AUS DEM LEHRSTUHL
FÜR PSYCHIATRIE UND PSYCHOTHERAPIE
PROF. DR. RAINER RUPPRECHT
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

FUNCTIONAL PARCELLATION OF THE HUMAN HIPPOCAMPUS

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

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German Summary

1. Einleitung und Hintergrund

Eine faszinierende Gemeinsamkeit zwischen einigen, auf den ersten Blick recht unterschiedlichen Krankheitsbildern wie Morbus Alzheimer, Schizophrenie, Depression und posttraumatischer Belastungsstörung ist die Beeinträchtigung derselben Gehirnregion im medialen Temporallappen: des Hippocampus (Abbildung 1). Es ist bemerkenswert, dass derart breit gefächerte und vielgestaltige Symptome, wie die der genannten Krankheiten, allesamt mit der gestörten Integrität einer gemeinsamen Region in Verbindung gebracht werden können. Diese hippocampale Involviertheit in einer Vielzahl von neuropsychiatrischen Erkrankungen spiegelt sich in dessen Teilnahme an zahlreichen unterschiedlichen Gehirnfunktionen wider: Der Hippocampus ist neben der Gedächtnisbildung auch in Funktionen wie räumliche Navigation und emotionale Verarbeitungsmechanismen involviert. Zusätzlich zu diesen weitgehend anerkannten Funktionen gibt es Hinweise auf eine Implikation des Hippocampus in verschiedenen weiteren Vorgängen, wie unter anderem Wahrnehmung und Vorstellungsvermögen.

Doch wie schafft es der Hippocampus als relativ kleine, strukturell weitgehend homogene Gehirnstruktur, eine solche Vielfalt an Funktionen und pathologischen Implikationen zu unterhalten? Bisherige Studien weisen darauf hin, dass sich die funktionelle Diversität in einer intrinsischen Organisation der hippocampalen Längsachse widerspiegelt. Demnach sind anteriorer und posteriorer Teil des Hippocampus in verschiedene Funktionen eingebunden, wobei der Übergang zwischen diesen beiden funktionell distinkten Polen bisher allerdings ungeklärt ist. Einerseits werden Modelle diskutiert, die einen kontinuierlichen funktionellen Gradienten vorschlagen, welche andererseits mit Hypothesen einer modularen Struktur in diskreten Untereinheiten konkurrieren (Abbildung 3). Die Klärung dieser Frage würde es langfristig nicht nur ermöglichen, den präzisen Einfluss des Hippocampus auf kognitive Vorgänge im gesunden Gehirn zu charakterisieren, sondern auch seine Rolle in der Pathophysiologie krankhafter Prozesse zu verstehen und womöglich bessere Therapieoptionen zu entwickeln.

Die vorliegende Arbeit knüpft an diese Fragestellung an und zielt darauf ab, das dominante Muster funktioneller Organisation innerhalb des Hippocampus zu identifizieren. Zu diesem Zweck wurde die Gehirnaktivität gesunder Probanden[1] mittels hochauflösender funktioneller Magnetresonanztomografie (fMRT) während eines Navigationsexperiments untersucht. Mithilfe eines kürzlich entwickelten Analysealgorithmus (Haak et al., 2018) wurde das funktionelle Verhalten des Hippocampus während einer Navigationsaufgabe in Beziehung zum Verhalten des restlichen Gehirns gestellt, um individuelle Interaktionsmuster hippocampaler Neuronenverbände zu ermitteln. Diese sogenannten Profile funktioneller Konnektivität wurden quantifiziert und untereinander verglichen, um die Topografie der Ähnlichkeit funktioneller Konnektivität innerhalb des Hippocampus zu identifizieren. Die berechnete Topografie entspricht dem dominanten Organisationsmuster funktioneller Konnektivität im Hippocampus und diente als Ausgangspunkt für eine Parzellierung des Hippocampus in funktionelle Untereinheiten.

2. Material und Methoden

Um die funktionelle Organisation im Hippocampus zu untersuchen, analysierten wir fMRT-Daten mit einer ultrahohen Auflösung im Submillimeterbereich mithilfe des sogenannten *connectopic mapping* Algorithmus. Der verwendete 7 Tesla fMRT-Datensatz wurde bereits für ein vorangegangenes Projekt erhoben und vorverarbeitet (Navarro Schröder et al., 2015).

2.1 Experimentelles Design

Der Datensatz besteht aus den fMRT-Scans von 22 gesunden, erwachsenen Probanden (Alters- und Geschlechtsverteilung in Abbildung 7). Da mithilfe dieser Daten Rückschlüsse über die funktionelle Organisation des Hippocampus gezogen werden sollten, war es notwendig, während des Scannens sicherzustellen, dass der Hippocampus der Probanden funktionell aktiv ist. Daher führten die

[1] Zur besseren Lesbarkeit wird in der folgenden Arbeit auf die gleichzeitige Nennung männlicher und weiblicher Sprachformen verzichtet. Es wird das generische Maskulinum verwendet, wobei alle Geschlechtsidentitäten gleichermaßen gemeint sind.

Versuchsteilnehmer im Scanner eine experimentelle Aufgabe durch, welche Navigationsverhalten und räumliches Gedächtnis erfordert und daher mit hoher Wahrscheinlichkeit die Aktivität des Hippocampus gewährleistet. Währenddessen wurde simultan die Gehirnaktivität mittels fMRT erfasst. Um während des Scannens ein Navigationsverhalten zu ermöglichen, bei dem die Versuchsteilnehmer in körperlicher Ruhe bleiben können, wurde die experimentelle Aufgabe mithilfe virtueller Realität umgesetzt (Doeller et al., 2008; 2010): Probanden benutzten einen Controller in ihrer Hand, um durch eine dreidimensionale, virtuelle Arena zu navigieren, welche auf einen Bildschirm innerhalb des Scanners in ihr direktes Blickfeld projiziert wurde. Sie hatten die Aufgabe, sechs verschiedene Objekte einzusammeln und sich deren assoziierte Positionen innerhalb der Arena zu merken. Nach dieser Phase des Einsammelns wurde ein visueller Stimulus in Form eines der zuvor eingesammelten Objekte präsentiert. Die Teilnehmer waren instruiert, daraufhin zu der Position zu navigieren, an der sie das jeweilige Objekt zuvor eingesammelt hatten, um dort eine bestimmte Taste auf dem Controller zu drücken. Unverzüglich erhielten die Probanden Rückmeldung mittels eines Emoticons (glücklich, neutral oder traurig), wie akkurat sie die mit dem Objekt assoziierte Position aufgesucht hatten, und sammelten danach das entsprechende Objekt erneut ein, um die Assoziation des Objektes mit der entsprechenden Position für folgende Versuche zu stärken (Abbildung 6).

2.2 Definition der *region of interest*

Für die Untersuchung neuronaler Aktivität im Hippocampus musste das gemessene fMRT-Signal innerhalb dieser Gehirnstruktur, der sogenannten *region of interest*, extrahiert werden. Dies erforderte eine dreidimensionale binäre Maske, welche die exakte Lokalisation und den Umriss des Hippocampus innerhalb unserer fMRT-Scans des gesamten Gehirns definiert. Eine solche Maske wurde unter sorgfältiger Befolgung der Instruktionen des Harmonisierten Protokolls für Hippocampale Segmentierung (Boccardi et al., 2015) auf der Basis unserer gruppenspezifischen fMRT-Bilder (sogenanntes *template*) manuell angefertigt (Abbildung 8).

2.3 Quantifizierung der Topografie funktioneller Konnektivität

Um mithilfe der fMRT-Daten die funktionelle Organisation des Hippocampus zu analysieren, verwendeten wir den kürzlich entwickelten *connectopic mapping* Algorithmus (Haak et al., 2018). Dieser Algorithmus verfolgt das Ziel, die Topografie der Ähnlichkeit funktioneller Konnektivität in einer Zielstruktur, hier im Hippocampus, zu quantifizieren, und kann in drei Schritte unterteilt werden.

Der erste Schritt des *connectopic mapping* Algorithmus diente dazu, das individuelle Muster funktioneller Konnektivität jedes einzelnen Voxels (= *volumetric pixel*, d.h. kleinste räumliche Einheit eines MRT-Datensatzes, für die ein Signal gemessen wird) innerhalb des Hippocampus mit dem restlichen Gehirn zu bestimmen. Funktionelle Konnektivität wird in der funktionellen Bildgebung als statistisches Maß dafür verwendet, wie ähnlich sich die Aktivität zweier Voxel ist und gibt somit Auskunft darüber, wie stark die beiden betrachteten Voxel an denselben funktionellen Vorgängen beteiligt oder unbeteiligt sind (schematisch illustriert in Abbildung 5). Dies lässt allgemeine Rückschlüsse darüber zu, ob die jeweiligen Voxel ein ähnliches oder unähnliches funktionelles Verhalten zeigen und damit zum gleichen funktionellen Netzwerk bzw. der gleichen funktionellen Einheit gehören oder nicht (Friston et al., 1993). In der fMRT ist die funktionelle Konnektivität als Korrelation der fMRT-Signalzeitfolgen zweier Voxel definiert und kann Werte zwischen -1 (entsprechend inverser Zeitfolgen, d.h. gegensätzliches Aktivitätsprofil) und 1 (entsprechend identischer Zeitfolgen, d.h. äquivalentes Aktivitätsprofil) annehmen. Der erste Schritt des *connectopic mapping* Algorithmus zielte darauf ab, die funktionelle Konnektivität eines jeden Voxels innerhalb der Hippocampusmaske mit jeweils allen extrahippocampalen Voxeln zu quantifizieren. Hierzu wurden für jedes hippocampale Voxel hunderte Korrelationswerte berechnet, welche jeweils die Beziehung seiner Aktivitätszeitfolge mit der Zeitfolge jedes extrahippocampalen Voxels angeben (Abbildung 9B). Dies ergab einen individuellen Satz an Korrelationswerten für jedes hippocampale Voxel, welcher das einzigartige Muster funktioneller Konnektivität des entsprechenden Voxels zum restlichen Gehirn charakterisiert und auch Fingerabdruck funktioneller Konnektivität genannt wird.

Mithilfe dieser Fingerabdrücke sollte nun das intrinsische Organisationsmuster funktioneller Konnektivität im Hippocampus identifiziert werden. Genauer gesagt hatte

der *connectopic mapping* Algorithmus das Ziel, die hippocampalen Voxel entsprechend der Ähnlichkeit ihrer Fingerabdrücke funktioneller Konnektivität zu „ordnen“ bzw. zu bewerten, um dann das zugrundeliegende Organisationsmuster der Ähnlichkeit funktioneller Konnektivität im Hippocampus zu visualisieren. Zu diesem Zweck wurde im zweiten Schritt des Algorithmus zunächst die Ähnlichkeit zwischen den Fingerabdrücken funktioneller Konnektivität quantifiziert. Hierzu wurde für jedes Paar an hippocampalen Fingerabdrücken ein Ähnlichkeitswert berechnet (mathematisch definiert als η^2-Koeffizient), der Werte zwischen 0 und 1 annimmt, was keiner bzw. hoher Ähnlichkeit der entsprechenden Fingerabdrücke entspricht. Diese Werte wurden in einer sogenannten Ähnlichkeitsmatrix mit *n* Zeilen und *n* Spalten gespeichert, wobei *n* der Anzahl der hippocampalen Voxel entspricht (Abbildung 10).

Die Ähnlichkeitsmatrix enthält prinzipiell die relevante Information, die für das Ableiten des intrinsischen hippocampalen Organisationsmusters notwendig ist. Allerdings ist die Interpretation dieser Matrix komplex, weswegen der dritte und letzte Schritt des *connectopic mapping* Algorithmus darin bestand, die Komplexität der Ähnlichkeitsmatrix mittels des sogenannten *Laplacian Eigenmaps* Algorithmus (Belkin & Niyogi, 2003) zu reduzieren und der enthaltenen Information wieder einen räumlichen Bezug zu verleihen (Abbildungen 11 und 12). Das finale Resultat dieses Schrittes war eine topografische Darstellung des Hippocampus, in welcher jedes einzelne hippocampale Voxel einen Wert innerhalb einer Skala von funktioneller Organisation zugeschrieben bekam. Statt also wie in der Ähnlichkeitsmatrix jedes Paar aus hippocampalen Voxeln mit einem Wert (d.h. η^2-Koeffizienten) zu charakterisieren, zielte dieser Schritt darauf ab, jedem individuellen Voxel einen Wert zuzuschreiben, der die Position dieses Voxels innerhalb der zugrundeliegenden Topografie der Ähnlichkeit funktioneller Konnektivität angibt. Diese Werte können dann als dreidimensionale Karte, sogenannte *connectopic map*, des Hippocampus dargestellt werden, welche die Beurteilung der dominanten, topografischen Organisationsstruktur der funktionellen Konnektivität aller hippocampalen Voxel ermöglichte.

Die beschriebenen Analyseschritte wurden für den Datensatz eines jeden Versuchsteilnehmers durchgeführt, sodass insgesamt 22 probandenspezifische *connectopic map*s resultierten. Zudem implementierten wir einen Mittelungsprozess, sodass für die Beurteilung der Gesamtheit aller Probanden eine gruppenspezifische *connectopic map* zur Verfügung stand.

2.4 Validierung einer potenziell modularen Organisation

Auf Basis der erhaltenen *connectopic maps* wollten wir herausfinden, ob die identifizierte hippocampale Organisation einem kontinuierlichen Gradienten folgt oder im Gegenteil klar abgrenzbare, modulare Untereinheiten enthält. Um dies im Detail zu untersuchen, wurden die *connectopic maps* als Histogramme abgebildet, in welchen die absolute Anzahl hippocampaler Voxel (y-Achse) gegen den jeweils attribuierten Wert innerhalb des Organisationsmusters funktioneller Konnektivität (x-Achse) aufgetragen wurde. Wie in Abbildung 13 schematisch illustriert, kann anhand des Verteilungsmusters im Histogramm auf eine kontinuierliche oder diskrete Organisation rückgeschlossen werden. Eine diskrete Organisation in funktionellen Modulen würde sich beispielsweise in einigen deutlichen Maxima im Histogramm zeigen, welche durch mehrere Histogrammklassen, deren y-Werte nahe Null liegen, getrennt wären. Bevor allerdings die Schlussfolgerung einer relevanten modularen Organisation aus der Existenz klar trennbarer Maxima in den Histogrammen gezogen und auf dieser Grundlage weitere Analysen durchgeführt würden, war es notwendig, die Validität dieses Rückschlusses sicherzustellen. Daher wollten wir testen, ob die Höhe der beobachteten Maxima in unserer Stichprobe tatsächlich einer biologisch relevanten Organisation entsprach oder möglicherweise auch durch Zufall entstehen könnte. Daher generierten wir eine Nullverteilung von Histogramm-Maxima, mit der wir die Maxima unserer Probanden verglichen. Dies wurde mithilfe einer zufälligen Durchmischungsprozedur auf Ebene der originalen fMRT-Datensätze verwirklicht: In den Daten aller Probanden wurden jeweils die Aktivitätszeitverläufe einzelner Voxel zufällig zeitlich und örtlich durchmischt, um die grundlegenden statistischen Eigenschaften von realen fMRT-Daten zu erhalten, aber die Integrität realer Zeitverläufe und damit funktioneller Organisation vollkommen zu zerstören. Die durch dieses Durchmischungsverfahren produzierten Datensätze wurden genauso wie die Probandendaten mittels des *connectopic mapping* Algorithmus prozessiert. Die resultierenden c*onnectopic maps* wurden anschließend als Histogramme geplottet. Um die Höhe der Maxima zwischen realen und simulierten Daten zu vergleichen, identifizierten wir die zehn höchsten Maxima innerhalb eines jeden realen und simulierten Histogramms: Aus jedem Histogramm wurden die Maximalwerte für das höchste Maximum, für das zweithöchste Maximum, usw. bis zum zehnthöchsten Maximum extrahiert. Somit erhielten wir für den Maximalwert jeder Größenordnung

(vom höchsten bis zum zehnthöchsten Maximum) eine Nullverteilung, mit der wir den entsprechenden gemittelten Wert der Histogramm-Maxima unserer Probanden verglichen. Dadurch wollten wir die Frage beantworten, ob bzw. welche Maxima innerhalb der Organisation funktioneller Konnektivität unserer Versuchsteilnehmer die durch Zufall erwartbaren Maximalwerte übersteigen.

2.5 Funktionelle Parzellierung des Hippocampus mittels *k*-Means-Algorithmus

Neben der Beleuchtung und Validierung des Musters funktioneller Organisation im Hippocampus hatten wir uns das weitere Ziel gesetzt, die *connectopic maps* im Falle einer enthüllten modularen Organisation als Basis für eine funktionelle Parzellierung zu verwenden, um potenziell klar abgrenzbare hippocampale Untereinheiten zu visualisieren. Daher wandten wir eine Clusteranalyse, den sogenannten *k*-Means-Algorithmus (implementiert in MATLAB (2017)), auf die erzielten *connectopic maps* an, um basierend auf einem potenziell modularen Organisationsmuster funktionelle Cluster innerhalb des Hippocampus zu identifizieren. Die Wahl der Anzahl der Cluster *k* wurde mithilfe der sogenannten Ellbogenmethode objektiviert (Kodinariya & Makwana, 2013), deren Details im englischen Kapitel 2.8 ausgeführt sind.

3. Ergebnisse

Die beschriebenen Analysen zielten auf die Beantwortung zweier Schlüsselfragen ab: Erstens, folgt die Organisation funktioneller Konnektivität des Hippocampus einem graduellen, gleichmäßigen Gradienten oder weist diese diskrete, klar abgrenzbare Untereinheiten auf? Falls unsere Ergebnisse die letztere Hypothese einer modularen Organisation bestätigten, wollten wir als Zweites herausfinden, wie viele hippocampale Untereinheiten unterschieden werden und ob die Module in einer kohärenten Parzellierung visualisiert werden können.

3.1 Funktioneller Gradient entlang der Längsachse im Gruppendurchschnitt

Um die erste Frage zu beantworten, betrachteten wir zunächst die *connectopic maps* unseres Gruppendurchschnitts, welcher die analysierten fMRT-Daten aller 22 Probanden beinhaltet. Abbildung 14 zeigt die entsprechenden *maps* des linken

sowie rechten Hippocampus im Sagittalschnitt. Die rot-gelbe Farbskala visualisiert die topografische Organisation der Profile funktioneller Konnektivität des jeweiligen hippocampalen Voxels zur grauen Masse des restlichen Gehirns. Bezüglich der Interpretation bedeutet dies, dass zwei Voxel mit verschiedenen Farben sehr unterschiedliche Fingerabdrücke funktioneller Konnektivität aufweisen, während umgekehrt Voxel mit sehr ähnlichen Farben ein sehr ähnliches Konnektivitätsprofil zeigen. Im Gruppendurchschnitt zeigt sich insofern in den Hippocampi beider Hemisphären ein funktioneller Gradient entlang der hippocampalen Längsachse, als dass sich die Profile funktioneller Konnektivität von Voxeln im anterioren Pol deutlich von denen im posterioren Pol unterscheiden. Nachdem wir somit die Längsachse als dominante Achse funktioneller Organisation identifiziert hatten, galt es konkret den Übergang von anteriorem zu posteriorem hippocampalen Pol zu untersuchen, um herauszufinden, ob der funktionelle Übergang als kontinuierlich oder diskret zu charakterisieren ist. Zu diesem Zweck wurden in Abbildung 15 die Werte innerhalb der Topografie funktioneller Konnektivität der *connectopic maps* auf der x-Achse aufgetragen, um in Form eines Histogramms auf der y-Achse zu visualisieren, wie vielen Voxeln der jeweilige Wert zugeschrieben wurde. Interessanterweise zeigt sich in den gruppenspezifischen Histogrammen weder ein eindeutig kontinuierliches noch ein klar diskretes Muster: Einerseits wurde jeder Wert an mindestens etwa 50 Voxel vergeben, was auf einen graduellen Übergang hindeutet, andererseits finden sich innerhalb dessen kleinere Spitzen, was möglicherweise mit funktionellen Modulen vereinbar wäre. Die Gruppenresultate liefern also keine klare Aussage bezüglich der oben formulierten ersten Forschungsfrage.

3.2 Diskrete Organisation einzelner Probandendaten

Es ist möglich, dass der Mittelungsprozess über 22 Probanden die eigentlichen Topografien einzelner Probanden verfälscht und somit der Gruppendurchschnitt der Individualität eines jeden Einzelnen nicht gerecht wird. Dies leuchtet insbesondere ein, wenn man bedenkt, dass im Falle einer modularen Organisation die Grenzen zwischen funktionellen Untereinheiten nicht zwingend für alle Individuen an der exakt gleichen Stelle liegen müssen. Daher ist es notwendig, die *connectopic maps* individueller Probanden zu untersuchen, welche in Abbildung 16 in Form der bereits eingeführten Histogrammdarstellung illustriert sind. In der Tat wird ein gänzlich anderes Muster als

im Gruppendurchschnitt deutlich: In beiden Hippocampi des exemplarisch abgebildeten Probanden zeigen sich klar abgrenzbare Maxima, welche durch Segmente, die nahezu auf der Nulllinie liegen, getrennt sind (Histogramme aller Probanden in Abbildung 22 A und B). Bezüglich unserer ersten Frage spricht dies dafür, dass die Topografie funktioneller Konnektivität in einzelnen Probanden diskret verläuft und in funktionellen Modulen organisiert sein könnte.

3.3 Validierung der Modularität

Die oben beschriebene Schlussfolgerung einer modularen hippocampalen Organisation basiert auf dem Vorhandensein abrupter Maxima in der Histogrammdarstellung der *connectopic maps*. Um die Validität dieser Schlussfolgerung sicherzustellen, ist es notwendig zu klären, ob solche beobachteten Maxima nicht auch durch Zufall entstehen könnten. Daher generierten wir mithilfe einer zufälligen Durchmischungsprozedur eine Nullverteilung von Histogramm-Maxima und verglichen die Maximalwerte der Histogramme unserer Probanden mit der 95sten Perzentile der entsprechenden Maxima in den Histogrammen der simulierten Daten (illustriert in Abbildung 17). Genauer gesagt führten wir *t*-Tests durch zwischen den Werten des höchsten Maximums, den Werten des zweithöchsten Maximums usw. bis zum zehnthöchsten Maximum. Diese Herangehensweise erlaubt uns nicht nur, die generelle Validität unserer Argumentation zu prüfen, sondern könnte auch einen Hinweis auf die Anzahl der hippocampalen Module geben, falls nicht alle Werte von höchstem bis zehnthöchstem Maximum signifikant höher sind als die Nullverteilung (oben formulierte Forschungsfrage 2). Wie Abbildung 18 sowie die *p*-Werte in Tabelle 1 verdeutlichen, zeigen sich signifikant höhere Maximalwerte in den Probandendaten für das höchste und zweithöchste Maximum in den Hippocampi beider Hemisphären. Der viert- bis zehnthöchste Peak in den realen Daten ist jeweils entweder nicht signifikant unterschiedlich bzw. signifikant niedriger als in den simulierten Daten. Beim dritthöchsten Peak allerdings unterscheiden sich die Ergebnisse insofern leicht zwischen linkem und rechtem Hippocampus, als dass links der dritthöchste Peak entsprechend der 5 %-Signifikanzschwelle gerade noch signifikant höher als die 95ste Perzentile der Nullverteilung ist, während dieser rechts knapp oberhalb der Schwelle und damit nicht signifikant höher ist. Daher lässt sich schlussfolgern, dass die beiden höchsten Maxima mit hoher Wahrscheinlichkeit nicht

durch Zufall entstanden sind und einer realen diskreten Organisation entsprechen, während die Höhe des vierten bis zehnten Maximums die zufällig erwartbaren Werte nicht übersteigen und daher wahrscheinlich keine biologische Relevanz aufweisen. Bezüglich des dritten Maximums differieren die Resultate zwischen linkem und rechtem Hippocampus. Somit ist an dieser Stelle unklar, wie viele Untereinheiten im Hippocampus abgrenzbar sind, wobei die Anzahl bereits auf entweder zwei oder drei eingegrenzt wurde.

