectum measured. Statistical analysis was performed by one-way ANOVA with Tukey's post-test. No significant differences occurred. For epididymal fat, two values had to be eliminated due to technical reason (one from control, one from probiotics group).

Malena dos Santos Guilherme

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