Angesichts dieser uneinheitlichen Ergebnisse führten wir eine zusätzliche Berechnung durch, um die Frage nach der Anzahl der funktionellen Module abschließend zu klären. Diese Herangehensweise involvierte die Quantifizierung der Anzahl der Maxima (im Gegensatz zur Höhe der Maxima in obiger Validierungsprozedur): In unserer Stichprobe von 22 Probanden lag die gemittelte Anzahl der Maxima im linken Hippocampus bei 2,81 und im rechten Hippocampus bei 2,91. Dies deutet auf eine hippocampale Organisation in insgesamt drei funktionellen Untereinheiten hin.

3.4 Funktionelle Parzellierung

Nachdem wir die funktionelle Organisation des Hippocampus entsprechend der Ähnlichkeit funktioneller Konnektivitätsprofile hippocampaler Voxel charakterisiert und ein diskretes Organisationsmuster in insgesamt drei funktionellen Modulen identifiziert hatten, war unsere nächste Frage, ob wir diese Organisation in einer funktionellen Parzellierung visualisieren könnten. Daher verwendeten wir einen populären und in vorherigen fMRT-Studien vielfach eingesetzten Parzellierungsalgorithmus, das sogenannte *k*-Means-Clustering in drei Cluster. Die Wahl der Anzahl der Cluster $k = 3$ basierte auf der gemittelten Anzahl von Histogramm-Maxima und wurde mithilfe der so genannten Ellbogenmethode objektiviert (Abbildung 19).

Die Clusteranalyse wurde auf die *connectopic maps* des Gruppendurchschnitts sowie jedes einzelnen Probanden angewandt und resultierte in einer Unterteilung des Hippocampus in drei longitudinal arrangierte Cluster (Abbildung 20). In den meisten Fällen (Probanden #1 – #15) waren diese Cluster klar voneinander abgrenzbar und kohärent angeordnet, während manche Probanden (Probanden #16 – #22) in den Hippocampi einer oder beider Hemisphären teils inkonsistente oder zersplitterte Cluster aufwiesen. Obwohl also aus noch unklaren Gründen die hippocampale

Organisation mancher Individuen durch *k*-Means-Clustering in drei Cluster nicht erfolgreich visualisiert werden konnte, war dies in der Mehrheit der Probanden der Fall. Bezüglich der Volumina war das posteriore Cluster stets am größten, gefolgt vom anterioren und intermediären Cluster (Abbildung 21).

4. Diskussion

4.1 Beurteilung potenzieller Limitationen

Bezüglich möglicher Unzulänglichkeiten der präsentierten Analysen und Ergebnisse wollen wir zunächst klären, ob der genutzte fMRT-Datensatz für unsere spezielle Fragestellung geeignet war. Bezüglich funktioneller Bildgebungsverfahren ist grundsätzlich zu bedenken, dass die räumliche Auflösung bisher nicht ausreicht, um Aktionspotentiale oder Erregungszustände auf der Ebene einzelner Neuronen darzustellen. Allerdings ist die Aussagekraft funktioneller fMRT-Studien deshalb nicht zwingend eingeschränkt, da die Fragestellungen in der Regel nicht individuelle Neurone betreffen, sondern vielmehr Erregungsmuster in funktionellen Netzwerken, d.h. größeren Zellverbänden, welche die Größenskala einzelner Zellen überschreiten. Analog waren wir in diesem Projekt nicht am einzelnen hippocampalen Neuron, sondern an der intrinsischen Organisation des gesamten Hippocampus interessiert. Um die Organisationsstruktur und eventuelle Untereinheiten identifizieren zu können, gilt die Voraussetzung, dass die räumliche Auflösung unserer Daten größer sein muss als die kleinste zu erwartende Untereinheit. Unsere fMRT-Daten wurden auf einem Scanner mit einer hohen magnetischen Feldstärke von 7 Tesla akquiriert, was eine Voxelgröße von etwa $0.9 \times 0.9 \times 0.9\ mm^3$ und damit eine räumliche Auflösung unterhalb der Millimetergrenze ermöglichte. Da potenzielle Module im Hippocampus auf wesentlich größeren Längenskalen liegen, müssten diese Daten grundsätzlich in der Lage sind, über die Organisationsstruktur im Hippocampus zuverlässig Auskunft zu geben. Ein weiterer Faktor, der die räumliche Auflösung maßgeblich mitbestimmt, ist jedoch das sogenannte *smoothing* im Rahmen der Vorverarbeitung. Dieses wird in den meisten modernen fMRT-Studien neben anderen Schritten standardmäßig durchgeführt, um unter anderem das Signal-Rausch-Verhältnis sowie die Generalisierbarkeit zwischen Probanden zu erhöhen (Friston, 2003). Allerdings bringt das *smoothing* auch Nachteile mit sich, wie beispielsweise eine Verschlechterung der

initialen räumlichen Auflösung. In diesem Sinne ist es möglich, dass die Anwendung eines *smoothing kernels* von 2.5 mm (*full-width at half-maximum*) in unseren Analysen dazu führte, dass potenziell scharfe Abgrenzungen zwischen hippocampalen Untereinheiten egalisiert werden und ursprünglich harte Grenzen verschwimmen. Daher ist der wahrscheinlichste Effekt, mit dem sich *smoothing* auf unsere Analysen auswirken könnte, ein Bias in Richtung eines gleichmäßigen funktionellen Gradienten, was in den präsentierten Resultaten nicht ausgeschlossen ist. Bemerkenswerterweise sprechen unsere Ergebnisse allerdings trotz dieser möglichen Verzerrung für eine diskrete Organisation.

Neben der Datenerhebung und Vorverarbeitung muss geklärt werden, ob der *connectopic mapping* Algorithmus voreingenommen sein könnte. Diesbezüglich ist insbesondere der letzte Schritt des Algorithmus, die Berechnung der sogenannten *Laplacian Eigenmaps*, hervorzuheben: Die Entwickler erklären in der Originalpublikation, dass die zugrundeliegenden mathematischen Berechnungen die natürlichen Cluster innerhalb der Eingangsdaten nachweisen und möglicherweise verstärkend hervorheben (Belkin & Niyogi, 2003). Daher könnte argumentiert werden, dass die detektierte diskrete Struktur des Hippocampus nicht in einer realen modularen Organisation begründet liegt, sondern durch den Algorithmus künstlich eingeführt wurde. Dies erscheint allerdings aus zweierlei Gründen nicht stichhaltig: Erstens wenden die Entwickler die *Laplacian Eigenmaps* Methode in der Originalpublikation exemplarisch auf einen simulierten Datensatz an und liefern eine akkurate Detektion der zugrundeliegenden kontinuierlichen Struktur der Beispieldaten. Zweitens wurde *connectopic mapping* bereits auf andere Gehirnregionen angewandt, beispielsweise den Motorcortex (Haak et al., 2018), und ergab ebenso einen kontinuierlichen Gradienten funktioneller Konnektivität, der die bekannte somatotopische Organisation des Motorcortex widerspiegelt. Daher ist mit keinem Bias in Richtung diskreter Übergänge innerhalb des *connectopic mapping* Algorithmus zu rechnen.

Eine mögliche Verbesserung unserer Methodik liegt zudem im Generieren der simulierten Datensätze für die Nullverteilung an Histogramm-Maxima. Um die reale Organisationsstruktur des Gehirns so weit wie möglich aufzulösen, aber gleichzeitig die fundamentalen statistischen Gegebenheiten von fMRT-Daten beizubehalten, implementierten wir ein zufälliges Durchmischungsverfahren. Dieses war insofern erfolgreich, als dass die inhärente hippocampale Organisation zerstört wurde,

allerdings wurde so sowohl die Integrität realer Aktivitätszeitverläufe als auch die natürlicherweise in fMRT-Daten vorkommende räumliche Autokorrelation aufgehoben. Obwohl unklar ist, wie genau dies unsere Ergebnisse beeinträchtigt haben könnte, wäre es für zukünftige Studien interessant, eine fairere Implementierung der Nullverteilung anzustreben. Um beispielsweise die räumliche Autokorrelation zu erhalten, würde sich eine Phasenverschiebungsmethode eignen, welche im Detail im englischen Kapitel 4.1.4 beschrieben ist.

Eine weitere Limitation entsteht durch die Verwendung von *k*-Means-Clustering, da diese Methode im Gegensatz zum *connectopic mapping* Algorithmus nicht vollständig datenbasiert ist, sondern die gewünschte Anzahl der Cluster *k* als Eingangsgröße vom Anwender festgelegt werden muss. Um den Wert von *k* so weit wie möglich zu objektivieren und ein personenbezogenes Bias bezüglich der Anzahl der Cluster zu vermeiden, quantifizierten wir die Höhe sowie Anzahl der Histogramm-Maxima der *connectopic maps* und wandten zudem die Ellbogenmethode an. Allerdings könnten zukünftige Studien von der Verwendung eines vollständig datenbasierten Clustering-Algorithmus profitieren, welcher nicht die Vorgabe von *k* benötigt, wodurch sich die genannten Analysen erübrigen würden, die in unserem Fall notwendig waren.

Im Zuge der Limitationen dieser Studie sei zudem die manuelle Definition der Hippocampusmaske als methodologische Schwierigkeit erwähnt. Trotz der sorgfältigen Befolgung der detaillierten Instruktionen im Harmonisierten Protokoll für Hippocampale Segmentierung (Boccardi et al., 2015) war insbesondere die Abgrenzung des posterioren Pols eine Herausforderung, da sich die hippocampale graue Masse an dieser Stelle verdünnt und fließend in das Indusium griseum übergeht (Abbildung 1). Hier entschieden wir uns insofern für eine liberale Segmentierung, als dass wir lieber alle hippocampalen Voxel und eventuell kleinere Mengen nicht-hippocampaler Voxel inkludierten, anstatt um jeden Preis extrahippocampale Masse und damit womöglich wesentliche hippocampale Masse auszuschließen. Daher ist es möglich, dass unsere Hippocampusmaske am posterioren Pol etwas zu voluminös ist. Dies spiegelt sich möglicherweise in dem Ergebnis, dass das Volumen des posterioren Clusters in der funktionellen Parzellierung signifikant höher war als das des intermediären und anterioren Clusters (Abbildung 21). Obwohl diese Frage in der Literatur noch nicht abschließend geklärt ist, wäre gemäß vorausgehenden Studien eher mit Untereinheiten von etwa gleicher Größe zu rechnen (Chase et al., 2015).

4.2 Konzeptuelle Implikationen unserer Ergebnisse

Mithilfe des *connectopic mapping* Algorithmus identifizierten wir die dominante Achse funktioneller Organisation hinsichtlich der Ähnlichkeit der Fingerabdrücke funktioneller Konnektivität aller hippocampaler Voxel zur restlichen grauen Substanz. Unsere Ergebnisse weisen darauf hin, dass die dominante Topografie funktioneller Konnektivität innerhalb des Hippocampus in klar abgrenzbaren Modulen entlang der Längsachse verläuft.

Dies wirft die interessante Frage auf, ob und inwiefern die identifizierten Module in verschiedene Gehirnfunktionen involviert sind. In bisherigen Studien hat sich im Laufe der Jahrzehnte die generelle Sicht verfestigt, dass der anteriore und posteriore hippocampale Pol tatsächlich unterschiedliche Funktionen ausführen (Fanselow & Dong, 2010). Bezüglich der konkreten funktionellen Aufgaben besteht in der aktuellen Literatur allerdings noch Uneinigkeit: Einige Studien deuten auf ein Modell hin, in welchem der anteriore Hippocampus in emotionale Vorgänge, wie Angstverhalten, die körpereigene Stressantwort und stressbedingte hormonelle Regelkreisläufe, eingebunden ist, während der posteriore Teil eher kognitiv betonte Funktionen vermittelt, darunter allen voran die Bildung räumlicher Erinnerungen und Navigation (Bannerman et al., 2004; Moser & Moser, 1998). Diese Hypothese wird von einigen Autoren insofern erweitert, als dass der posteriore Hippocampus nicht nur für die Verarbeitung räumlicher, sondern auch deklarativer Gedächtnisinhalte zuständig sein könnte (Fanselow & Dong, 2010). Allerdings wurde diese Theorie durch andere Untersuchungsergebnisse infrage gestellt, welche nahelegen, dass die kognitive Repräsentation von Gedächtnis entlang der gesamten hippocampalen Längsachse und insbesondere auch im anterioren Teil stattfindet (Chase et al., 2015; Zeidman & Maguire, 2016). Allerdings stehen solche Erkenntnisse nicht zwingend im Widerspruch zu einer funktionellen Differenzierung, da möglicherweise die Modalität verarbeiteter Information entlang der Längsachse variiert: Auch wenn Neuronen entlang der gesamten Länge des Hippocampus an der Gedächtnisbildung beteiligt sind, könnte der anteriore Teil eher selbstzentrierte (egozentrische) und der posteriore Teil eher weltbezogene (allozentrische) Informationen verarbeiten (Plachti et al., 2019). Zudem deuten weitere Studien auf eine Differenzierung hinsichtlich der Detailliertheit codierter Erinnerungen hin, wonach der anteriore Pol eher grobe Inhalte

und der posteriore Teil eher detaillierte Informationen verarbeite (Brunec et al., 2018; Evensmoen et al., 2013; Poppenk et al., 2013; Sekeres et al., 2018).

Obwohl zukünftige Studien nötig sind, um diese verschiedenen Theorien in Einklang zu bringen, besteht aus aktueller Sicht kaum Zweifel an der grundsätzlichen Existenz einer anterior-posterioren Differenzierung. Eine viel weniger untersuchte, aber nicht minder relevante Frage bezieht sich auf die präzise Topografie des Übergangs zwischen den beiden funktionell distinkten hippocampalen Polen. Die wenigen Studien, welche diesen Zusammenhang explizit ansprechen, liefern teils gegensätzliche Ergebnisse (Abbildung 3): Auf der einen Seite sprechen einige funktionelle Bildgebungsstudien für eine Organisation der hippocampalen Längsachse in klar abgrenzbaren funktionellen Untereinheiten. Die Anzahl der identifizierten Module variiert je nach Studie zwischen zwei bis zu fünf Untereinheiten (Plachti et al., 2019; Robinson et al., 2015), wobei die Mehrzahl allerdings eine dreigeteilte Organisation in einem anterioren, intermediären und posterioren Cluster vorschlägt (Chase et al., 2015; Zarei et al., 2013). Unterstützung findet eine solche funktionelle Dreiteilung beispielsweise auch in Untersuchungen struktureller Charakteristika, wie etwa der Genexpression oder elektrophysiologischer Eigenschaften (Dong et al., 2009; Kenney & Manahan-Vaughan, 2013; Thompson et al., 2008). Im Gegensatz zu einem möglicherweise modularen Organisationsmuster stehen Erkenntnisse, welche einen gleichmäßigen funktionellen Gradienten entlang der hippocampalen Längsachse postulieren. Diese fußen ebenfalls sowohl auf funktionellen Bildgebungsdaten (Masouleh et al., 2020; Przeździk et al., 2019) als auch auf der Untersuchung struktureller Parameter (Kjelstrup et al., 2008; Witter et al., 1989).

Die in dieser Dissertation präsentierten Ergebnisse lassen sich eindeutig der ersteren Gruppe zuordnen, da die hier identifizierte Topografie funktioneller Konnektivität abrupte Änderungen entlang der Längsachse aufweist und in den meisten Probanden drei funktionelle Module klar abgegrenzt werden können. Während unsere Resultate deutlich für eine modulare Organisation sprechen, spiegeln sie doch in einem gewissen Maß die Unklarheit in der Literatur bezüglich der Anzahl der Untereinheiten wider (z.B. uneinheitliche Ergebnisse der *t*-Tests). Obwohl unsere Ergebnisse auf den ersten Blick im Widerspruch zu anderen Bildgebungsstudien stehen, welche eine graduelle Änderung der Konnektivitätsprofile berichten, könnte diese Diskrepanz durch fundamentale Unterschiede zwischen den entsprechenden Studien erklärt

werden. Im Falle der Studie von Przeździk et al. (2019) beispielsweise, in welcher ebenfalls den *connectopic mapping* Algorithmus auf den Hippocampus angewandt wird, basieren die fMRT-Daten auf einem sogenannten *resting-state* Experiment, d.h. die Probanden wurden gebeten, während des Scannens keiner geistigen Beschäftigung nachzugehen, sondern möglichst gedankenlos, aber wach zu verweilen. Im Gegensatz dazu wurde in den hier präsentierten Daten explizit darauf geachtet, die Gehirnaktivität der Versuchsteilnehmer während einer hippocampusabhängigen experimentellen Aufgabe zu scannen. Es erscheint also möglich, dass die hippocampale Längsachse abhängig von der gegenwärtigen kognitiven Beschäftigung ein unterschiedliches Organisationsmuster hervorbringt (Robinson et al., 2016). Zudem verwenden Przeździk et al. als Ausgangspunkt der Analysen nicht die Konnektivität hippocampaler Voxel zur gesamten extrahippocampalen grauen Masse, wie in der hier präsentierten Studie, sondern berücksichtigen ausschließlich neokortikale Regionen. Da allerdings gezeigt wurde, dass der Hippocampus auch eine hohe funktionelle Konnektivität mit verschiedenen subkortikalen Kerngebieten aufweist (Kahn & Shohamy, 2013), ist wenig verwunderlich, dass die Konnektivitätsprofile variieren, je nachdem ob diese Areale miteingeschlossen sind oder nicht. Vor diesem Hintergrund wird klar, dass die Studie von Przeździk et al. und unsere Resultate sich nicht zwingend widersprechen, sondern sich vielmehr zu einem interessanten Gesamtbild zusammenfügen und Fragen für zukünftige Experimente aufwerfen. Möglicherweise sind verschiedene Organisationsmuster von funktioneller Konnektivität entlang der hippocampalen Längsachse überlagert, welche je nach geistiger Involvierung bzw. je nach Berücksichtigung verschiedener Gehirnareale zu Tage treten (Strange et al., 2014).

4.3 Klinische Anwendungen

Wie eingangs erwähnt, zeichnet sich eine Vielzahl neuropsychiatrischer Erkrankungen durch eine massive Störung der funktionellen bzw. strukturellen hippocampalen Integrität aus, weswegen verschiedenste klinische Forschungsbereiche derzeit die Auswirkungen pathologischer Vorgänge auf den Hippocampus betrachten. Mehrere Studien zeigen, dass sich die Anfälligkeit für spezifische Krankheiten entlang der hippocampalen Längsachse unterscheidet, weswegen der Untersuchung dieser eine große Bedeutung für klinische Fragestellungen zukommt (Lladó et al., 2018;

Ranganath & Ritchey, 2012; Vogel et al., 2020). Allerdings ist die Vergleichbarkeit zwischen derzeitigen Studien aufgrund der variierenden Methodik stark eingeschränkt. Mögliche Inkonsistenzen ergeben sich insbesondere aufgrund der Vielzahl existierender Protokolle zur Unterteilung longitudinaler Untereinheiten. Dies könnte durch eine gemeinsame Herangehensweise verbessert werden. Als solche universelle und automatisierte Segmentierungsmethode könnte der in dieser Arbeit präsentierte Ansatz, *connectopic mapping* kombiniert mit *k*-Means Clustering, zum Einsatz kommen. Auf der Basis der vorgeschlagenen Methodik könnten zudem unbegrenzte weitere Fragestellungen bearbeitet werden, die nicht auf den Hippocampus begrenzt sein müssen, da als *region of interest* jegliches Gehirnareal definiert werden kann.

Zusätzlich zu den breiten Anwendungsmöglichkeiten in der neurowissenschaftlichen Forschung könnte die hier verwendete Herangehensweise in der Zukunft auch als prädiktives Tool Einzug in den praktischen Alltag in der Klinik finden. Da Studien zufolge die funktionelle Konnektivität des Hippocampus insbesondere bei Patienten mit neurodegenerativen Erkrankungen wie etwa Morbus Alzheimer stark verändert ist, könnte der Quantifizierung funktioneller Konnektivität sowie einer konnektivitätsbasierten Parzellierung zukünftig eine größere Bedeutung in der Diagnostik, Verlaufsbeurteilung oder dem Monitoring des Therapieansprechens zukommen (Zarei et al., 2013; Zhang et al., 2010). Obwohl weitere Studien benötigt werden, um den präzisen Nutzen der hier verwendeten Methodik für eine Anwendung in der klinischen Praxis zu etablieren, sind die denkbaren Vorteile enorm, da die fMRT als nichtinvasive, schmerzfreie und nahezu risikofreie Untersuchung mit keinen relevanten unerwünschten Nebenwirkungen einhergeht.

Somit liefern die hier präsentierten Ergebnisse nicht nur interessante Erkenntnisse in die faszinierende Organisationsstruktur der hippocampalen Längsachse, sondern bieten auch eine methodische Grundlage für vielfältige zukünftige Anwendungen. Diese umfassen insbesondere den Einsatz der vorgestellten Methodik als universell einsetzbares, automatisiertes Segmentierungstool der hippocampalen Längsachse in der neurowissenschaftlichen Forschung sowie als wertvolles, nahezu risikofreies prädiktives Tool bei neurodegenerativen Erkrankungen in der klinischen Praxis.

1. Introduction

1.1 What a seahorse has to do with navigation systems

In the milliseconds before starting to read this text, the unusual title of this section may have caused different memories to cross the reader's mind, perhaps including a past visit at 'Sea Life', facts and figures from a marine biology textbook, or potentially even the specific frustrating experience of intending to reach a destination with an outdated navigation device. If not before reading this, perhaps by now similar memories may have been elicited. This capability of the mind to instantaneously recollect previously acquired information or events and vividly relive past experiences may be best described as a form of mental time travel, which is an intriguing ability of the human brain. Interestingly, the addressed entities, namely the formation of memory (textbook knowledge or personal experience), spatial navigation (attempting to reach a destination), and emotional processing (frustration with a technical device), have all been linked to a circumscribed brain structure in the medial temporal lobe, the hippocampus, which – being the Latin word for seahorse – explains the connection hinted at in the title. As this thesis is dedicated to the functional organization of that fascinating brain structure, it is important to first provide a brief overview of its gross anatomy and functional implications. After describing the structure and function of the hippocampus in a physiological context, the hippocampal role in pathological conditions shall be pointed out, specifically intending to captivate readers whose general curiosity is focused on the clinical rather than fundamental sciences. Hopefully having sparked all readers' interest in the functional organization of the hippocampus, subsection 1.5 will introduce an efficient means to investigate organizational patterns of the human brain in-vivo, namely functional magnetic resonance imaging. With the relevant background information given, the Introduction will be concluded by an outline of the precise goal of this study.

1.2 Topological and structural neuroanatomy of the hippocampus

The human hippocampus is a bilateral, circumscribed structure in the medial temporal lobe that partly forms the floor of the lateral ventricle (Duvernoy, 2005). In addition to its resemblance with a seahorse, the shape of the hippocampus could also be described as a large cashew nut, with a dilated anterior portion forming the so-called hippocampal head and an elongated hippocampal tail that is located posteriorly (illustrated in Figure 1). The anterior and posterior poles of the hippocampus are connected by the hippocampal body, the orientation of which follows a sagittal direction. Note that the entire length of the hippocampus features several elongate humps, also called hippocampal digitations, which are separated by smaller sulci. These digitations correlate to cortical folding, similar to gyrification in the neocortex, and are most prominent in the anterior hippocampus.

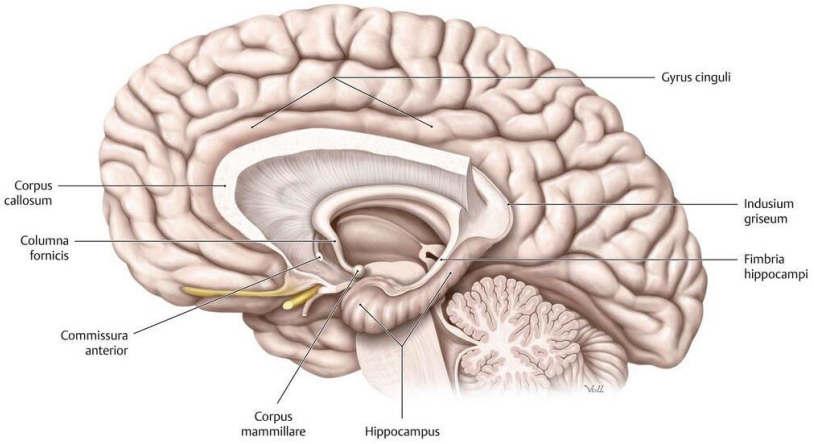

Figure 1: Topology of the left hippocampus

Viewed from a lateral position, the left cerebral hemisphere is dissected to reveal the left hippocampus, which is usually not visible from the outside as it is covered by temporal lobe matter. The anterior pole of the hippocampus thickens to form the hippocampal head, whereas the posterior pole thins out into the indusium griseum. The fimbria hippocampi, a fine strip of white matter, continues into the crus fornicis.

Illustration adopted from Schünke et al.(2012), p. 322.

In terms of topology, the hippocampal head anteriorly neighbors the amygdala (not visible in Figure 1 due to removal for illustration purposes) and posteriorly, the hippocampus elongates towards the caudal end of the corpus callosum, also termed splenium (Schünke et al., 2012). At this position, the gray matter of the hippocampus merges into the indusium griseum, which is a thin layer of gray matter closely following the cranial surface of the corpus callosum. The hippocampus, mostly composed of gray matter, is accompanied by a thin, fibrous strip of white matter, the so-called fimbria, which posteriorly continues into the crus of the fornix (Trepel, 2008). Structurally, the hippocampus is part of a particular subtype of cerebral cortex termed archicortex, which remarkably constitutes the phylogenetically oldest region of the cerebral cortex (Frotscher & Seress, 2007). As a part of this system, the hippocampus is thought to be highly conserved across (especially mammalian) species (Amaral & Lavenex, 2007).

1.3 Functional neuroanatomy of the hippocampus

The anatomical characteristics outlined above have been known for a long time, as already studies in the late 19th and early 20th century have focused on the topological and morphological anatomy of the hippocampus (Ramón y Cajal, 1893). However, it was not until the 1950s that research questions regarding hippocampal function gained sweeping popularity.

1.3.1 A brief history of research on hippocampal functions

The interest in hippocampal function was sparked by a groundbreaking discovery based on the case of Henry Molaison, by now famously referred to as ‘patient H. M.’ This patient suffered from severe epilepsy that was resistant to all treatment options available at the time. At the age of 27, as patient H. M. was utterly incapacitated by his recurring seizures, he underwent an experimental surgery as a last resort (Scoville & Milner, 1957): Since attending physicians suspected the known epileptogenic potential within the medial temporal lobe of causing the condition, surgeons generously resected large parts of the patient’s bilateral medial temporal lobes, including the majority of the hippocampus (Augustinack et al., 2014). Unexpectedly at the time, the seminal finding stemming from this procedure did not

concern the antiepileptic effect of the surgery – although both incidence and severity of seizures were reduced – but rather the fact that after bilateral temporal lobe removal, patient H. M. experienced severe memory impairment (Squire, 2009). Specifically, the patient suffered from anterograde amnesia, i.e., he was not able to form new memories, while he was still able to recall memories that had been encoded before the surgery. This discovery led to the conclusion that structures within the medial temporal lobe, including the hippocampus, are essential for the formation of memory. Interestingly, however, further experiments involving patient H. M. after his surgery revealed that not all kinds of memory depend on the medial temporal lobe (Cohen & Squire, 1980): For instance, in a multi-day drawing task patient H. M. was asked to trace the outline of a five-pointed star with a pencil, while he could only see his hand and the drawing paper reflected in a mirror. Interestingly, the patient's motor skill of 'mirror-drawing' improved significantly and was stable for several days, yet at every new trial the patient had absolutely no awareness that he had performed the task before (Milner, 1962).

These intriguing insights originating from the unique case of patient H. M. boosted interest in hippocampal research. In subsequent years, intensive studies progressively corroborated the pivotal role of the hippocampus for the formation of declarative memory (Squire et al., 1993). This specific type of memory usually coincides with the use of 'memory' in everyday language, i.e., conscious knowledge of facts (semantic memory) and events or episodes (episodic memory) and is distinct from procedural memory which typically encompasses unconscious skill-based knowledge, including complex motor skills like the above-mentioned mirror-drawing task.

In parallel, however, a different line of evidence emerged from the new-found interest in hippocampal function, suggesting a hippocampal role for cognition beyond declarative memory: Lesion studies in rodents demonstrated that hippocampal damage resulted in major impairment of spatial memory (Morris et al., 1982). Moreover, the breakthrough discovery of 'the brain's navigational system', which was later awarded the Nobel Prize for Physiology or Medicine (The Nobel Committee for Physiology or Medicine, 2014), further corroborated a hippocampal role in spatial navigation: The rodent hippocampus was shown to contain specialized neurons, so-called place cells, that respond to specific, circumscribed locations in space with an

increased firing pattern (O'Keefe & Dostrovsky, 1971). Hence, hippocampal place cells are thought to provide a mapping of the environment around the individual, which led to the view that the mental representation of space is situated in the hippocampus and organized as a 'cognitive map' (O'Keefe & Nadel, 1978). Although these experiments, based on in-vivo cell recordings via implanted electrodes, have not been replicated in humans for obvious ethical reasons, noninvasive functional imaging studies have provided evidence for the existence of place cells in humans (Epstein et al., 2017).

As detailed above, decades of hippocampal research have established two key functions of the hippocampus, namely declarative memory and spatial navigation. However, more recent work has extended the functional role of the hippocampus to additionally take part in emotional processing, including anxiety or fear (Kjelstrup et al., 2002), and stress-related behaviors (Franklin et al., 2012). This suggested emotional component of hippocampal function is supported not only by behavioral studies, but also by evidence indicating a regulatory influence of the hippocampus for the hypothalamic-pituitary-adrenal-axis (Jacobson & Sapolsky, 1991). Furthermore, a hippocampal role in perception (Lee et al., 2012), decision-making (McCormick et al., 2016), and imagination of objects and fictitious events (Karapanagiotidis et al., 2017) has been proposed, although these hypotheses are not yet corroborated by a large body of evidence.

In sum, it is to date widely established that the hippocampus takes part in a multitude of complex brain functions. These include most prominently the formation of declarative memory, spatial navigation, and emotional processing but may extend to even more roles that are still under investigation.

1.3.2 Current view: Two axes of functional organization

The involvement of the hippocampus in a multiplicity of brain functions gives rise to the question how this functional diversity is sustained by an underlying hippocampal organization. Although this question is not yet entirely resolved, an established view assumes that the hippocampus exhibits two axes of functional organization that are perpendicular to each other (Vos de Wael et al., 2018).

The first axis has been identified long before patient H. M.'s case was studied, when Spanish histologist Santiago Ramón y Cajal and his student Rafael Lorente de Nó published the first detailed and comparative studies on morphology and neuronal connectivity of the hippocampus (Lorente De Nó, 1934; Ramón y Cajal, 1893). Specifically, they discovered delicate differences in cytoarchitectonic properties, based on which they divided the hippocampus into histological subfields that are until now integral part of neuroanatomy textbooks: These subfields include the so-called dentate gyrus, cornu Ammonis fields CA1, CA2 and CA3, and the subiculum (illustrated in Figure 2). Subsequent studies have extended the original nomenclature and suggested subdomains on an even smaller scale, like a division of the subiculum into pre-, para-, prosubiculum, and subiculum proper (Duvernoy, 2005). Critically, the identified histological subfields are arranged along the transversal axis of the hippocampus and are in this thesis henceforth referred to as transversal subfields.

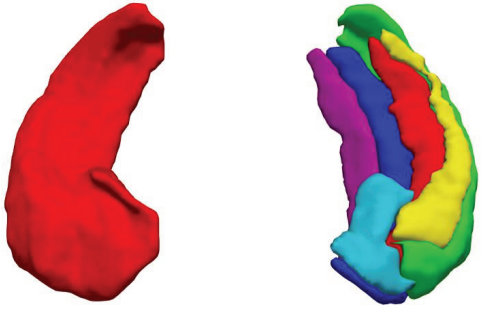

Figure 2: Transversal subfields of the hippocampus

According to delicate cytoarchitectonic differences, the hippocampus can be divided into several histological subfields. In this view onto the hippocampi of both hemispheres from an antero-ventral angle, the left hippocampus shows the outline and orientation of the transversal subfields, as defined in the segmentation protocol by Dalton et al. (2017) Although the precise nomenclature and number of subfields differ between protocols, these subfields are generally arranged along the transversal axis of the hippocampus (except for the uncus, which is not consistently mentioned in different segmentation protocols).
Magenta: pre- and parasubiculum, dark blue: prosubiculum and subiculum, green: CA1, yellow: CA2/3, red: CA4 and dentate gyrus, turquoise: uncus.

Regarding the functional relevance of these microscopically discriminable subfields, more recent work has suggested that transversal hippocampal subfields may be implicated in different cognitive functions (Carr et al., 2010), for example the encoding versus retrieval of a memory (Suthana et al., 2011). Hence, one aspect of hippocampal functional organization may be sustained by a functional distinction along its transversal axis.

In addition to the long-standing notion of a transversal axis of organization, which has first been identified using microstructural characteristics but moreover may be functionally relevant, more recent studies have proposed a functional differentiation along the longitudinal axis of the hippocampus (Poppenk et al., 2013). Hitherto this organization is not underpinned by clearly defined cytoarchitectonic properties that can be thoroughly assessed using microscopy. Thus, its exact morphology is more ambiguous and previous studies have produced different hypotheses regarding the hippocampal long-axis organization (schematically illustrated in Figure 3): Some studies suggest a dichotomous organization in which the anterior one-third, roughly covering the hippocampal head, is functionally different than the posterior two-thirds. This is mostly based on studies of intrinsic anatomical connectivity of the hippocampus that revealed an abrupt divergence of neuronal projections between the ventral one-third and dorsal two-thirds of the hippocampus (Kondo et al., 2009). Extending this discrete view, gene expression (Dong et al., 2009), electrophysiological (Kenney & Manahan-Vaughan, 2013), and behavioral (Bast et al., 2009) studies point towards a modular organization in three distinct, nonoverlapping subunits. In contrast, a third set of evidence suggests a smooth functional gradient spanning from the anterior to the posterior hippocampal pole with continuous transitions of functional specialization (Kjelstrup et al., 2008; Przeździk et al., 2019). Evidently, the question regarding the precise functional organization of the hippocampal long-axis is not yet entirely resolved (Strange et al., 2014).

Figure 3: Hippocampal long-axis organization

The functional organization along the hippocampal long-axis is not yet entirely resolved. Previous studies have proposed different models, which can be grouped into three partly opposing hypotheses: A dichotomous view with a discrete functional distinction between the anterior one-third and the posterior two-thirds (left sketch), a tripartite model with three sharply demarcated subunits (middle), or a continuous gradient of functional differentiation (right).

Illustration inspired by Strange et al. (2014)

1.4 Why should clinicians care about the hippocampal organization?

At this point, it may be of interest to consider why a clinician should at all be bothered with the hippocampal (long-axis) organization. As established above, decades of research have revealed that the hippocampus is critically involved in an abundance of important neurophysiological functions. Yet this functional multiplicity reflects only one side of a coin, as analogously it was shown that the hippocampus not only sustains healthy brain function, but also shows major impairment in pathological conditions (Small et al., 2011). These include a variety of neuropsychiatric diseases, for instance neurodegenerative illnesses, like most prominently Alzheimer's disease (La Joie et al., 2014) and frontotemporal dementia (Vogel et al., 2020), but also schizophrenia (Lewis & Lieberman, 2000), bipolar disease (Altshuler et al., 2000), major depressive disorder (Kemmotsu et al., 2013), anxiety (Cha et al., 2016), and post-traumatic stress disorder (Karl et al., 2006). Some of these links between disease and hippocampal alteration have been long established and are currently widely accepted, for instance a hippocampal involvement in Alzheimer's disease and other forms of dementia, whereas other connections are rather recent and may require further testing. Besides, correlation does not automatically imply causation and a possible confounder may relate to comorbidity (Cha et al., 2016), therefore one should

not over-interpret findings at the current stage. Nonetheless, the multitude of neuropsychiatric diseases in which the hippocampus may play a role is remarkable, especially given the diversity of the mentioned medical conditions, ranging from impaired memory and spatial orientation in dementia to different instances of emotional imbalance in depression or anxiety. Therefore, fundamental research investigating how the multiplicity of cognitive functions is sustained by the hippocampal organization will eventually help to advance our clinical understanding of how such different symptoms can be caused by the damaged integrity of a single circumscribed brain region. In fact, the link between functional transitions along the hippocampal long-axis (matter of fundamental research) and the relevance of such organizational patterns in disease (clinical relevance) was already indicated by recent studies: Not only the distribution of brain functions changes along the long-axis, but also the vulnerability to disease, as shown for Alzheimer's and other forms of dementia (Lladó et al., 2018; Ranganath & Ritchey, 2012; Vogel et al., 2020). In the long term, such insights can promote a more accurate model of a disease's pathophysiology and therein help to discover new avenues for better, earlier, and potentially more targeted treatment options.

1.5 Mapping the functional organization of the human brain

As outlined above, the functional organization of the hippocampus is an unresolved question, and its illumination may help to understand the fundamental role of the hippocampus in health and disease and thus pave the way for better treatment options for various conditions. Since many previous findings stem from animal research and their generalizability to humans is not entirely evident, it is important to investigate the hippocampal organization directly in humans. Furthermore, to derive information regarding functional rather than structural aspects, analyses should be performed in alive humans, as postmortem studies are better suited to address the structural anatomy of brain tissue but often not directly conferrable to functional neuroanatomy.

1.5.1 FMRI as an efficient tool for indirect quantification of neuronal activation

Generally, for research questions regarding the functional behavior of human brain regions, ethical concerns naturally forbid numerous invasive techniques that are useful

in animal research. Hence, a noninvasive, riskless, and ideally high-resolution tool for mapping human brain function in-vivo is needed. Such an approach was rendered possible by the development of functional imaging techniques, including among others functional magnetic resonance imaging (fMRI). Against a common misconception, fMRI does not provide a means to directly quantify brain activity but in fact constitutes an indirect measure of neuronal activity as it detects the physiological reaction of the cerebral vascular system following neuronal activation (Huettel et al., 2009). To understand this, it is first important to deconstruct what the term 'neuronal activation' precisely implies: On a cellular level, neuronal activity is reflected by numerous biochemical reactions, including for instance axonal transmission of action potentials or synaptic release of neurotransmitters (Glover, 2011). These processes require energy, which is usually provided in the form of adenosine triphosphate, produced by intracellular oxidative glycolysis. The main substrates needed for this reaction, glucose and oxygen (O_2), are supplied via the blood stream, which conversely also removes accumulating metabolites like carbon dioxide (CO_2). Note that whereas CO_2 is in large parts dissolved directly in the blood, O_2 usually requires hemoglobin for transportation. Neuronal activation, i.e., upregulation of energy-consuming cellular processes above baseline, leads to a higher substrate demand, which is met by changes in blood supply after a short delay of a few seconds (Roy & Sherrington, 1993), the so-called hemodynamic response. A major consequence of this hemodynamic response is a local change in the concentrations of oxygenated and deoxygenated hemoglobin: As oxygen is transported into cells for energy production, hemoglobin molecules are increasingly depleted of their carried O_2 and change from a so-called oxygenated to a deoxygenated state. Shortly after, however, the hemodynamic system responds to this increased oxygen depletion with an overshoot of blood flow and volume so that the concentrations of oxygenated and deoxygenated hemoglobin reverse.

Having outlined the hemodynamic response to neuronal activation, the question emerges as to how an fMRI scanner is able to detect these hemodynamic changes and thereby indirectly measure brain activity. Without detailing the physical concepts underlying the generation and detection of MR signal, it is worth noting that MRI is technically designed to detect specific changes in spin orientation and precession frequency of hydrogen atomic nuclei located in a strong magnetic field, induced by excitation of the nuclei via high-energy electromagnetic waves, so-called

radiofrequency pulses (Huettel et al., 2009). Thus, the MR signal fundamentally relies on the magnetic characteristics of the examined tissue. Critically, the hemoglobin molecule exhibits substantially different magnetic properties depending on whether it currently carries oxygen or is in its deoxygenated state (Pauling & Coryell, 1936). Due to this difference in magnetic susceptibility, deoxygenated hemoglobin interferes with and distorts the MR signal more than oxygenated hemoglobin (Thulborn et al., 1982). Hence, local changes in the amount of deoxygenated hemoglobin can be detected using MRI, which requires the use of a specific contrast (Ogawa et al., 1990; Ogawa & Lee, 1990). This so-called blood-oxygen-level-dependent (BOLD) contrast relies on changes in deoxygenated hemoglobin over time, which – as we have established above – are caused by the cerebral hemodynamic response which in turn mirrors local neuronal activity. Therefore, BOLD fMRI not only maps the concentrations of differentially loaded hemoglobin molecules but importantly allows for conclusions about the underlying neuronal activation. Importantly, this neuronal activity is quantified over time, in contrast to structural MRI for instance, which is commonly used in clinical contexts to produce a momentary snapshot of the brain's structural composition. In functional MRI, BOLD signal is measured at thousands of time-points throughout the duration of an experiment, thereby quantifying neuronal activation with a temporal resolution. At each time-point, the fMRI scanner measures the current BOLD signal from the entire brain, producing a three-dimensional reconstruction of the signal intensity values at that specific time-point, a so-called brain volume (illustrated as stacks of brain images in Figure 4, top left panel). For technical reasons, most scanners acquire a complete brain volume by assembling two-dimensional, axial brain slices (bottom left panel). Each of these slices, and thus each brain volume is virtually dissected into small cubes with an edge length on the millimeter scale, which constitute the smallest spatial units for which BOLD signal is recorded (bottom right panel). These cubes are called voxels and can be described as three-dimensional pixels, or volumetric pixels (hence the compound term vo-xel). A voxel's acquired BOLD signal over time can be represented as a voxel-specific time-course of BOLD signal, sometimes referred to as activity time-course (top right panel).

As voxels constitute the smallest spatial units of signal acquisition, voxel size determines the spatial resolution of the obtained images. It can be influenced by numerous factors, most notably by the employed magnetic field strength. Usually, fMRI

studies employ scanners operating at a field strength of 1.5 or 3 Tesla, resulting in a voxel edge length measuring a few millimeters. With ultra-high-field scanners at 7 or very rarely 9.4 Tesla, an even higher sub-millimeter resolution can be achieved.

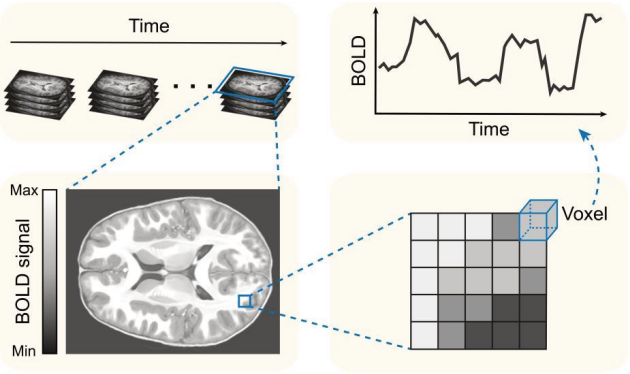

Figure 4: Basic structure of fMRI data

In fMRI, three-dimensional brain volumes are acquired over time (top left panel). Due to the typical acquisition technique, brain volumes are acquired as several two-dimensional axial brain slices (bottom left). The smallest entity within each brain slice, and thus in the entire brain volume, is a small, three-dimensional 'volumetric pixel', i.e., voxel (bottom right). For each voxel, BOLD signal is detected over time, yielding voxel-wise time-courses of BOLD signal (top right).

1.5.2 Functional connectivity for investigating functional networks

Based on acquired fMRI data, abundant approaches for further analyses and statistical testing have been developed. For instance, experimenters can choose to analyze each voxel's time-series independently, constituting the concept of so-called massive univariate testing, which is to date commonly used. A univariate approach typically involves fitting a linear model to the BOLD signal time-course of every voxel using a set of covariates. This method can for instance provide information about the overall activation level in voxels of a brain region during different conditions of an experimental design, for example the activation during performing a task versus during rest.

Thus, while the question may be answered to what extent different regions take part in an experimental task, strictly univariate approaches are usually not suited to derive information beyond the absolute activation of distinct brain regions. However, given accumulating evidence for a complex hierarchy and organization in the human brain, it is a question of increasing interest to identify and analyze functional brain networks. Especially when addressing a question regarding the functional organization of a circumscribed brain region it may be useful to investigate whether different subunits of the region of interest may or may not belong to different functional brain networks.

One way to address this question, i.e., to assess how functionally connected brain regions are, is to determine the extent of coactivation between them. This is based upon the assumption that regions that belong to the same functional entity take part in similar tasks and are therefore active at the same time (and vice versa inactive at the same time). This concept, more precisely the amount of coactivation of two brain areas, can be quantified as the similarity, i.e., correlation, between the respective brain regions' or voxels' BOLD signal time-courses, which is termed functional connectivity (Friston, 2003). Produced values of functional connectivity range from one to negative one, indicating an identical or inverse activation pattern, respectively (schematically illustrated in Figure 5). In other words, two voxels with very similar BOLD time-series will have a high value of functional connectivity (gray and blue voxels in Figure 5), whereas a pair of voxels in which one is consistently active when the other one is inactive will be characterized by a more negative value of functional connectivity (gray and red voxels in Figure 5).

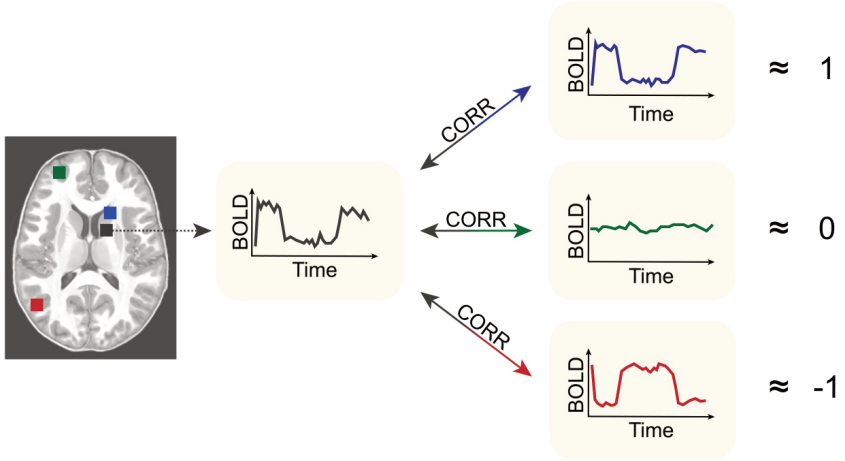

Figure 5: Principle of functional connectivity

One way to investigate patterns of functional brain organization consists in comparing activity time-courses across different brain regions. This can be done using functional connectivity, which measures the correlation of BOLD time-courses of different brain regions. In this example, we regard the time-series of an exemplary voxel in the head of the caudate nucleus (dark gray square). As the time-series of another caudate nucleus voxel (blue square) features a very similar temporal activation pattern, the correlation of their time-courses will yield a high value close to 1. These two voxels exhibit a high functional connectivity. Conversely, an almost inverse time-course (here: red cortical voxel) will result in a negative correlation, i.e., functional connectivity, close to -1. A voxel with a time-course neither identical nor inverse (green cortical voxel) will lie in between.

1.6 Aim of this study

As outlined above, the precise functional organization of the human hippocampus is a topic of much debate and until now remains elusive. The involvement of the hippocampus in a multitude of intriguing cognitive functions, including but most likely not limited to memory formation and mental representation of space, motivated us to investigate how this multiplicity is sustained by an underlying functional organization. Illuminating this would not only provide fundamental insights into the mechanisms of such complex brain functions and thereby considerably advance the field of fundamental neuroscience but may also improve our understanding of the pathophysiology of the abundance of neuropsychiatric diseases that the hippocampus

is involved in. Therefore, we set out to investigate the functional organization of the human hippocampus in healthy adults based on an ultra-high-resolution fMRI dataset acquired at a magnetic field strength of 7 Tesla. Specifically, we aimed to identify the dominant axis of functional organization within the hippocampus. Furthermore, we tackled the question whether the pattern of functional organization along the dominant organizational axis revealed step-like or smooth transitions. Moreover, if we revealed a step-like pattern, we intended to determine the number of functional modules within the hippocampus.

To answer these questions, we considered the functional connectivity of the hippocampus to the rest of the brain. More precisely, we employed a recently developed, data-driven analysis algorithm, connectopic mapping, to identify the dominant topography of functional connectivity, or connectopy, within the hippocampus (Haak et al., 2018). The applied algorithm involved several computational steps but essentially, we compared functional connectivity profiles across hippocampal voxels and processed this information to determine the overall pattern of functional connectivity similarity within the hippocampus. Our first focus of interest was whether the detected organizational pattern revealed either a smooth functional gradient or a step-like, modular organization. In the latter case, the second question we aimed to address was the precise number of hippocampal subunits. Thus, we eventually performed participant-specific hippocampal parcellations based on the identified topography of functional connectivity similarity.

2. Materials and methods

With the above-mentioned goals in mind, this section provides a detailed description of the applied methods. The first subsections focus on design-related and technical specifications of the fMRI data acquisition and preprocessing. Note that the fMRI data processed here had been acquired and preprocessed for a previous project (Navarro Schröder et al., 2015). Subsection 2.5 is dedicated to a detailed and illustrated description of the applied connectopic mapping algorithm. Finally, our validation and parcellation approaches will be outlined.

2.1 Experimental design

To achieve the goal of illuminating the functional organization of the hippocampal long-axis in humans, a set of 22 healthy participants was investigated. As we aimed to obtain information about the organization of the hippocampus while it is functionally active, we deemed it necessary to engage participants in a hippocampus-dependent task while simultaneously recording their brain activity using functional magnetic resonance imaging (fMRI). Specifically, participants performed a navigation task involving spatial memory, which in our rationale should ensure a general functional engagement of the hippocampus, since it is – as established above – pivotally implicated in the processing of spatial memory. At the same time, brain activity was mapped using ultra-high-resolution fMRI, acquired at a magnetic field strength of 7 Tesla. During fMRI acquisition it is vital that participants remain as still and stationary as possible to reduce motion-induced artifacts such as blur and transient amplitude changes. Thus, to collect high quality fMRI data on participants who engaged in a spatial navigation task we employed a virtual reality setup. Participants used a controller in their hand to navigate a three-dimensional virtual arena, which was projected onto a screen in their direct field of view. In the object-location memory task adapted from Doeller et al. (2008; 2010), participants collected and relocated six everyday objects while freely navigating the circular arena (illustrated in Figure 6): In an initial trial, participants collected each object from a specific location within the arena and had been instructed to memorize each object's associated location. In each subsequent trial, they saw an image of one of the previously collected objects in the

upper part of the screen. Upon this cue, they had to navigate to the location where they had initially collected the item and press a button. After each object relocation phase, participants received immediate feedback on how well they did in navigating to the object's associated location (via a happy, neutral, or sad emoticon). Subsequently, the object reappeared at its correct location and participants had to recollect it to reinforce the memory. To separate sets of trials, after an average of three trials (range two to four trials) a fixation cross on a gray background was presented for four seconds.

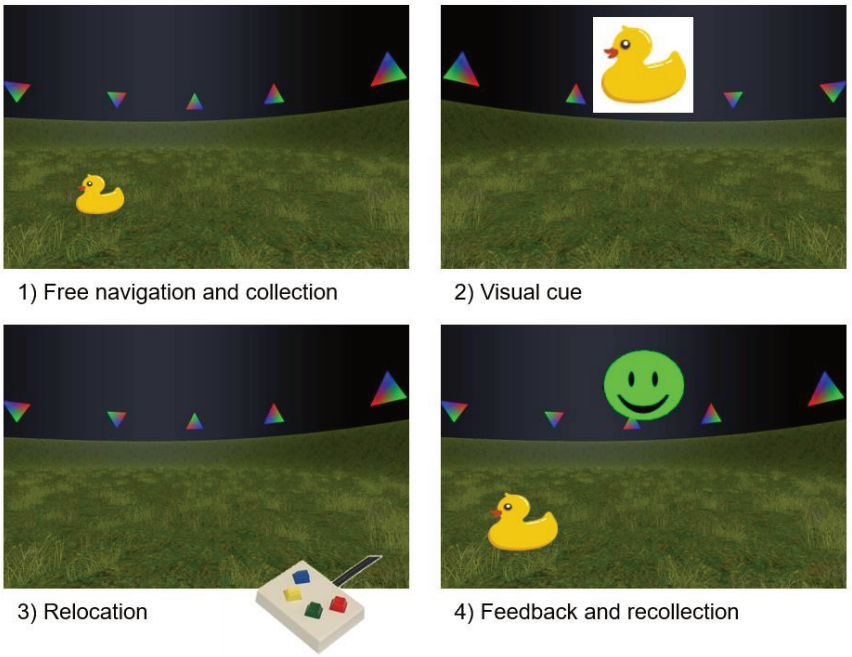

Figure 6: Object-location memory task in virtual reality

During fMRI acquisition, participants performed an object-location memory task in a three-dimensional virtual arena, the edges of which were equipped with colored triangles to facilitate spatial orientation. While freely navigating the circular arena using a manual controller, participants collected six everyday objects (1) and had been instructed to remember their associated location. Upon a visual cue of a previously collected object (2), they were to navigate back to the object's associated location and press a button on the controller (3). After relocation, the participants received immediate feedback regarding the accuracy of the remembered location and recollected the item (4).

Each fMRI scanning session was subdivided into acquisition runs of 210 brain volumes. Participants underwent an average of five runs (± 1) and the total number of runs across all participants was 108. In total, the dataset consisted of 26 healthy participants (11 females and 15 males, age 19-36, mean 23 years, illustrated in Figure 7). The data of 22 participants entered the analyses, as four participants had to be excluded due to excessive movement during scanning (number of instantaneous movements > 0.5 mm (Power et al., 2012) exceeded the mean plus 1 standard deviation).

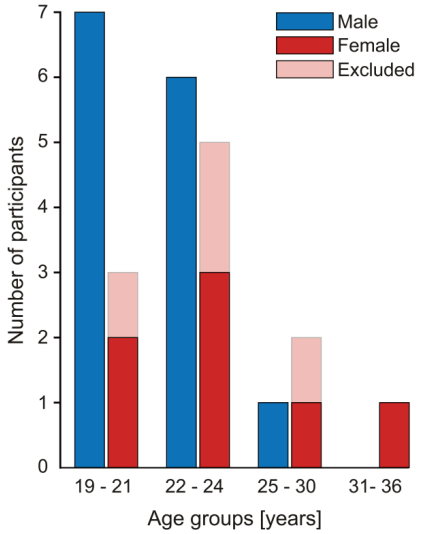

Figure 7: Age and sex distribution of the participants

From the original dataset, containing fMRI scanning sessions of 26 healthy participants, four participants had to be excluded due to excessive movement during scanning (transparent bars). Coincidently, all excluded subjects were female. This resulted in a definitive sample of 22 subjects entering the analyses, including eight female and 14 male participants. Note, however, that one female subject (that also entered the analyses) could not be included in this diagram due to lacking age information.

Materials and methods were approved by the local research ethics committee (CMO University Duisburg-Essen, Germany and CMO region Arnhem-Nijmegen, NL). Written informed consent was obtained from each participant for data analysis and publication of the study results. Note that this dataset had been acquired and preprocessed for a previous project. Detailed technical specifics can therefore be found in the published work of Navarro Schröder et al. (2015) and are only briefly outlined in subsections 2.2 and 2.3. This information is included here for the sake of completeness, but for all readers who are not experts in fMRI analyses the following two subsections are not vital for understanding the main analyses and results of this project.

2.2 FMRI acquisition

The fMRI data was collected on a Siemens MAGNETOM scanner operating at a magnetic field strength of 7 Tesla (Siemens Healthcare, Erlangen, Germany), providing ultra-high-resolution brain images at sub-millimeter resolution. The blood-oxygen-level-dependent (BOLD) T2*-weighted functional images were acquired using a three dimensional echo-planar imaging (EPI) pulse sequence (Poser et al., 2010) and a 32-channel surface coil: Repetition time TR = 2.7 s, echo time TE = 20 ms, flip angle = 14°, slice thickness = 0.92 mm, slice oversampling = 8.3%, in-plane resolution $(0.9\ \text{mm})^2$, field of view = 210 mm in each direction, 96 slices, phase encoding acceleration factor = 4, 3D acceleration factor = 2. To allow for T1 equilibration, the first five volumes of the scan were discarded.

Magnetic resonance images are naturally subject to spatial distortions due to local inhomogeneities of the magnetic field, which can lead to lower quality images and an inaccurate registration between the functional and structural scans. Common sources of field inhomogeneity are differing magnetic susceptibilities of neighboring tissues, such as regions where air-filled sinuses border with bone, like around the temporal lobe (Ojemann et al., 1997). As our region of interest, the hippocampus, is located in the very medial temporal lobe, it was especially important to account for distortion artifacts. Hence, distortion correction of the acquired EPI images was performed using a field map, which was recorded using a gradient echo sequence.

2.3 FMRI data preprocessing

Preprocessing was implemented using the automatic analysis library (Cusack et al., 2015; http://automaticanalysis.org) and included motion correction in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and data denoising with the FIX artifact removal procedure implemented in FSL 5.0.4 (Salimi-Khorshidi et al., 2014; Griffanti et al., 2014; http://fsl.fmrib.ox.ac.uk/fsl) that was trained manually on ten out of 22 participants. Furthermore, nonlinear normalization to a group-specific EPI template was performed with the help of the Advanced Neuroimaging Toolbox (Avants et al., 2011; http://www.picsl.upenn.edu/ANTS). The use of a group-specific template was

feasible because of the anatomical detail provided by the sub-millimeter resolution of our dataset. This rendered the registration to structural images unnecessary, which eliminated a potential source of noise. The functional data were smoothed with a full-width at half-maximum (FWHM) Gaussian kernel of 2.5 mm (roughly 2.5 times the voxel size) to increase the signal-to-noise ratio and to improve independent-component-analysis (ICA) based data denoising with the FIX procedure while maintaining high spatial resolution. Correction for residual motion artifacts was performed with AROMA (automated removal of motion artifacts), another ICA-based method, which has been shown to outperform motion scrubbing and spike-regression methods both in terms of reproducibility of resting-state networks and conservation of temporal degrees of freedom (Pruim et al., 2015a; Pruim et al., 2015b). In addition, brain extraction (Smith, 2002), tissue segmentation (Zhang et al., 2001) and high-pass filtering with a 128 s cut-off were carried out using FSL 5.0.4.

2.4 Region of interest definition

To investigate the neuronal activity within the hippocampus, it was necessary to extract the BOLD signal measurements of our region of interest from the whole-brain fMRI scans. This required a three-dimensional binary mask defining the location and outline of the hippocampus within our participants' brains. Therefore, the hippocampus was manually delineated on the group-specific template using ITK-SNAP 3.6.0 (Yushkevich et al., 2006; www.itksnap.org) according to the instructions provided by the Harmonized Protocol for Manual Hippocampal Segmentation (Boccardi et al., 2015; Frisoni et al., 2015; www.hippocampal-protocol.net). This unified protocol was jointly developed by the European Alzheimer's Disease Consortium (EADC) and the Alzheimer's Disease Neuroimaging Initiative (ADNI). Figure 8 illustrates the resulting hippocampal mask.

One might think that it may be faulty to use the exact same mask for all participants, as acquired scans can vary in brain size and head orientation across individuals. However, before applying the mask and performing the analyses described below, we transformed every participant's fMRI dataset into the same group-specific space. In other words, we adjusted individual participants' brain volumes to match the same coordinates as our group-specific template. This so-called nonlinear normalization to

our group-specific template was part of the preprocessing (subsection 2.3). As therefore all our data referred to the same space and coordinates, it was sufficient to define the hippocampal outline only once on the group-specific template for the mask to universally fit all participants. In total, the binary mask covering the left hippocampus contained 4664 nonzero voxels and the mask for the right hippocampus contained 5112 nonzero voxels.

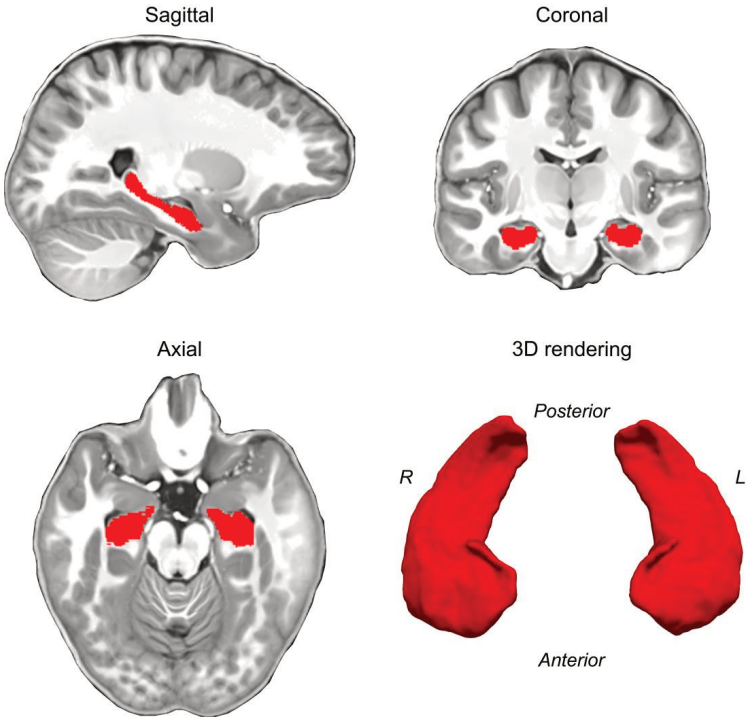

Figure 8: Hippocampal masks

Depicted are manually delineated hippocampal masks of both hemispheres overlayed onto sagittal, coronal, and axial slices of the group-specific brain template. Furthermore, a 3D rendering of the masks is included, corresponding to a view from an antero-ventral direction.

2.5 Quantification of hippocampal functional connectivity topography

To deduce the functional organization of the hippocampus based on the acquired and preprocessed fMRI data, we employed a recently developed analysis algorithm, connectopic mapping (Haak et al., 2018), that has proven successful in previous fMRI studies (Navarro Schröder et al., 2015; Przeździk et al., 2019). As this entirely data-driven algorithm constitutes the heart of the analyses in this project, its three main steps are described below in more detail. The underlying Python code is openly accessible at https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/OtherSoftware, section 'Congrads', and was implemented using Python 3.6.3 (Van Rossum & Drake Jr, 1995).

2.5.1 Computation of hippocampal functional connectivity fingerprints

As a first step of the algorithm, we aimed to quantify the functional connectivity of the hippocampus with the rest of the brain. Generally, functional connectivity indicates the level of coactivation of different brain regions and is thus defined as the temporal correlation between measured activity signals from those brain regions (Friston et al., 1993). Here, we were interested in voxel-wise functional connectivity profiles of the hippocampus, i.e., we aimed to determine how strongly neuronal activity of each hippocampal voxel correlated with activity from the rest of the brain.

To this end, we first processed each participant's fMRI dataset individually in the following way (details on obtaining group-level results in subsection 2.5.4): We extracted the neuronal activity measurements, i.e., time-courses of BOLD signal intensity, from all voxels within the hippocampus of one hemisphere, using our manually delineated hippocampal mask. Note that we performed the analyses separately for the left and right hippocampus. In addition, we extracted the time-courses of all extrahippocampal gray matter voxels, using a group-specific gray matter mask. Restricting the analysis to gray matter voxels corresponds to the goal of identifying mere firing patterns of neuronal cell bodies (gray matter) without introducing the potential bias of recording delayed activation in their axons (white matter). Thanks to the sub-millimeter resolution provided by the 7 Tesla fMRI scanner, the binary gray matter mask comprised roughly 800.000 nonzero voxels. Thus, the matrix storing the time-courses of extrahippocampal gray matter voxels contained 800.000 entries for

each time-point. Processing a matrix of that size would render the following analyses highly expensive in terms of computational resources and time. This is a common challenge in big data processing, which we overcame by reducing the dimensionality of the dataset using singular value decomposition (SVD). Generally, dimensionality reduction procedures like SVD transform a high-dimensional source matrix into an output matrix of lower dimensionality by extracting the ‘essence’, or principle components, of the original matrix. Importantly, dimensionality reduction using SVD is lossless because the data in the high-dimensional source matrix typically features many redundancies in its pattern, which do not contribute new or unique information, but just elongate evaluation of the data. Here, we decomposed the initial matrix, containing the time-courses of 800.000 extrahippocampal gray matter voxels, into 210 principle components, containing the same information and explaining 100% of the source variance but allowing for better computational manageability (Figure 9, SVD). Intuitively, these principle components can be thought of as ‘principle time-series’ of the extrahippocampal gray-matter data, from which theoretically all original time-courses could be reconstructed.

At that point, we had extracted on the one hand the time-courses of BOLD signal for all hippocampal voxels and on the other hand the 210 ‘principle time-series’ of BOLD signal from all extrahippocampal gray matter voxels. Based on this, we aimed to deduce a functional connectivity profile for each hippocampal voxel denoting how strongly the respective hippocampal voxel’s BOLD signal correlated with each of the 210 principle patterns of extrahippocampal BOLD signal (Figure 9, CORR). In other words, we determined how closely a hippocampal voxel’s time-course resembled each of the 210 principle components extracted from the extrahippocampal gray matter time-courses. As a mathematical measure of resemblance, the code uses the pairwise Pearson correlation, yielding correlation values ranging from negative one (minimal resemblance) to one (maximal resemblance). This provided a set, or vector, of 210 correlation values for each hippocampal voxel, denoting the respective voxel’s individual fingerprint in terms of coactivation with the rest of the brain, thus termed a functional connectivity fingerprint.

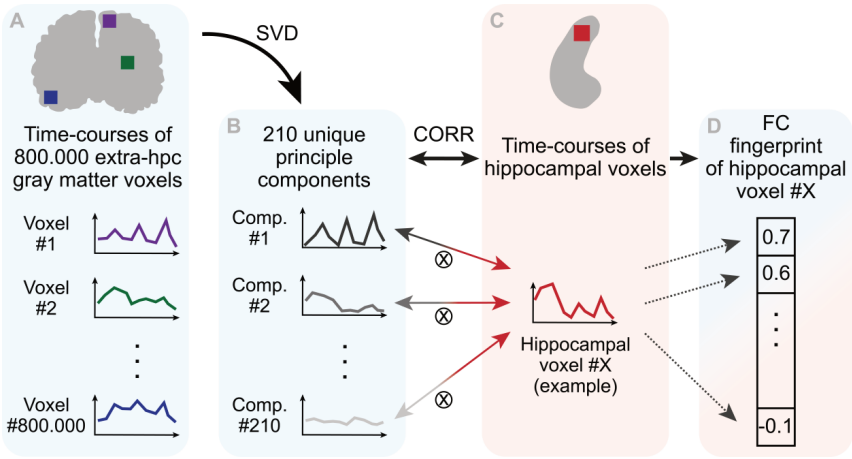

Figure 9: Dimensionality reduction and computation of functional connectivity fingerprints

SVD: In the 7 Tesla fMRI data, each brain volume contains roughly 800.000 extrahippocampal (extra-hpc) gray matter voxels (panel A). We used singular value decomposition (SVD) to remove redundancies in this data and to extract only the 210 unique principle components of the gray matter data (panel B). Thereby, the dimensionality of the gray matter data was reduced from 800.000 voxels x 210 time-points to 210 components x 210 time-points.

CORR: As the first main step of the connectopic mapping algorithm, we aimed to determine how strongly each of the 'principle time-series' from the gray matter data was reflected in a hippocampal voxel's time-course. This was measured as the pairwise Pearson correlation (CORR) between a hippocampal voxel's time-course (panel C) and every single principle component (panel B), respectively (each performed correlation indicated by ⊗). This yielded a vector with 210 correlation values for each hippocampal voxel (panel D shows the correlation vector for hippocampal voxel #X). Each of these vectors can be thought of as the respective hippocampal voxel's functional connectivity (FC) fingerprint with respect to the rest of the brain.

2.5.2 Quantification of fingerprint similarity

Using the computations above, we obtained a functional connectivity fingerprint for each hippocampal voxel, respectively. However, we were not solely interested in these vectors quantifying hippocampal functional connectivity, but ultimately intended to identify an inherent organizational pattern of functional connectivity similarity within the

hippocampus. Therefore, as a second step in the algorithm, the code determined how similar or dissimilar the individual functional connectivity fingerprints were across hippocampal voxels. To this end, we iterated through hippocampal voxels, compared one-by-one the fingerprint of one hippocampal voxel with every fingerprint of the remaining hippocampal voxels and stored a measure of the similarity between the respective two fingerprints in a so-called similarity matrix (Figure 10).

As a mathematical measure of similarity, we employed the η^2 coefficient, which generally quantifies the similarity between two signals on a point-by-point basis (mathematical definition provided in Cohen et al. (2008), equation 1). Briefly, at every position of a vector, the η^2 coefficient incorporates the distances of the value in one vector (here e.g., fingerprint of hippocampal voxel #1) and the value in a second vector (e.g., fingerprint of hippocampal voxel #2) to the mean value of the two vectors at that position. The sum of squared distances is then inverted and normalized to range from zero to one, indicating no or high similarity of the two vectors, respectively. In our analysis pipeline, an η^2 coefficient was calculated for every pair of hippocampal voxels. More precisely, functional connectivity fingerprints of two hippocampal voxels were compared on a point-by-point basis, producing one η^2 coefficient per pair of hippocampal voxels, which characterizes the similarity between these two voxels' functional connectivity fingerprints. The ensuing η^2 values were stored in a similarity matrix with the size $n \times n$, where n equals the number of hippocampal voxels. The more similar two hippocampal voxels' fingerprints are, the higher the η^2 coefficient between them, ranging from zero (entirely dissimilar) to one (identical).

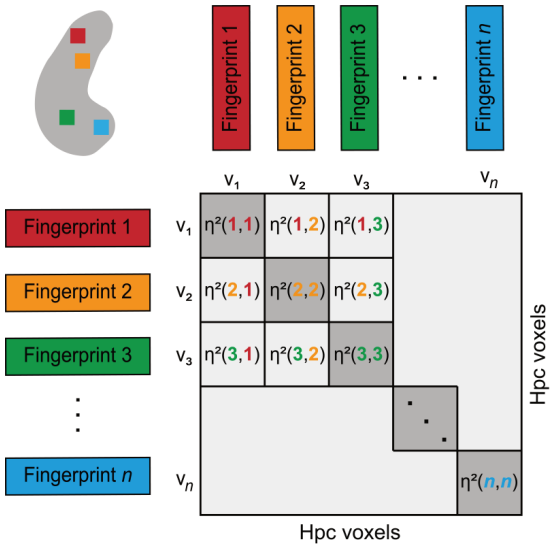

Figure 10: Matrix of fingerprint similarity between hippocampal voxels

As a second step of the connectopic mapping algorithm, we compared how similar or dissimilar functional connectivity fingerprints were across hippocampal (hpc) voxels. To this end, we generated a similarity matrix with *n* rows and *n* columns, where *n* equals the number of hippocampal voxels. As a measure of similarity between two fingerprints, we computed an η^2 coefficient $\eta^2(x, y)$ for each pair of hippocampal voxels x and y. The η^2 values reached from zero to one, indicating no or high similarity, respectively. Thus, elements on the diagonal (dark gray squares) are filled with ones as comparing a voxel's fingerprint to itself yields $\eta^2 = 1$ (maximal similarity). Furthermore, the similarity matrix is symmetrical along the diagonal because $\eta^2(x, y)$ equals $\eta^2(y, x)$.

2.5.3 Obtaining hippocampal maps using Laplacian Eigenmaps

We have now obtained a matrix that fully describes the similarity between functional connectivity fingerprints across hippocampal voxels. In fact, this similarity matrix contained all information required to derive an intrinsic organizational pattern of functional connectivity similarity for the hippocampus, which was our eventual goal. However, for a visual representation, it would be preferable to produce a map of the

hippocampus with a single value per each voxel (instead of a similarity coefficient per each pair of hippocampal voxels). The exact value that would be assigned would represent an arbitrary measure of functional behavior, so that voxels with similar behavior would be assigned similar values. Such a hippocampal map would accurately map the spatial arrangement of the identified organization and allow for easier interpretation of the underlying organizational pattern.

To achieve this and to transform the similarity matrix into a hippocampal map, we first built a graph from the similarity data. Importantly, in the mathematical field of graph theory, a graph serves as an abstract representation of a dataset and consists of datapoints (nodes) which are interconnected by links (edges). Per definition, a graph is 'connected' if there exists a path from each node to any other node via an arbitrary number of edges. In our graph representation of the similarity matrix, each node represented a hippocampal voxel and edges between nodes corresponded to the similarity of the respective two voxels' functional connectivity fingerprints (Figure 11). This means that edges between two nodes were not binary – with the value of either zero (no connection) or one (connection) – but weighted according to the similarity, i.e., η^2 value, of the respective two voxels. This resulted in a graph with 'stronger' and 'weaker' edges (connections) between nodes (voxels). Importantly, at this point we reduced the complexity of the data in that weighted edges were not blindly established between all nodes. We disregarded all edges that were 'too weak', which in this regard was defined as 'not essential to form a connected graph'. This concept was implemented using an iterative procedure, in which we initially created links only between the voxels with the highest similarity values. Then we checked if the resulting graph is connected and if not, we moved the threshold further down in that edges were established for the next lower similarity values. This process was repeated until we first obtained a connected graph. Ensuring a connected graph accords with the concept that we aimed to find an organizational pattern covering the entire hippocampus rather than multiple mappings that each might cover only a part of the region of interest. Finally, the ensuing graph featured a network of edges that were weighted according to the functional connectivity similarity but thinned out in that unnecessary small similarities between nodes were disregarded.

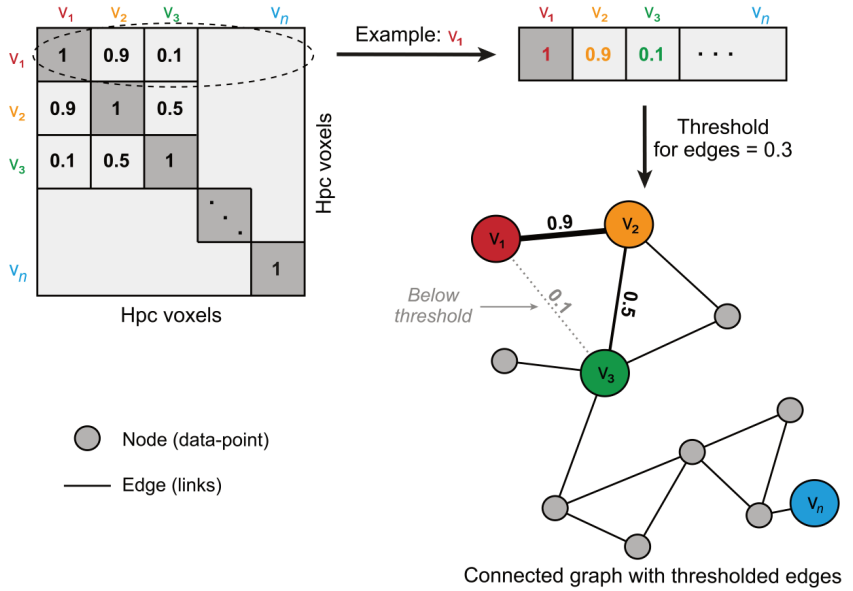

Figure 11: Graph representation of the similarity matrix

The similarity matrix (top left) was transformed into a graph (bottom right). In this graph, every node represents a hippocampal voxel (for illustration purposes, nodes corresponding to voxels that are described in the similarity matrix were highlighted). Each edge between two nodes represents the similarity of the respective two voxels' fingerprints. Therefore, edges were weighted according to the η^2 values between the two respective voxels' fingerprints. In addition, edges were only established between voxels whose fingerprint similarity exceeded a certain threshold (in this example: $\eta^2 > 0.3$). The threshold was chosen so that the ensuing graph was connected, i.e., there was a path from every node to any other node via an arbitrary number of edges. The thresholding process is illustrated for the highlighted voxels $v_1 - v_3$: η^2 values above 0.3 were translated into weighted edges (hence: strong connection between v_1 and v_2; weaker but still relevant connection between v_2 and v_3). In contrast, η^2 coefficients below 0.3 were not necessary for the graph to be connected and were disregarded (hence: no connection between v_1 and v_3).

Now this graph constitutes a data representation in which hippocampal voxels with highly similar functional connectivity profiles are more strongly connected, or closer together, than voxels with entirely dissimilar fingerprints. Hence, it is already a representation of the functional organization within the hippocampus, yet we needed to transform it into a map of the hippocampus to be able to visualize the data and to facilitate interpretation. This was rendered possible by the use of the so-called

Laplacian Eigenmaps algorithm, developed by Belkin and Niyogi (2003). Conceptually, this algorithm is a means of nonlinear dimensionality reduction, which relies on a similar concept as SVD (see above, subsection 2.5.1) in that the underlying problem is a dataset of unnecessarily high dimensionality that complicates data analysis and interpretation (Tenenbaum et al., 2000). The Laplacian Eigenmaps algorithm assumes that the dimensionality of the data is only artificially high, and the representation of the data of interest can be simplified. More precisely, the original data can be described as a low-dimensional representation (so-called manifold) embedded in a higher-dimensional space. Importantly, the Laplacian Eigenmaps algorithm computes a lower-dimensional representation of the data that at the same time optimally preserves local neighborhood information. This results in a representation map that naturally arises from the geometry of the manifold.

In our implementation, the input for the Laplacian Eigenmaps algorithm was the graph in which nodes represented voxels that were connected by stronger or weaker edges according to the similarity of their functional connectivity fingerprints. In other words, voxels with similar functional connectivity profiles stayed closer together than voxels with no similarity. The Laplacian Eigenmaps algorithm pertained this neighborhood information and transformed the graph into a mapping in which every hippocampal voxel was assigned a value between zero and one according to its location within the topography of functional connectivity similarity (illustrated in Figure 12). That means that voxels with similar fingerprints were assigned a similar value, whereas voxels with dissimilar fingerprints remained farther away from each other in this mapping. Thus, importantly, the absolute value on this scale is not equal to a measure of similarity in which zero indicates no similarity and one indicates high similarity (as was true for η^2 values). Instead, one voxel's value on this arbitrary scale is only meaningful with respect to values of the other hippocampal voxels. The resulting mapping conveys the dominant topography of hippocampal functional connectivity similarity, i.e., a functional connectivity topography or 'connectopy', and is henceforth referred to as connectopic map.

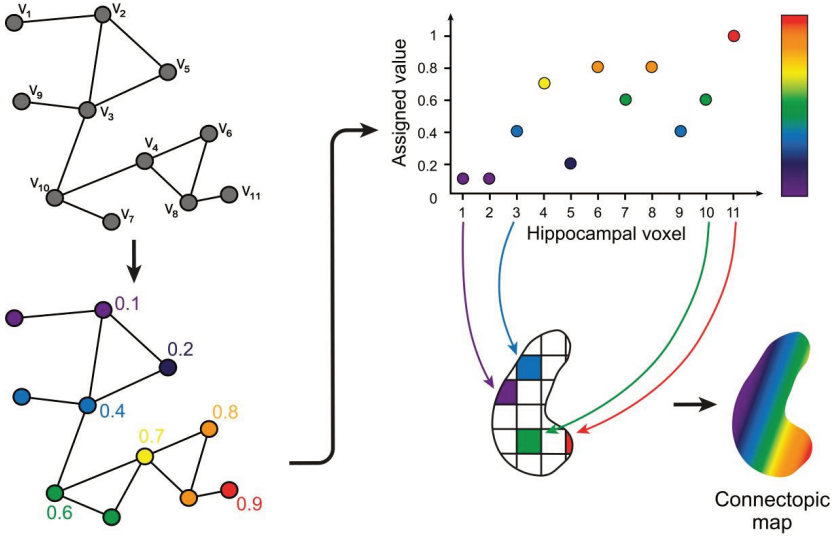

Figure 12: Mapping of functional connectivity similarity onto hippocampal map

Using the Laplacian Eigenmaps algorithm, a means of nonlinear dimensionality reduction, we transformed the graph, representing the proximity of hippocampal voxels in terms of their functional connectivity fingerprints, into a hippocampal map. For illustration purposes, the depicted graph only consists of eleven hippocampal voxels, whereas in reality this graph comprised all voxels within the hippocampus. Importantly, this algorithm optimally preserves the underlying neighborhood information in the resulting mapping. The ensuing connectopic map captures the topography of functional connectivity similarity as values on an arbitrary scale between zero and one and projects this scale back onto hippocampal voxels. Note that the connectopic map illustrated here constitutes only a fictitious example corresponding to a theoretical transversal gradient.

For the sake of completeness, it should be mentioned that as a result of the mathematical computations within the Laplacian Eigenmaps algorithm – or more specifically because an eigenvalue problem typically has several solutions – several connectopic maps were obtained. However, as suggested by Belkin and Niyogi (2003) and concordant with previous implementations (Haak et al., 2018; Navarro Schröder et al., 2015; Przezdzik et al., 2018), the eigenvector with the smallest nonzero eigenvalue was considered the dominant connectopic map, which explained most of the data's variance. All results presented below refer to this first, dominant topography of functional organization.

On a side note, technically building the graph representation of the similarity matrix can be viewed as the first step of the Laplacian Eigenmaps algorithm but was described separately here for didactic purposes. Those interested in the mathematical details and calculations underlying the Laplacian Eigenmaps approach may find the description in Belkin's doctoral thesis helpful (Belkin, 2003), complemented by the examples in the publication by Belkin and Niyogi (2003).

2.5.4 Single-subject and group-level averaging

In this study, we planned to consider connectopic maps on a single-subject level as well as on the group-level. Thus, we aimed to compute on the one hand a connectopic map for each individual participant and on the other hand a group-specific connectopic map. As each participant's fMRI scanning session was divided into several acquisition runs, we applied the first two steps of connectopic mapping (computation of connectivity fingerprints and fingerprint similarity) to every run of each participant's data separately, yielding one similarity matrix for each acquisition run. Then, we merged all runs of one participant by first averaging the η^2 values over all runs of this participant, yielding a participant-specific similarity matrix. Then, the third step of the algorithm (Laplacian Eigenmaps) was applied to the participant-specific similarity matrix to eventually produce a participant-specific connectopic map, which will be reported on below as the single-subject level results. Additionally, group-level results were obtained by averaging the 22 participant-specific similarity matrices and applying a final Laplacian Eigenmaps algorithm to yield one group-specific connectopic map.

2.6 Quantification of the modularity in connectopic maps

Using the obtained connectopic maps, we aimed to determine whether the dominant functional organization in the hippocampus either followed a smooth gradient or a modular organization. To answer this, connectopic maps were plotted as histograms with 75 bins, depicting the number of voxels (y-axis) that were assigned a certain value within the topography of functional connectivity similarity (x-axis). In theory, a smooth functional gradient would show as a horizontal line of bins in the histogram (Figure 13, left panel). In contrast, a modular organization would manifest as several clusters of

voxels with very similar functional connectivity profiles, corresponding to several abrupt peaks in the histogram (Figure 13, right panel). Therefore, to determine the modularity of our participants' histograms, we counted how many clusters of adjacent bins exceeded the mean number of voxels per bin in the respective histogram. To favor global rather than local peaks, thus preventing a large number of local false positives, we defined a minimum peak distance such that clusters of adjacent bins above the mean had to be separated by at least four bins (illustrated in the right panel in Figure 13). This peak finding algorithm was implemented in MATLAB (2017).

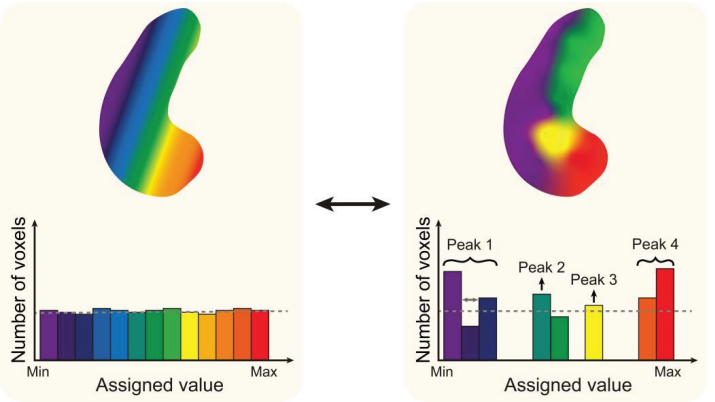

Figure 13: Histograms of a smooth versus modular organization

Hippocampal connectopic maps were plotted as histograms, using 75 bins (for illustration purposes, this sketch contains only 13 bins). Each histogram depicts the absolute number of voxels that were assigned a certain value within the topography of functional connectivity similarity, ranging from zero (Min) to one (Max). In a strictly smooth pattern, every assigned value would be present in the histogram, resulting in a horizontal line (left panel). In contrast, in case of a modular organization, the histogram would consist of discrete similarity peaks and some values may not be present at all (right panel). To assess the modularity in the histograms, a peak identification algorithm was implemented such that sets of adjacent bins above the mean (indicated by the dotted line) were defined as peaks. However, cases like the purple cluster, where bins exceeding the mean are separated but pertain to the same cluster, would be identified as two peaks if peak identification was applied without additional parameters. To overcome this, we implemented a criterion of minimum peak distance (MPD) between peaks (in reality: MPD = 4, in this illustration: MPD = 2). Thus, the fictitious example in this illustration contains four peaks of functional connectivity similarity.

2.7. Validation of a potentially modular organization

As outlined above, we identified the number of peaks in the histograms to deduce information about the modularity of the underlying connectivity topography. However, this was only a valid conclusion if the identified peaks were higher than peaks that would correspond to a nonmodular organization. Therefore, it was necessary to find out whether the ‘peakiness’ in our data represented a meaningful functional organization or could in fact be caused by chance. To this end, we compared the connectopic maps of our participants to a simulated set of random connectopic maps with regards to their respective ‘peakiness’.

2.7.1 Creation of simulated connectopic maps

To simulate connectopic maps, we aimed to preserve the integrity of the analyses as much as possible. Thus, to introduce the simulation at the most upstream point of our analysis pipeline, we simulated random fMRI datasets. These were based on our 22 participants’ datasets to preserve the fundamental statistical properties of real fMRI data. First, we applied a random spatial and temporal shuffling procedure to our participants’ fMRI acquisition runs, using MATLAB. More precisely, for each run we separately shuffled the BOLD signal intensity values of all hippocampal voxels on the one hand and all extrahippocampal gray matter voxels on the other hand. This shuffling procedure was repeated ten times for all runs of each participant, thus yielding 1080 simulated fMRI datasets in total. Note that although this random shuffling approach was performed with the intention of preserving the fundamental statistical properties of real fMRI data, it has likely disrupted the naturally occurring level of spatial autocorrelation (potentially arising bias discussed in detail in subsection 4.1.4).

Next, like it was done with the participants’ datasets as part of the preprocessing, the shuffled datasets were smoothed with a FWHM Gaussian kernel of 2.5 mm using SPM 12. After that, they were each processed using the connectopic mapping algorithm described above. Thus, each shuffled dataset yielded a hippocampal map of functional connectivity similarity, which could be plotted as a histogram.

2.7.2 Generation of a null distribution of peak heights

Based on these histograms originating from simulated datasets, it was our goal to create a reliable null distribution of peak heights. Therefore, we ran a peak identification algorithm implemented in MATLAB to determine the values of the ten highest peaks in each histogram. Analogous to the peak identification in real participants' data above, we defined a minimum peak distance criterion so that peaks had to be separated by at least four bins (illustration in Figure 13). To imitate the real sample size of 22 participants, peak identification was performed on 22 histograms that had been randomly selected out of the 1080 shuffled histograms. Next, the 22 identified peak height values were averaged for each peak order, ranging from one to ten, i.e., we calculated the mean height of the highest peak in the 22 selected histograms, the mean height of the second highest peak in the 22 selected histograms and so on. This random selection (22 out of 1080 histograms) and averaging of highest through tenth highest peaks were repeated 1000 times, yielding one null distribution of peak heights for every peak order, each incorporating 1000 datapoints. To assess the probability of histogram peaks with a magnitude as in our sample of 22 participants, we then compared the mean peak heights of the participants to the 95th percentile of the null distribution. Thanks to this analysis, we were able to assess whether applying connectopic mapping to a randomly shuffled dataset could yield topographies that were equally modular as the observed data.

2.8 Functional parcellation using *k*-means clustering

In addition to identifying the dominant functional connectivity topography of the hippocampus, we planned to tackle a second question: Given a modular hippocampal organization, we aimed to establish a functional parcellation into subunits based on the revealed discrete pattern of functional connectivity similarity. To this end, we applied *k*-means clustering, implemented in MATLAB, to each participant's connectopic map separately as well as to the group-averaged map. *K*-means clustering is a popular clustering algorithm that attempts to split a given dataset into a predefined number of clusters *k* (Hartigan & Wong, 1979). Methodologically, *k*-means clustering employs an iterative refinement technique, which can be described as follows: Initially, a number

(*k*) of so-called centroids are chosen randomly. A centroid is the specific datapoint (real or imaginary) that is located at the center of a cluster. Having defined centroids, each datapoint is assigned to the cluster with the closest centroid, providing an initial, preliminary parcellation. Subsequently, the centroid is adjusted in that it is redefined as the arithmetic mean of all assigned datapoints. These two steps of datapoint assignment and centroid adjustment are repeated until the centroids stabilize and assignments no longer change. Having outlaid this principle, it becomes apparent that in some cases there can be more than one equally good clustering solutions and the algorithm leads to slightly different parcellations upon repetition. In those cases, we repeated *k*-means clustering of the respective dataset 1000 times and chose the cluster assignments that were most frequently obtained. This was necessary in three participants for the left hippocampus and two participants for the right hippocampus.

We chose *k*-means clustering for its simplicity as well as speed and because it performed well in previous fMRI studies performing functional parcellations (e.g., (Chase et al., 2015; Plachti et al., 2019; Robinson et al., 2015; Vos de Wael et al., 2018). However, there are certain drawbacks, including for instance the necessity to predefine the number of clusters *k*. Hence, to corroborate our choice of *k*, we aimed to identify the optimal number of clusters using the so-called elbow method (Kodinariya & Makwana, 2013), an iterative procedure consisting of the following steps: *k*-means clustering was repeated for different numbers of clusters *k* (1 – 15) for each participant. Note that this was done separately for the left and right hippocampus. For each *k*, the total clustering error was calculated as the sum of squared Euclidian distances from all data points of a cluster to the respective cluster's centroid (sum of squared errors). The calculated clustering errors were then plotted as a function of *k*. Since our goal was a parcellation that is consistent across our sample of 22 participants, we calculated the overall mean of all participant-specific curves, yielding one group-level elbow curve for the left and right hippocampus, respectively. According to the elbow method, the optimal number of clusters was then identified as the starting point of the plateau in the resulting curve. The underlying rationale is that at the point of the curve where the plateau starts, increasing *k* would not yield a significant reduction of the total clustering error, but decreasing *k* would result in a considerable augmentation of this error. In other, less abstract words, imagine that clustering with the minimum number of clusters $k = 1$ yields a certain clustering error,

which can be considered the maximally possible error. In contrast, clustering with the maximum number of clusters, here k = 15 (hypothetically the number of datapoints is considered the maximum k), yields a certain clustering error, which is considered the minimally possible error. The maximally achievable reduction of clustering error within the boundaries of k = 1 and k = 15 (hypothetically number of datapoints) is now used as a reference to determine the optimal number of clusters for the dataset: The ideal k, coinciding with the starting point of the elbow curve's plateau, is identified as the first k where more than 90% of the maximally possible reduction of clustering error is achieved (MATLAB code made available by Bao (2021)). It should be mentioned that in our analyses, the optimal k determined using the elbow method with a maximum k of 15 clusters was equal to the results obtained when the number of datapoints (i.e., number of hippocampal voxels) was considered the maximum k, since the clustering error very quickly asymptotically approaches the x-axis with increasing k (visible in Figure 19).

3. Results

In this project, we used ultra-high-resolution fMRI to map healthy participants' brain activity while they performed a hippocampus-dependent spatial navigation task. By applying a recently developed data-driven analysis algorithm, connectopic mapping, we aimed to illuminate the organizational pattern of functional connectivity similarity. Based on the revealed organization, we furthermore performed *k*-means clustering to identify functional subdivisions within the hippocampus.

Using these analyses, the two key questions we strived to answer are (1) whether the topography of functional connectivity similarity either follows a smooth gradient or exhibits step-like transitions. In case of an identified modular pattern, we planned to tackle the question (2) regarding the number of functional hippocampal subunits.

3.1 Functional long-axis gradient in group-level average

To answer question (1), we first consider the group-level connectopic maps of our sample of 22 participants, which are shown in Figure 14. Note that the analyses were performed for the left and right hippocampus separately (left and right panel, respectively). At the depicted sagittal plane, the hippocampus extends in its full length, at one level with parts of the striatum, the trigone of the third ventricle and the amygdala (Trepel, 2008). Each panel shows an overlay of the group-averaged connectopic map onto our group-specific brain template. These hippocampal maps represent the topography of functional connectivity similarity, based on the respective hippocampal voxel's functional connectivity with the rest of the brain. Note that similar values (i.e., colors in Figure 14) within the topography indicate similar functional connectivity fingerprints of the respective voxels. On the group-level, the pattern of functional connectivity similarity clearly follows a longitudinal gradient, in that the most anterior portion of the hippocampus is entirely distinct from the most posterior portion. This confirms the notion established by previous work that the anterior and posterior parts of the hippocampus are implicated in different brain functions (e.g., Fanselow and Dong, 2010).

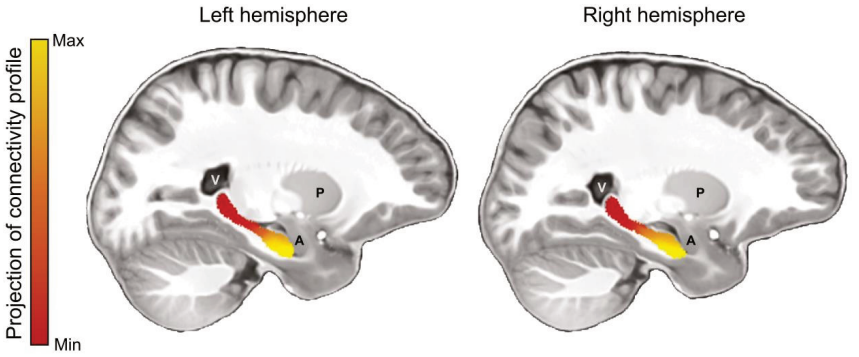

Figure 14: Functional long-axis differentiation in group-level connectopic maps

Here, group-level connectopic maps for the left and right hippocampus (left and right panel, respectively) are overlayed onto sagittal plains of our group-specific brain template. At this position, the hippocampus extends in its full length, with the posterior pole adjacent to the trigone of the lateral ventricle (marked with V), the anterior pole neighboring parts of the amygdala (A) and in one plain with the putamen (P). The colormap of the hippocampal connectopic map displays the pattern of functional connectivity similarity, wherein similar colors indicate similar functional connectivity fingerprints of the respective voxels. In line with the established notion of an anterior-to-posterior functional distinction, the pattern of functional connectivity similarity within both the left and right hippocampus points towards a functional differentiation between the anterior and posterior hippocampal pole.

Having identified the long-axis as the dominant organizational axis of functional connectivity similarity in the hippocampus, we next aimed to address question (1) regarding the nature of the transition between the anterior and posterior hippocampal pole. In the mere projection of the connectopic maps (Figure 14), the transition is somewhat blurred and cannot be pinpointed. Therefore, Figure 15 shows the group-level connectopic maps plotted as histograms with 75 bins, depicting the absolute number of hippocampal voxels that are assigned a certain value within the projection of functional connectivity similarity. Importantly, the x-axis displays a projection of hippocampal voxels' functional connectivity fingerprints independent of their spatial information, as the connectopic mapping algorithm assigns the values solely depending on the closeness of the respective voxels in terms of their functional connectivity fingerprints (details in subsection 2.5.3). Interestingly, as in our case the topography of functional connectivity similarity clearly follows a longitudinal gradient

(Figure 14), the x-axis of the pertaining histograms roughly corresponds to the hippocampal long-axis. Hence, histogram patterns allow for the precise assessment of functional transitions along the long-axis organization. Theoretically, in case of a perfectly smooth gradient regarding the anterior-to-posterior transition of functional connectivity similarity, one would expect no significant clusters of voxels with a similar value, i.e., no peaks of functional connectivity similarity. Therefore, a smooth gradient of functional long-axis differentiation would correspond to a horizontal line in the histogram (illustration in Figure 13, left panel). In contrast, a strictly modular hippocampal organization would present itself in a histogram pattern with several abrupt peaks separated by a flat line (Figure 13, right panel). Interestingly, the group-level histograms of both the left and right hippocampus neither reveal a horizontal line nor any radical peaks, but a rather irregular slope. On the one hand every bin contains at least around 50 hippocampal voxels indicating a gradual change, but on the other hand minor peaks point towards some granularity within this gradient. Moreover, in both group-level histograms a major peak emerges at the posterior hippocampal pole (reflected by the left edge of the histogram). Thus, the group-averaged data suggest a rather blended topography of functional connectivity similarity with several peaks superimposed on a yet not perfectly smooth gradient.

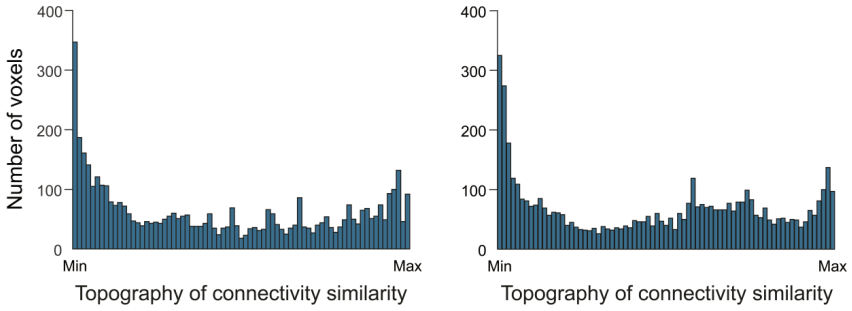

Figure 15: Discrete pattern superimposed on gradient in group-level histograms

To assess the transition of an anterior-to-posterior functional differentiation in more detail, the group-level connectopic maps of the left and right hippocampus (left and right panel, respectively) are plotted as histograms with 75 bins. Each bin corresponds to the absolute number of voxels that are assigned a certain value within the projection of functional connectivity similarity. The emerging pattern reveals an irregular slope with several peaks superimposed on a functional gradient.

3.2 Discrete organization of single-subject connectopic maps

However, it is possible that group averaging across 22 subjects obscured the true individual organizational patterns of every single participant. In theory, given a smooth organization in all participants, the average would perhaps not change much in comparison to each individual map. But assuming a modular organization, the location of transitions between putative hippocampal modules are likely to differ between participants. Thus, averaging might have smoothed out potentially discrete subject-specific patterns, making it necessary to analyze the connectopic maps on a single-subject level. Hence, Figure 16 shows the histograms of an exemplary participant with the same binning as Figure 15. Strikingly, both hippocampi of this participant indeed exhibit a highly discretized pattern of functional connectivity similarity with several clearly demarcated peaks, separated by a nearly flat line. This pattern is especially pronounced in the right hippocampus. Note that similarly to the group-level histograms there is a peak of functional connectivity similarity at the very posterior end of the hippocampus (corresponding to the left-hand side of the histogram). For illustration purposes, the mean number of voxels per bin is overlayed

onto the histogram as a dotted line. Once again, in case of a smooth, continuous gradient of functional connectivity similarity, the slope of the histogram would resemble the course of this line, as all bins would contain the same number of voxels. This is not the case in the exemplary participant.

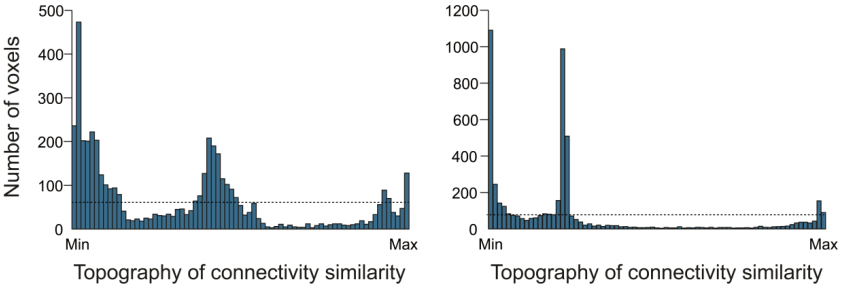

Figure 16: Discretized single-subject histograms

This illustration shows the histograms of the left and right hippocampus (left and right panel, respectively) of an exemplary participant (henceforth referenced as participant #1). Both histograms reveal a discretized pattern of functional connectivity similarity, which is more pronounced in the right hippocampus. Dotted lines indicate the mean number of voxels per bin (left hippocampus: 62.2 voxels, right hippocampus: 68.2 voxels).

Most of the other participants exhibit a similar topography with clearly discretized peaks of functional connectivity similarity, however there is variability regarding the number of peaks (all histograms in Figures 22a and 22b). With respect to research question (1), this clearly points towards a modular functional organization in distinct subunits.

3.3 Validation of the modularity with respect to the null distribution

The next logical question emerging from this evidence, our research question (2), is of course how many subunits the hippocampus is composed of. However, before we scrutinize the single-subject histograms with regards to the number of subunits, it is important to validate our underlying inference: If we base the conclusion of a discretized functional organization on the occurrence of peaks in the respective histograms, it is necessary to test whether the extent of ‘peakiness’ in our data could

be expected based on chance, i.e., could be caused by random effects without an actual underlying modular functional organization. Therefore, we generated a null distribution of peak heights (details in subsection 2.7.1). Briefly, this null distribution originates from 1080 simulated fMRI datasets, in which any functional integrity of activity time-courses, and therefore hippocampal organization, is destroyed, while the inherent fundamental statistical properties remain. These random datasets were processed using the identical analysis pipeline as for our participants, eventually yielding 1080 random connectopic maps. To assess the probability of obtaining histogram peaks with a magnitude as in our sample of 22 participants, we measured peak heights in our participants' data as well as in the random data and compared the ensuing quantified peak heights. Figure 17 illustrates this concept by showing the mean height of the highest histogram peak in our 22 participants in comparison to the 95^{th} percentile of the null distribution, reflecting the height of the highest peak in randomly obtained histograms. Clearly, peak heights of the highest peak observed in our sample of 22 participants by far exceed the 95^{th} percentile of the null distribution. This implies that the highest peak in our participants is not caused purely by chance and likely corresponds to an underlying discrete organizational pattern in contrast to a smooth functional gradient.

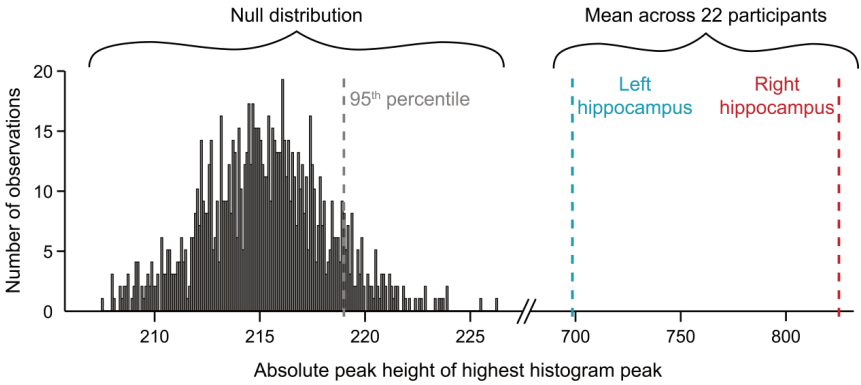

Figure 17: Null distribution of peak heights and observed mean peak heights (peak order 1)
To test whether the height of the observed histogram peaks could be expected based on chance, we created a null distribution of peak heights using a random shuffling procedure (black histogram). The aim of this analysis is to compare the observed peak height averaged across 22 participants for both the left and right hippocampus (blue and red dashed lines, respectively) to the 95th percentile of the null distribution (gray dotted line). The chart illustrates that for the highest peak the observed peak heights across 22 participants clearly exceed the null distribution.

This finding raises the question how many peaks in the participants' data exceed the 95th percentile of the null distribution. Answering this could not only provide further validation of our results by indicating which peaks cannot be caused purely by chance but potentially also point towards the number of hippocampal subunits that are biologically meaningful, thereby providing evidence regarding our research question (2). Specifically, only those observed peaks that are significantly higher than the peaks based on random data most likely correspond to meaningful functional modules. In contrast, the ones that are not significantly different or even lower than the peaks originating from random data can probably be disregarded in the search for a biologically meaningful functional parcellation.

Therefore, we examine the peak heights of not only the highest peak, but for several peak orders, reaching from one (highest peak, see above) to ten (tenth highest peak). Again, we compare the mean peak heights of our participants' histograms to the 95th percentile of the null distribution of peak heights obtained from random histograms.

However, instead of showing these comparisons as ten separate plots following the logic of Figure 17, we merge all these into one illustration (Figure 18), in which peak heights of observed as well as random data are plotted as a function of peak order. Each of the ten vertical sets of data in Figure 18 (consisting of three datapoints: Mean peak height for left and right hippocampus, respectively, and the 95th percentile of the null distribution) can be viewed as a condensed version of a plot like Figure 17. For visualization purposes, the observed peak heights of each peak order are normalized to the respective 95th percentile of the null distribution by division.

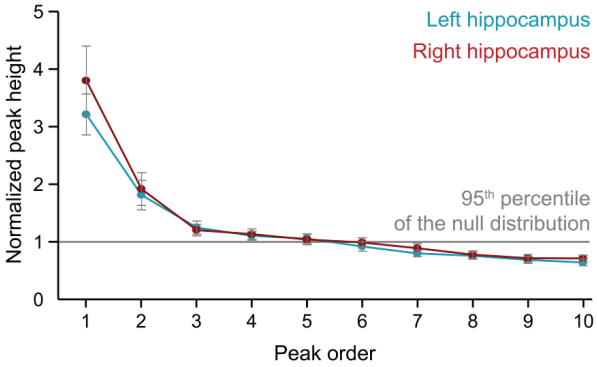

Figure 18: Participants' mean peak heights vs. null distribution of peak heights

Random shuffling and peak averaging were performed for the highest ten histogram peaks, yielding one null distribution for each peak order (highest, second highest etc., details in subsection 2.7). This figure displays the observed peak heights of the left (blue line) and right (red line) hippocampus averaged across 22 participants for all considered peak orders in relation to the 95th percentile (gray line) of the respective null distribution. For better comparison, the peak heights of the observed data are normalized to the 95th percentile of the respective null distribution by division. Error bars depict the standard error of the mean across 22 participants.

Upon visual inspection, it appears that the mean peak heights of both the left and right hippocampus exceed the 95th percentile in the highest up to fourth or fifth highest peaks. To assess this statistically, we performed a *t*-test for each peak order in MATLAB to test the null hypothesis of no difference between participants' mean peak heights and 95th percentile of the null distribution. All *p* values are reported in Table 1.

Table 1: Testing the null hypothesis of no difference between participants' mean peak heights and 95th percentile of the null distribution using *t*-tests: *p* values for peak orders 1 – 10 (significant *p* values at the 5% level in bold print, *t* values in parentheses, degrees of freedom: 21)

	1	2	3	4	5
Left hpc	**3.571×10^{-4}** (t = 6.224)	**0.005** (t = 3.170)	**0.041** (t = 2.180)	0.173 (t = 1.411)	0.494 (t = 0.697)
Right hpc	**1.579×10^{-4}** (t = 4.592)	**0.004** (t = 3.226)	0.054 (t = 2.044)	0.156 (t = 1.470)	0.653 (t = 0.456)

	6	7	8	9	10
Left hpc	0.334 (t = -0.990)	**0.002** (t = -3.491)	**6.714×10^{-4}** (t = -3.986)	**2.936×10^{-4}** (t = -5.304)	**8.947×10^{-4}** (t = -6.002)
Right hpc	0.906 (t = -0.120)	0.175 (t = -1.402)	**0.003** (t = -3.405)	**2.941×10^{-4}** (t = -4.332)	**1.300×10^{-4}** (t = -4.781)

These statistical tests point towards three preliminary conclusions: First, the highest and second highest peaks of functional connectivity similarity in the participants' data may likely correspond to biologically meaningful functional modules in the hippocampus. This is statistically based upon the observation that for the first and second peak orders the mean absolute peak height of the participants' data significantly exceeds the 95th percentile of the null distribution in the hippocampi of both hemispheres. Second, in contrast, the fourth through tenth highest peaks in the participants' topographies of functional connectivity similarity may not correspond to biologically meaningful modules in the hippocampus of either hemisphere, as for peak orders four through ten, the peaks in the participants' data are either not significantly different from or even significantly lower than the 95th percentile of the null distribution in terms of their absolute height. Third, however, for peak order three the statistical tests yield different results between the left and right hippocampus: In the left hippocampus, the third highest peak in the participants' data is marginally significantly higher than the 95th percentile of the null distribution in terms of the absolute peak height ($t(21) = 2.180$, $p = 0.041$). However, in the right hippocampus the difference in peak heights of participants' data and 95th percentile of the null distribution is marginally

not significant, as the reported *p* value does not survive a strict significance level of 5% ($t(21) = 2.044$, $p = 0.054$). Given this tight proximity of *p* values to the 0.05 significance level for peak order three, we reason that perhaps our analyses do not provide sufficient power to produce statistically reliable results, which may in part be due to our restricted sample size of 22 participants. Therefore, the statistical analyses not yet provide an exact answer to our question (2) regarding the number of subunits along the hippocampal long-axis. However, they indicate that the highest and second highest peaks, but not the fourth through tenth highest peaks may be biologically meaningful, potentially pointing towards at least two distinct modules, whereas the importance of the third highest peak remains ambiguous.

To further investigate the number of hippocampal subunits, we employed a different, quite straightforward approach. Since functional clusters of hippocampal voxels correspond to histogram peaks, we implemented a procedure to quantify the number of peaks in each individual participant's histograms. Specifically, we counted how many clusters of adjacent bins exceeded the mean number of voxels per bin in the respective histogram (details in subsection 2.6). Figure 16 in subsection 3.2 illustrates this concept for an exemplary participant: In participant #1, three clusters of bins exceed the mean in both hippocampi, implying three clusters of functional connectivity similarity. In the entire sample of 22 participants, the mean number of peaks, identified as adjacent bins above the mean, is 2.81 (standard deviation 1.01) for the left and 2.91 (standard deviation 0.87) for the right hippocampus. Regarding research question (2), this suggests an organization in overall three functional modules.

3.4 Functional parcellation

Having characterized the topography of functional connectivity similarity within the hippocampus as a discretized pattern of functional organization with overall three peaks, we next tackle our intent to provide a parcellation based on the identified pattern. To this end, we applied *k*-means clustering to the participants' connectopic maps.

3.4.1 Determining the optimal number of clusters

K-means clustering is a widely used algorithm to group datapoints of a dataset into separate clusters. Before applying *k*-means clustering, the user is required to specify the desired number of clusters *k*. In fact, we already identified three as the number of significant peaks in our participants' connectopic maps, therefore suggesting a clustering into *k* = 3 subunits. Nevertheless, we attempted to additionally corroborate the choice of *k* in a data-driven manner. To this end, we applied the so-called elbow method to compare the clustering errors of different numbers of clusters (Kodinariya & Makwana, 2013). More precisely, we repeated *k*-means clustering for different numbers of clusters *k* (1 – 15) and plotted the mean clustering error as a function of *k* (details in subsection 2.8). Figure 19 shows the resulting curve.

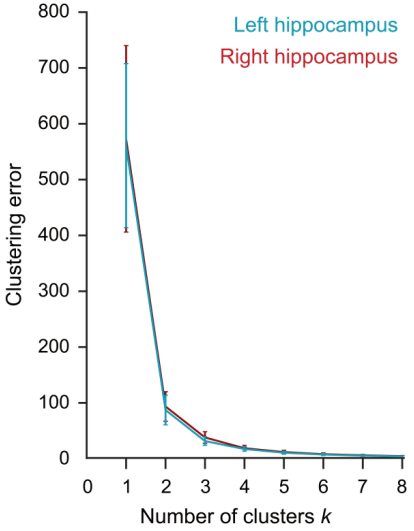

Figure 19: Determining the optimal number of clusters using the elbow method

The so-called elbow method was applied to find the optimal number of clusters for a parcellation based on the connectopic maps. This figure displays a section of the elbow curve showing the clustering error of the left (blue line) and right (red line) hippocampus as a function of the number of clusters *k*. According to the elbow method, the optimal number of clusters can be approximated by the starting point of the plateau, which lies at *k* = 3.

According to the elbow method, the ideal number of clusters is given by the x-coordinate of the point where the curve begins to reach its plateau ('elbow' of the curve). This plateau point of the curve is calculated as the specific *k* where increasing the number of clusters would not yield a considerable improvement of the clustering error, while reducing the number of clusters would result in a significantly increased

error (computational details in subsection 2.8). Following this rationale, $k = 3$ was identified as the optimal number of clusters for the left as well as right hippocampus. Taken together with the analyses above including the *t*-tests of peak heights in participants' data versus 95th percentile of the null distribution and more importantly the number of peaks across 22 participants, we opted for $k = 3$ in the definitive clustering procedure. This choice is furthermore supported by the fact that the bulk of hippocampal parcellation studies suggesting a modular organization point towards a tripartite functional long-axis organization. These findings include gene expression studies (Thompson et al., 2008; Dong et al., 2009), behavioral experiments (Bast et al., 2009), studies on synaptic plasticity (Kenney and Manahan-Vaughan, 2013) and previous functional imaging analyses (Chase et al., 2015; Plachti et al., 2019; Robinson et al., 2015, 2016).

3.4.2 Description of the parcellation

We therefore performed *k*-means clustering into three clusters on the group-level connectopic maps (box in Figure 20) as well as on the subject-specific maps of our 22 participants (remaining renderings in Figure 20).

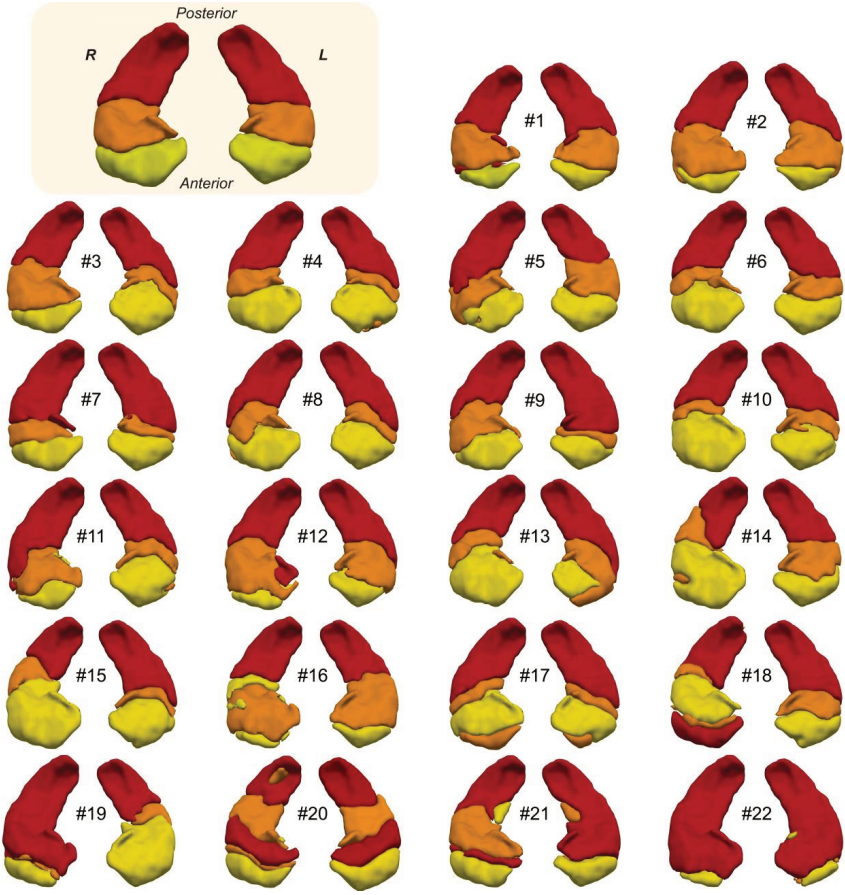

Figure 20: Functional parcellation into three subunits using *k*-means clustering

Functional parcellations were obtained for the group-level connectopic maps (yellow box) and all individual participants (rest, numbered 1 – 22). Orientation labels provided in the group-level parcellation are analogous for all single-subject renderings. The colormap was chosen such that changes from yellow to orange to red correspond to transitions from anterior to intermediate to posterior parcels in most participants. The renderings were manually arranged so that the parcellations yielding clearly longitudinally arranged subunits are shown in the upper four rows, while the lower two rows depict less consistent parcellations.

In most cases (e.g., participants #1 – #15), the three clusters are arranged along the longitudinal axis to form an anterior, intermediate, and posterior functional subunit, respectively. Regarding the volume of subunits, the posterior parcel generally appears to be the biggest (with the mean number of voxels per parcel around 2500), followed by the anterior (roughly 1500 voxels) and intermediate parcel (roughly 1000 voxels). The exact numbers of voxels per parcel, indicating the volumes of subunits, are depicted in Figure 21 for the left and right hippocampus, separately.

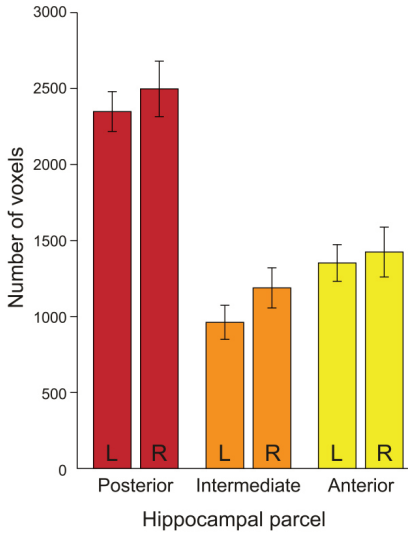

Figure 21: Volume of hippocampal subunits

This figure displays the volumes of hippocampal parcels, as yielded by *k*-means clustering. Parcel volumes are calculated as the total number of voxels within a subunit for the left (L) and right (R) hippocampus, respectively. Note that in the hippocampi of both hemispheres the posterior parcel is the biggest, followed by the anterior and intermediate subunit. The error bars depict the standard error of the mean over 22 participants. The color scale corresponds to the color scheme in Figure 20.

Interestingly, in terms of the location of boundaries, the parcellations of the left and right hippocampus are very similar within a participant, indicating a high intra-subject consistency across hemispheres. However, there is quite some variability across parcellations when comparing different participants. Besides, for some participants (mainly #16 – #22) *k*-means clustering has apparently not been successful in capturing a clearly arranged long-axis organization. Especially considering these partly unsuccessful parcellations, it is interesting to view the individual participants' histograms in relation with the boundaries that the clustering algorithm established. This way, it may be possible to decide whether either the clustering algorithm did not successfully capture the underlying histogram peaks or in contrast the clustering

algorithm did perform well, however the histograms of the affected participants are simply not as clear. Therefore, Figures 22a and 22b illustrate all participants' histograms with the color scheme indicating the boundaries between subunits according to *k*-means clustering. The colors correspond to the color code from parcellations in Figure 20 in that red histogram bars were integrated into the red (posterior) parcel, orange bars into the orange (intermediate) parcel and yellow bars into the yellow (anterior) parcel. Notably, in some histograms the pattern of functional connectivity similarity is composed of a very high peak and several smaller peaks. Thus, to show all peaks the histograms in Figures 22a and 22b are normalized and enlarged so that the depicted area on the y-axis reaches from zero to the mean plus one standard deviation. Hence, the tops of very high peaks are clipped and the visibility of the remaining pattern of 'peakiness' is enhanced.

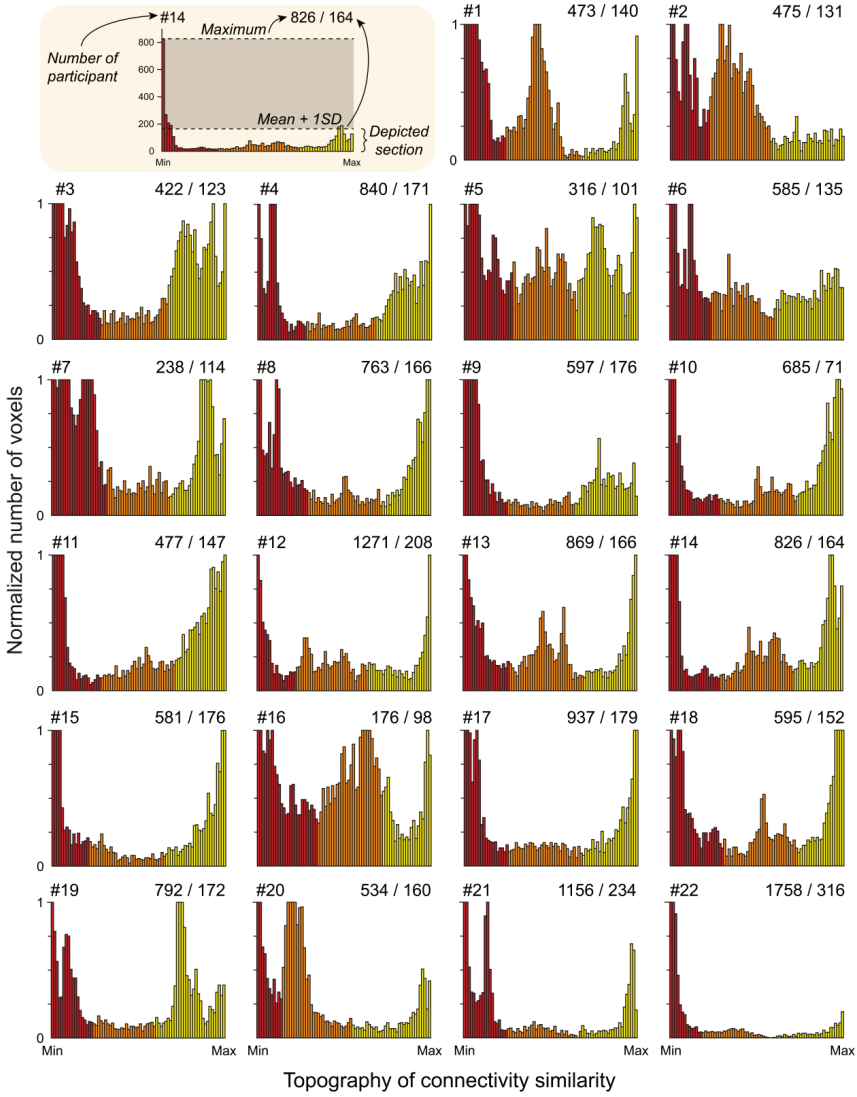

Figure 22a: Single-subject histograms of all 22 participants (left hippocampus)

Illustrated are all participants' histograms depicting the normalized numbers of hippocampal voxels per similarity value. The absolute number of voxels is normalized so that only the area below the mean + 1 standard deviation (SD) is plotted (illustrated in yellow box).

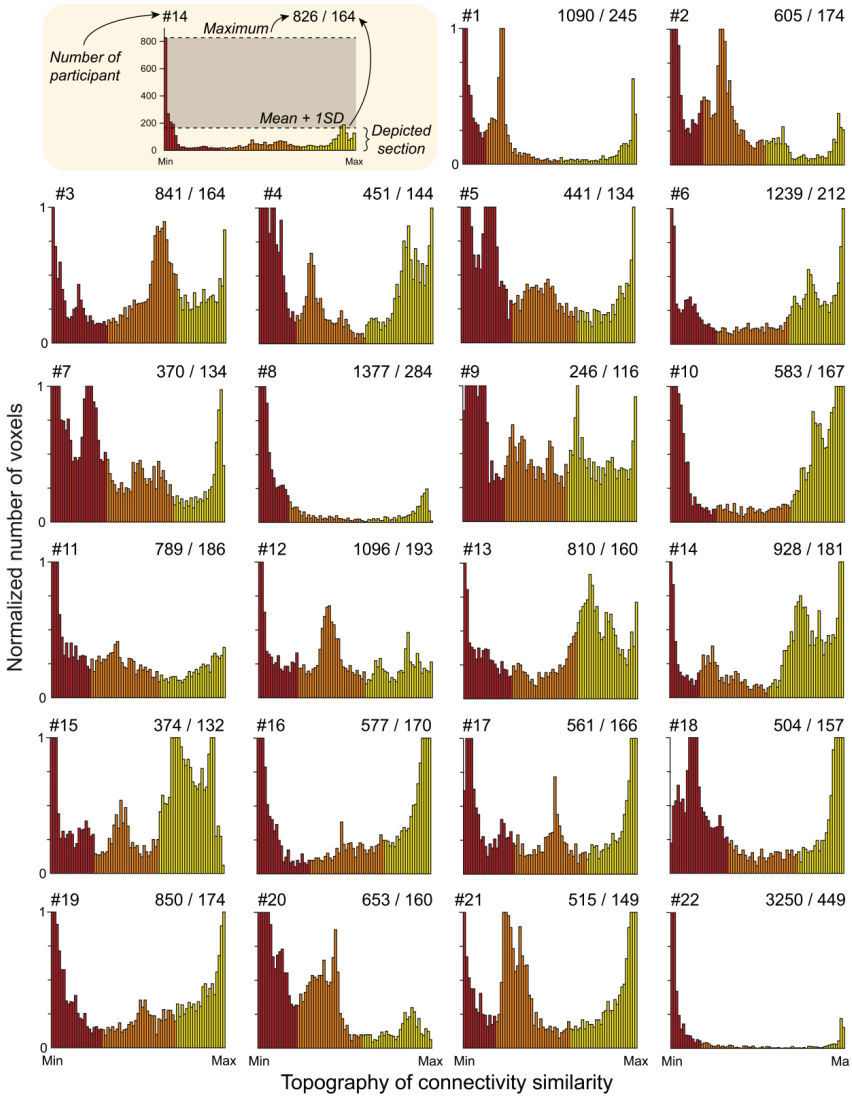

Figure 22b: Single-subject histograms of all 22 participants (right hippocampus)

Histograms depicting the normalized numbers of hippocampal voxels per similarity value in the right hippocampus. Normalization, visualization, and axes are identical to Figure 22a.

It becomes apparent that *k*-means clustering was overall successful in capturing major peaks of functional connectivity similarity, both in subjects with a clear parcellation (#1 – #15) and the ones with inconclusive subunits (#16 – #22). However, in both groups, there are histograms in which some peaks have been taken together into the same cluster (e.g., #19 left or #15 right). Overall, it cannot be concluded that unsuccessful parcellations are due to the clustering algorithm not capturing the underlying peaks.

3.5 Summary of the results

The aim of this project was to investigate the functional long-axis organization of the hippocampus in alive humans. We therefore analyzed an ultra-high-resolution fMRI dataset that had been acquired on a 7 Tesla fMRI scanner. During scanning, the 22 participants performed a self-paced object-location memory task, which was facilitated by a 3D virtual reality setup. This fMRI data was processed using the recently developed, fully data-driven analysis algorithm connectopic mapping (Haak et al., 2018). Using this method, we quantified the intra-hippocampal similarity of functional connectivity, based on the functional connectivity of the hippocampus to the rest of the brain, and then applied a nonlinear dimensionality reduction approach to extract the dominant topography of functional connectivity similarity. This approach yielded 22 participant-specific connectopic maps and a group-averaged connectopic map for the left and right hippocampus, respectively.

The group-level connectopic maps confirm the previously established notion of an anterior-posterior differentiation in that the anterior portion of the hippocampus is entirely distinct from the posterior portion with respect to the topography of functional connectivity similarity (Figure 14). To investigate the nature of the transition between the two functionally distinct hippocampal poles, we moved on to the single-subject results. Interestingly, the participant-specific connectopic maps display clearly discretized patterns of functional connectivity similarity, pointing towards a modular hippocampal organization in several longitudinal subunits (Figure 16).

Next, we aimed to determine the number of hippocampal modules by assessing whether and which peaks of functional connectivity similarity could or could not have

occurred by chance. To this end, we compared our observations in the participants' data to a simulated null distribution of connectopic maps and find that the observed peaks of functional connectivity similarity are significantly higher in terms of their absolute height than the 95th percentile of the null distribution for peak orders one and two (Figure 18). Statistically, the *t*-tests addressing the null hypothesis of no difference between participants' mean peak heights and the 95th percentile of the null distribution yielded the following results for the left hippocampus: $t(21) = 6.224$, $p = 3.571 \times 10^{-4}$ (highest peak), $t(21) = 3.170$, $p = 0.005$ (second highest peak) and for the right hippocampus: $t(21) = 4.592$, $p = 1.579 \times 10^{-4}$ (highest peak), $t(21) = 3.226$, $p = 0.004$ (second highest peak). In contrast, the observed peaks are not significantly different or even significantly lower than the null distribution for peak orders four to ten at the 5% significance level (Table 1). However, for peak order three, the left hippocampus yields a significant difference ($t(21) = 2.180$, $p = 0.041$), whereas the right hippocampus yields no significant difference between observed and simulated data at the 5% significance level ($t(21) = 2.044$, $p = 0.054$). Given this inconsistency, we implemented an additional approach to determine the number of functional subunits within the hippocampus and quantified the peaks in each participant's connectopic map as sets of adjacent bins above the mean of the respective histogram. The number of peaks, identified as described and averaged across all 22 participants, is 2.81 (standard deviation 1.01) for the left and 2.91 (standard deviation 0.87) for the right hippocampus, suggesting an overall rounded mean of three functional modules. To further corroborate the optimal number of clusters in the participants' connectopic maps, we applied the so-called elbow method (Kodinariya & Makwana, 2013), which also points towards three as the ideal number of clusters (Figure 19).

With the goal of capturing the identified tripartite pattern of functional connectivity similarity, we then parcellated each participant's connectopic map into three clusters using *k*-means clustering. Out of the 22 yielded parcellations, 15 exhibit clearly longitudinally arranged subunits, whereas seven are rather problematic in that clustering did not yield consistently arranged clusters in both hemispheres (Figure 20). Parcellations exhibit a high within-participant consistency across hemispheres, but a high variability between participants. In general, the posterior parcel is the biggest across all participants (roughly containing 2500 voxels), followed by the anterior (~ 1500 voxels) and intermediate (~ 1000 voxels) parcels (Figure 21).

4. Discussion

In this section, our findings shall be thoroughly discussed with regards to their significance in relation to previous studies, but also regarding their potential shortcomings. To this end, the first main subsection will highlight whether and at what point within the analysis pipeline potential biases or limitations may have arisen. The second, larger subsection will then put our results in perspective with major unresolved questions addressing the hippocampal long-axis organization and discuss their standing with previous literature. Herein, the focus will lie on human research, mostly emerging from functional imaging studies, but for the bigger picture, some influential studies from rodent and nonhuman primate research will be considered as well. However, given the general popularity of hippocampal research and the limited scope of this thesis, no claim for completeness is made. To conclude, subsection 4.3 will briefly point out potential clinical applications of both our methodology and findings. This will hopefully build a bridge from our conclusions, originating in fundamental neuroscientific research, to the demands of the applied medical community regarding the practical importance of our findings.

4.1 Potential methodological limitations and inconsistencies

Applying a data-driven analysis algorithm on ultra-high resolution fMRI data, we provide evidence for a discretized pattern of functional connectivity similarity along the hippocampal long-axis. Furthermore, using a validation approach based on randomly generated data and a peak identification procedure, we argue for a modular functional organization in overall three longitudinal subunits. Given the conclusion of a modular organization, however, it is important to review our analyses in terms of potential biases towards discrete results. Therefore, this subsection reconsiders several aspects of the applied methodology, discusses potential limitations and highlights possible improvements for future studies.

4.1.1 Spatial resolution of 7 Tesla fMRI data

To investigate the functional organization of a brain structure using functional imaging, it should be assured that the spatial resolution of the acquired data exceeds the smallest expected organizational entity. Thus, it is a warrantable question whether our fMRI data offers sufficient resolution to be able to capture the true topography of functional organization within the hippocampus in an unbiased manner.

Generally, the spatial resolution in (f)MRI is determined by the magnetic field strength of the employed scanner: Higher magnetic field strengths provide higher resolution. Typical applications in the clinical routine, like structural MRI, but also an abundance of neuroscientific functional MRI studies employ scanners operating at 1.5 or 3 Tesla. In comparison, the dataset used for this thesis was acquired at a magnetic field strength of 7 Tesla, yielding ultra-high, sub-millimeter resolution with a voxel size of roughly 0.9 x 0.9 x 0.9 mm^3. Although our dataset thus relies on state-of-the-art technology and provides higher resolution than numerous previous imaging studies, the critical reader may argue that even sub-millimeter resolution does not allow for measuring the activity of individual neurons on a single-cell level, which is at the micrometer scale. However, critically, functional imaging studies do not pursue the goal of scrutinizing the firing patterns of individual neurons. In contrast, fMRI approaches like ours usually aim to capture a larger-scale organizational pattern by visualizing hemodynamic changes associated with the functional behavior of a set of neurons that are combined into a voxel (for a more precise description of the underlying principles see Introduction). Given this background, our analyses should be able to identify potential hippocampal subunits under the condition that the size of these subunits exceeds the voxel size. As a simple demonstration, an extreme case can be considered: If hypothetically an imaging technique yielded such a low resolution that the hippocampal long-axis was only mapped onto two voxels, analyses based on such a dataset would not allow for conclusions regarding long-axis transitions. However, in our case, the length of the hippocampal long-axis measures more than 40 voxels in the sagittal plane, which by far exceeds the expected number of biologically meaningful hippocampal subunits. Hence, we believe that in general the ultra-high resolution of our employed fMRI data should be sufficient for mapping the true hippocampal long-axis organization and does not introduce a significant bias. Note, however, that

the effective spatial precision of functional imaging analyses is not purely determined by the resolution of the original fMRI data but is also influenced by the preprocessing procedure, particularly by spatial smoothing, which will be discussed in the following subsection.

4.1.2 Influences of preprocessing

In general, preprocessing is a standard procedure in functional imaging studies to, among other purposes, enhance the quality and validity of fMRI data by removing noise. Potential sources of noise include head motion, other non-neuronal physiological effects like respiratory motion, and scanner artifacts (Salimi-Khorshidi et al., 2014). The mentioned effects are all accounted for in our study (procedural details in subsection 2.3).

In addition, most fMRI studies apply spatial smoothing as part of the preprocessing to achieve certain improvements, including increasing the signal-to-noise ratio and allowing for better inference across individuals as the influence of between-subject-variability is reduced (Friston, 2003). However, smoothing comes with certain disadvantages as it can decrease effective spatial resolution, blur activation in adjacent areas, and merge adjacent peaks of activation (for a comprehensive review see Mikl et al., 2008). Therefore, the smoothing kernel should be carefully considered and adapted to the intended analyses. In the context of massive univariate testing, which is to date a popular statistical approach for analyzing fMRI data, a smoothing kernel measuring at least twice the voxel size typically serves as an expedient recommendation, as previously established by Worsley and Friston (1995). As the 7 Tesla fMRI dataset used for the analyses reported in this dissertation had been acquired and preprocessed before the outset of the project presented here, the smoothing kernel was chosen according to the mentioned recommendation as roughly 2.5 times the voxel size (measuring 2.5 mm). However, in the specific context of the hippocampal functional connectivity analyses performed in this project, it is possible that smoothing blurred putatively discrete transitions between hippocampal subunits and thereby obscured a modular organization. Hence, the applied smoothing may have introduced a bias towards gradual transitions, thus limiting our ability to detect modular, sharply demarcated clusters of functional similarity. Perhaps some of

the ambiguities reported above, including for instance the inconsistent results regarding the comparison of the participants' data to the null distribution for peak order three (subsection 3.3), may partly relate to this potential bias. Thus, it may be worthwhile for future studies to consider performing the analyses on unsmoothed data or even processing two sets of the same source fMRI data, one smoothed and one unsmoothed, to determine the precise effect that can be attributed to spatial smoothing.

Besides the potential effect of smoothing, an additional source of artificial smoothness may have been introduced by the nonlinear normalization of every participant's dataset onto a group-specific brain template that was performed as part of the preprocessing. The employed nonlinear normalization procedure involved the transformation and reshaping of all participants' individual brain scans into a uniform sample-specific space with consistent coordinates. Such normalization processes not only render the registration to structural MRI scans unnecessary, thereby eliminating a potential source of noise, but can also allow for more efficient processing, for example by facilitating the application of a uniform mask to all participants' fMRI scans. Despite the advantages, however, it is possible that this normalization process blurred interindividual differences in terms of the hippocampal topography of functional connectivity similarity, thereby favoring a smooth functional gradient. Thus, to overcome this potential bias and characterize its magnitude, future projects may consider abstaining from nonlinear normalization and instead perform the analyses with participant-specific hippocampal masks to preserve any interindividual differences.

4.1.3 Potential bias within the connectopic mapping algorithm

Next, it is important to consider potential sources of bias within the applied connectopic mapping approach, especially given the data-driven and complex nature of the algorithm. One possibility where a bias could have been introduced is the Laplacian Eigenmaps algorithm (subsection 2.5.3). Briefly, the Laplacian Eigenmaps implementation constitutes the final step of the algorithm, in which the graph representation of functional connectivity fingerprint similarity between hippocampal voxels is remapped onto a hippocampal map (subsection 2.5.3, especially Figure 12).

According to the developers of Laplacian Eigenmaps (Belkin & Niyogi, 2003), the algorithm implicitly emphasizes natural clusters that are inherent to the input data, as neighborhood information of the source graph is optimally preserved in the ensuing mapping. Therefore, a critical mind may argue that such an algorithm is prone to over-emphasize clusters, even if a true topography was gradual. However, such a bias is unlikely given the examples provided in the original publication: Belkin and Niyogi apply Laplacian Eigenmaps to a so-called 'Swiss roll' (Belkin & Niyogi, 2003; Figures 1 and 2), which is a spiraled two-dimensional structure embedded in a three-dimensional space. In this example, the organization of datapoints on said Swiss roll exhibits rather smooth transitions as opposed to step-like clusters. Importantly, the ensuing mapping of the Swiss roll's organization, produced by the Laplacian Eigenmaps algorithm, is continuous and not artificially modular. In addition, a previously published implementation of the connectopic mapping algorithm, applied on the motor cortex, reports on a continuous pattern of gradual functional connectivity change, mirroring the somatotopic organization of the motor cortex (Haak et al., 2018). Therefore, we are convinced that connectopic mapping is an impartial means to analyze data without an algorithm-inherent bias towards modular patterns.

4.1.4 Possible improvement of the null model

Furthermore, we implemented a validation approach to test whether the observed peaks of functional connectivity similarity in fact corresponded to a modular organization or could be caused by chance. To this end, we compared the yielded peaks in the connectopic maps of our 22 participants to a null distribution of peak heights originating from connectopic maps of random data (subsection 3.3). However, our approach for generating random data needs to be addressed to decide whether the performed comparison was fair. To preserve the fundamental statistical properties of real fMRI data, we randomly shuffled hippocampal and extrahippocampal gray matter voxels, respectively, and importantly, applied this shuffling throughout the spatial and temporal dimensions (details in subsection 2.7.1). This disrupted the integrity of hippocampal organization, as was intended and required to simulate a random organizational pattern, but arguably, it may also have destroyed the integrity of BOLD signal time-courses altogether. Although it is unclear in what way, if at all, this

may have biased our results, it can be argued that a different approach may have been fairer with regards to creating a random hippocampal organization but at the same time pertaining realistic time-courses. Hence, for future studies a phase shuffling approach may be an option to consider for the generation of random out of real fMRI data. Briefly, in such an approach one could disintegrate the original time-courses using a fast Fourier transform (FFT), which decomposes a given signal into a set of components that are characterized by two characteristics, namely frequency and magnitude. Importantly, the obtained frequencies could be randomly shifted, or 'shuffled', in phase. Then, the original data structure, namely time-courses of BOLD signal, could be reconstructed using the shuffled phases and the original magnitudes via an inverse FFT. This approach would preserve the original amount of spatial autocorrelation, which is an inherent feature in fMRI data, and thus represent a more realistic, therefore fairer random dataset.

4.1.5 Threefold approach to finding the *k* in *k*-means

A final limitation potentially decreasing the validity of our results is the applied *k*-means clustering. Chosen for its simplicity, speed, and good performance in previous functional connectivity based parcellation studies, *k*-means clustering is not entirely data-driven in that it requires the user to specify the desired number of clusters. Clearly, if applied without careful consideration, this could provide a major bias, given that hypothetically negligent experimenters may choose the number of clusters entirely to their personal liking. To overcome this, we attempted to determine the optimal number of clusters in three ways (details in subsections 3.3 and 3.4.1). In a histogram visualization of the participants' connectopic maps, functional clusters are represented by histogram peaks. Therefore, first, we set out to statistically determine which peaks are significantly higher than the peaks originating from randomly shuffled data. Given the inconsistent results across hemispheres and the potential shortcomings of our random data generation (discussed above), we applied a second approach to identify the optimal number of clusters, namely the elbow method, which points towards three as the optimal number of clusters. Third, we further aimed to corroborate the number of hippocampal subunits by scrutinizing the histograms with regards to the number of peaks, which coincidingly points towards an overall mean of three peaks of functional

connectivity similarity across participants. Hence, we opted for parcellations into three functional modules, which in most cases yield longitudinally arranged subunits. However, it should be noted that the quantification of peaks, which we reasoned to be a criterion that was as close to the data as possible, was not entirely data-driven in that we applied the criterion of minimum peak distance (MPD, subsection 2.6). Although we aimed to make as little assumptions about the data as possible, introducing a MPD was necessary to be able to capture global peaks but conversely not over-emphasize local peaks that are likely to pertain to the same functional cluster. In other words, without the MPD criterion, our peak identification approach would have counted every local peak as a separate functional unit, which would not correspond to the overall underlying organization pattern (illustration in Figure 13). However, of course, the choice of four bins as a MPD was somewhat arbitrary, thus future research may benefit from a more data-driven way to quantify data peaks.

4.1.6 Challenges in hippocampal mask delineation

In addition to the potential biases discussed above it is worth considering the validity of our hippocampal mask. The hippocampi of individuals may vary in terms of size, shape, orientation, and position within the brain. However, as the hippocampus is a highly conserved structure across mammalian species (Manns & Eichenbaum, 2006) and furthermore its boundaries are more clearly delimitable than for instance cortical areas, it likely exhibits only minor differences between participants. Furthermore, the effective amount of inter-subject variability is reduced by transforming and reshaping all participants' brains into the same space and coordinates, so that global differences in head orientation and brain size should have diminished. However, one may argue that even if all brain volumes are universalized to the same size, maybe the size of individual hippocampi still varies. We attempted to overcome this problem by applying a rather liberal segmentation approach: Closely following the Harmonized Protocol for Manual Hippocampal Segmentation (Boccardi et al., 2015), there is still some ambiguity that leaves room for interpretation. In cases where the precise boundary of the hippocampus was rather ambiguous, we opted for a more liberal segmentation in that we rather included voxels that may not fully pertain to the hippocampus, instead of risking to lose any hippocampal voxels. Therefore, despite interindividual differences

our mask covered the hippocampi of participants overall well, which was additionally confirmed by visual inspection.

In addition, all manual segmentation approaches based on MRI data accommodate certain delineation challenges, especially in areas where extrahippocampal gray matter voxels directly neighbor hippocampal gray matter voxels. Given the lacking difference in signal intensity in these cases, the delineation is usually facilitated by the use of anatomical landmarks as orientations. Thanks to the detailed and abundantly illustrated instructions provided in the Harmonized Protocol, these challenges were scarce. However, it is worthwhile to mention that hippocampal delineation was particularly challenging in the area around the posterior pole. In the final coronal slices where the hippocampus is visible, it directly neighbors the indusium griseum, a gray matter structure adjacent to the corpus callosum (anatomical illustration in Figure 1). Around this position, demarcation was rather ambiguous, and the help provided by landmarks limited. Therefore, it is possible that at the posterior hippocampal pole our mask covers gray matter voxels that may not pertain to the hippocampus. This provides a potential explanation for the fact that the volume of the posterior hippocampal subunit was consistently larger than the intermediate and anterior parcels (Figure 21), especially given previous research pointing towards subunits of rather equal size (Chase et al., 2015).

Additionally, an interesting consideration concerns the interhemispheric difference of hippocampal masks. The hippocampal masks of each hemisphere were separately delineated by the same experimenter, using the identical protocol, and with equal careful attentiveness. Nonetheless, the masks exhibit a considerable difference in volume, precisely of 448 voxels (4664 voxels in the left and 5112 voxels in the right hippocampus). Although to our knowledge this has not been specifically addressed in previous literature, it is interesting that many precursor studies report an analogous difference in that the left hippocampal mask was larger than the right, although not as considerable as in our case (Masouleh et al., 2020; Plachti et al., 2019; Robinson et al., 2015). Of course, a definitive conclusion would require statistical testing, but interestingly, this may suggest that the left hippocampus tends to be larger than the right hippocampus.

4.1.7 Plausibility of interhemispheric differences

If the above-mentioned interhemispheric difference in hippocampal volume would be corroborated, the question emerges as to whether such differences may extend to the organization and even implication in different functions. Indeed, previous studies have suggested different functional roles (Burgess et al., 2002) and even a different anterior-posterior organization for the left and right hippocampus (Robinson et al., 2015). Hence, it is worthwhile to discuss to what extent our results support the hypothesis of interhemispheric differences. In addition to the difference in hippocampal mask volume (see above), another aspect that differs between the left and right hippocampus regards the *t*-tests performed to compare the observed peak heights to the 95th percentile of the null distribution (subsection 3.3, Table 1). To briefly recapitulate, for the third highest peak, the peak heights of the participants' data were significantly higher than the null distribution in the left hippocampus, whereas the difference in peak heights was marginally not significant in the right hippocampus. However, since we did not perform a statistical test specifically addressing the differences between left and right hippocampus, the reported results cannot be interpreted as evidence for or against an interhemispheric difference in the number of longitudinal hippocampal subunits. Previous studies explicitly addressing this question did find such evidence, including for example a report by Robinson et al. (2015), who for the first time suggested that the left hippocampus may be organized in three distinct clusters, whereas the right hippocampus is organized in either two or five clusters. Given this ambiguity, the authors argue that the right hippocampus may in fact have a more gradual organization, opening the interesting perspective of different organizational patterns between hemispheres. However, as Robinson and colleagues state, this finding should be interpreted with caution due to insufficient power of the underlying analyses. Regarding our results, the difference in *t*-statistics may possibly relate to some interhemispheric difference, but it should be clarified that the observed difference in significance is rather minor and was not explicitly statistically tested.

In contrast, several aspects of our results are in fact quite consistent across hemispheres. First, the rounded mean number of peaks across 22 participants equals three in the hippocampi of both hemispheres. Second, the elbow method yields nearly identical results and lastly, the single-subject parcellations exhibit a high

intra-individual consistency across hemispheres (with few exceptions, like participants #3, #19, and #21 in Figure 20). However, although loosely pointing towards the notion of high similarity between the left and right hippocampus, our analyses do not allow for statistically backed conclusions regarding interhemispheric differences, which therefore remains an interesting topic for future research.

4.1.8 Relevance of interindividual differences

Another interesting question emerges from the differences of functional parcellations across participants (Figure 20). Although the parcellations of some participants exhibit a striking similarity in that three clearly demarcated subunits are arranged along the long-axis (e.g., participants #1 – #15), the boundaries between long-axis subunits vary across subjects. This interindividual variability in terms of boundary locations most likely is the cause for the observed blurring effect of the group-level connectopic maps (detailed in subsection 3.2, patterns of group-level (Figure 15) versus single-subject level histograms (Figure 16)). Besides these rather minor differences in the outlines of subunits, there is an additional, larger amount of interindividual variability in that some participants do not exhibit a consistent hippocampal parcellation into clearly arranged subunits in both hippocampi (e.g., participants #16 – 22). In light of previous studies, it is imaginable that the hippocampi of different participants exhibit different functional organizations. This was suggested for example by Plachti et al. (2019) who argue that depending on personal talents and prerequisites, every individual may have its own distinct functional organization. One possible anatomical correlate of such interindividual differences may be the so-called hippocampal digitations, referring to irregularly folded bulges, similar to gyrification in the neocortex. These digitations, which are present along the entire hippocampal long-axis but most prominent in the anterior pole, have been shown to vary considerably between subjects and therefore potentially explain interindividual differences (Chang et al., 2018; DeKraker et al., 2018; Ding & Van Hoesen, 2015). In this study, we aimed to identify a coherent, universal parcellation scheme across our entire sample of 22 participants and thus performed the identical analysis pipeline on all participants. However, this may not have done justice to a potentially existing individuality regarding the number of

hippocampal subunits and may motivate future studies to perform parcellations into different numbers of subunits for different participants.

4.2 Conceptual implications and further questions arising from our findings

Having outlined potential shortcomings of our methodology, this subsection focuses on the conceptual implications of our findings and highlights how our results fit in with previous models. To this end, we will discuss our main conclusions in relation to the current state of research and review previous studies on the hippocampal long-axis.

4.2.1 Evidence for a hippocampal anterior-posterior differentiation

One of our main findings is an anterior-posterior functional distinction of the hippocampus. Specifically, within the dominant topography of functional connectivity similarity, identified using connectopic mapping, the posterior pole of the hippocampus is very different from the anterior hippocampal pole in terms of the underlying functional connectivity to the rest of the brain's gray matter (subsection 3.1).

4.2.1.1 Long-axis versus transversal axis

Before relating this finding to other studies and addressing potential functional correlates of a functional long-axis differentiation, it shall be discussed whether the mere detection of an anterior-posterior distinction as the dominant axis of organization in itself may constitute an interesting outcome.

In theory, the connectopic mapping approach applied here is an entirely data-driven algorithm with the pure goal of identifying the dominant pattern of organization based on neuronal functional connectivity. In other words, nowhere did we specify the long-axis as our axis of interest beforehand. Therefore, on first sight the fact that the algorithm detected the long-axis organization may imply that the longitudinal axis of the hippocampus acts as its main functionally meaningful axis of organization. However, it should be highlighted that despite the sub-millimeter resolution of our 7 Tesla fMRI dataset, the spatial smoothing applied within the preprocessing

procedure has likely reduced this spatial precision and thus the sensitivity to detect fine-grained modular changes within the hippocampal functional connectivity topography (discussed in depth in subsection 4.1.2). Given the small scale of transversal hippocampal subfields, typically measuring only a few millimeters in diameter, our spatial resolution was likely insufficient to examine differences along the transversal axis. Therefore, it would be rash to conclude solely based on our analyses that the hippocampal long-axis is more functionally relevant than its transversal axis. In fact, given the multi-dimensional scale of hippocampal organization (Amaral & Witter, 1989), the transversal axis may equally come into question as the dominant axis of functional organization. As outlined in the Introduction (subsection 1.3.2), the microstructure of the hippocampus is intricately organized along its transversal axis, allowing for the identification of several histologically sharply demarcated transversal subfields (including dentate gyrus, Ammon's horn, and subiculum, illustrated in Figure 2). Interestingly, not only microscopically detectable structural properties, like the morphology of neurons (Lorente De Nó, 1934; Ramón y Cajal, 1893), sustain this transversal organization, but in recent years additional functional evidence has emerged: Advances in functional imaging techniques allowing for fMRI data acquisition at ultra-high-resolution enabled the in-vivo delineation of transversal subfields in the human hippocampus (Wisse et al., 2012) and paved the way for a bulk of studies investigating functional differences between transversal subfields. For example, transversal hippocampal subfields seem to be differentially implicated in the encoding versus retrieval of a memory in healthy humans (Carr et al., 2010; Suthana et al., 2011). Moreover, such differences may not only play a role in health, but also in pathological conditions, including temporal lobe epilepsy and Alzheimer's disease (for comprehensive reviews see de Flores et al., 2015 and Small et al., 2011). Specifically, transversal subfields have been shown to exhibit different activation patterns (Das et al., 2011), variability in volume (Mueller et al., 2011) and vulnerability to damage (Schobel et al., 2009). Hence, the transversal axis remains an interesting candidate for the main axis of hippocampal functional organization.

In addition to the issue of insufficient spatial precision, another challenge regarding the examination of transversal subfields using functional imaging arises from the anatomical complexity of the hippocampus: Its folded anatomy, especially around the

anterior pole, renders analyses of the transversal axis challenging in conventional data-driven analyses (DeKraker et al., 2018; Duvernoy, 2005). However, recently, Vos de Wael et al. (2018) overcame this difficulty using a computational approach to virtually unfold the hippocampus for functional analyses, and interestingly identified both the longitudinal as well as the transversal axis as two equal axes of functional connectivity transitions. These insights open up the possibility that there is no hierarchy between the transversal and the longitudinal hippocampal axes, although this hypothesis requires further testing in the future.

In sum, our analyses provide interesting insights into the functional topography of the hippocampal long-axis but are not able to precisely address questions regarding the transversal axis. Thus, more studies are needed to fairly assess the transversal organization of the hippocampus in order to make claims with respect to the question which axis serves as the dominant, functionally meaningful axis of organization. Nonetheless, despite the remaining ambiguity regarding the hierarchy of longitudinal and transversal organization, it is uncontroversial that the long-axis does indeed sustain functional differences (Fanselow & Dong, 2010; Strange et al., 2014).

4.2.1.2 Functional correlates of an anterior-posterior differentiation

The notion of a functional differentiation along the hippocampal long-axis has been well established and widely accepted over the last decades of hippocampal research. In contrast, an emerging question of much debate concerns the functional correlates of such an anterior-posterior differentiation. In other words, the scientific community currently seems to agree on the fact that the anterior and posterior poles of the hippocampus are implicated in different brain functions, however it is unclear what concrete functions are sustained by anterior or posterior hippocampal portion, respectively.

One model suggests an ‘emotional versus spatial’ distinction in that the anterior hippocampus is implicated in emotional processing, including anxiety and stress-related behaviors, whereas the posterior hippocampus mediates spatial processing involving spatial memory (Bannerman et al., 2004; Moser & Moser, 1998). Initial evidence for this theory stems from lesion studies in rodents showing impaired spatial learning after damage to the dorsal, but not ventral hippocampus (Moser et al., 1993; Moser et al., 1995), and vice versa altered (neuroendocrine) stress responses

and fear behavior after damage to the ventral, but not dorsal hippocampus (Henke, 1990; Kjelstrup et al., 2002). Note that the ventral-dorsal axis in rodents is homolog to the anterior-posterior axis in primates (Sasaki et al., 2004; Strange et al., 2014). Although originated in rodent research, this model likely translates to primates, which was suggested by electrophysiological studies in monkeys (Colombo et al., 1998) as well as structural and functional imaging studies in humans (Maguire et al., 2000; Nadel et al., 2013).

Some authors extend this 'emotional versus spatial' model towards a more general view of 'emotion versus cognition', postulating that the posterior portion preferentially takes on cognitive tasks including the formation of declarative memory (not only spatial memory) as compared to the anterior hippocampus being involved in emotions (Fanselow & Dong, 2010). In contrast, other studies suggest that memory is represented along the entire length of the hippocampal long-axis (Chase et al., 2015). It has even been proposed that the anterior hippocampus exerts a special role in high-level cognitive functions including the recall of scenes and events (Zeidman & Maguire, 2016), which challenges a purely dichotomic 'emotion versus cognition' model.

However, even if the entire long-axis of the hippocampus takes part in mnemonic processing, there might still be an anterior-posterior differentiation in terms of subordinate aspects of memory. For example, Plachti et al. (2019) provided evidence for a model in which the entire hippocampal long-axis is implicated in memory, but intriguingly the center of information differs in that the anterior hippocampus processes rather self-centered memories, such as personal or autobiographic information, whereas the posterior hippocampus is involved in world-centered processing. This 'self versus world' view would even support the above-mentioned notion of an 'emotional versus spatial processing' distinction in that emotions usually relate to processes within our inner self, while space is typically referring to the world and landmarks around us. In addition, it has been proposed that not only the modality or perspective of mnemonic processing changes along the hippocampal long-axis, but also the scale of representation. This idea is underpinned by an influential study by Kjelstrup et al. (2008), who studied so-called place cells in the rodent hippocampus. Hippocampal place cells are pyramidal neurons that represent the spatial environment around an individual in that each place cell only fires when the subject is located at a

specific position within the environment (O'Keefe, 1976). Therefore, every place cell maps a specific location in space, and this associated location, triggering a place cell to fire, is termed the place cell's associated 'place field'. Strikingly, Kjelstrup and colleagues discovered that place cells, that can in fact be found along the entire hippocampal long-axis (Jung et al., 1994), differ in terms of their spatial resolution. Specifically, place fields pertaining to place cells in the anterior hippocampus were significantly larger than the ones of place cells in the posterior hippocampus. This suggests a model in which the anterior hippocampus is involved in the representation of coarse spatial information, whereas the posterior hippocampus processes fine-grained spatial information (Poppenk et al., 2013; Sekeres et al., 2018). Translating these insights across species, a 'coarse versus fine-grained' differentiation model is supported by several functional imaging studies in humans, pointing towards a coarse, 'gist-like' representation of context anteriorly and precise spatial details posteriorly (Brunec et al., 2018; Evensmoen et al., 2013; Nadel et al., 2013). Theoretically, this model could also be in line with an 'emotional versus spatial processing' view in that on the one hand (posteriorly), spatial navigation typically requires knowledge of precise landmarks for successfully reaching a destination. On the other hand (anteriorly), emotions can often be associated with rather broad spatial contexts, which for instance fits with the observation that the feeling of distress in patients with anxiety or post-traumatic stress disorder is often generalized to broader contexts. In addition, from an evolutionary point of view, it would make sense for the anterior hippocampus to represent space at a more coarse scale, considering its implication in stress and emotion related behaviors: Detecting danger from as far away as possible obviously is evolutionarily advantageous (Strange et al., 2014).

Having summarized the prevailing models regarding functional correlates of a hippocampal long-axis segregation, it is arguable to which extent our results may be able to confirm or refute these hypotheses. Specifically, the question remains whether our experimental setup generally allows for conclusions regarding different aspects of hippocampal function. Our ability to relate the detected discrete transitions of functional connectivity similarity to specific functional correlates is limited mainly due to the choice of our experimental task during fMRI acquisition: Since the employed spatial navigation memory task (subsection 2.1) primarily requires spatial processing without for instance specifically triggering emotional responses, the emotional component of hippocampal

function may likely be underrepresented. Similarly, neither the difference between self-related and world-centered nor between fine-grained and coarse (spatial) processing was particularly addressed in the employed task and in our analyses. This renders inferences regarding precise functional correlates of the detected functional long-axis modules difficult. Hence, future studies with more sophisticated task designs may be needed to precisely address this question.

4.2.1.3 Why an anterior-posterior distinction makes sense

We have now introduced several models regarding concrete functional correlates of an anterior-posterior differentiation of the hippocampus. Although even more views have been alluded in previous research, the described 'emotional versus spatial', 'self versus world' and 'coarse versus fine-grained' models are the most prevalent notions discussed in the current literature. However, one might pose the question why there is a functional differentiation within the hippocampus at all, as opposed to having such different brain functions – e.g., emotion on the one hand, memory on the other hand – incorporated in entirely distinct brain regions.

Generally, the view of a hippocampal functional differentiation is biologically coherent as it is possible that the hippocampus once was a functionally uniform structure processing only one kind of information. In the course of evolution, this single processing mechanism might have been preserved, yet adapted to different instances of information (Robinson et al., 2016). The consequential question is which present-day hippocampal function constitutes the original, primary instance of processed information and which hippocampal functions have come along later through evolutionary development. A possible answer suggests (spatial) memory as the original hippocampal function, since mnemonic processing and spatial navigation have been shown to be hippocampus-dependent in various species, including mammals (Manns & Eichenbaum, 2006) but also nonmammalian species, like reptiles (Striedter, 2016). Interestingly, reptiles and mammals phylogenetically originate from one branch of a bifurcation from a common ancestor roughly 320 million years ago (Reiter et al., 2017). Therefore, a functional analogy between ancient reptiles and humans would make a compelling case for the notion that the hippocampal implication in memory and spatial navigation has developed early on in evolution and has been phylogenetically preserved throughout the reptilian and mammalian lineages.

However, it is difficult to confirm such hypotheses as today's modern reptilian species have of course also undergone independent evolution, therefore the hippocampus of a given extant reptile is not necessarily identical to its ancestor's.

Despite such methodological challenges, it is interesting to consider potential implications of the suggested evolutionary model: Perhaps the hippocampus, initially specialized solely for spatial navigation and memory, has developed over the millennia towards more efficient emotional processing, which is necessary for mastering the challenges of increasingly more complex social environments (Robinson et al., 2016). Considering the current model of an 'emotional versus spatial' distinction, this would imply that the posterior hippocampus corresponds to the phylogenetically conserved memory-system, whereas today's anterior hippocampus represents the substrate for emotional processing that has developed later on. Interestingly, this view may be underpinned by differences in hippocampal size and shape across species: Comprehensive phylogenetic analyses demonstrate that the volume of the human hippocampus significantly exceeds predicted values based on an extrapolation from analyses of hippocampal volume in apes (Barger et al., 2014). In addition, morphologically, the human hippocampus exhibits a more pronounced anterior pole, the so-called hippocampal head, which is not as prominent in other species like rodents, birds, or reptiles, whose hippocampi are in contrast characterized by a rather elongate, bent shape (Reiter et al., 2017; Strange et al., 2014). These findings may underpin the hypothesis that especially the anterior hippocampus has evolved and taken on additional roles in humans, as compared to other species. Such a view, however, is challenged by findings suggesting a hippocampal role beyond memory and spatial navigation in other species (Bingman, 1992), for instance the involvement of the avian hippocampus in anxiety and stress-related behaviors (Smulders, 2017). Although these novel insights provide fascinating material for speculation, more cross-species studies are needed to thoroughly illuminate the evolutionary story of the hippocampus.

4.2.2 Transitions between anterior and posterior functional poles

As established above, an anterior-posterior functional differentiation of the hippocampus is based on a substantial body of evidence and thus to date widely

accepted. In contrast, the nature of the transition between the anterior and posterior poles until now remains elusive. Previous approaches tackling this question can methodologically be grouped into on the one hand studies that examine functional properties, referring to the relevance of an organization for executing functions, and on the other hand research relying on structural properties, corresponding to the underlying anatomical and molecular characteristics. After we first outline three important methodological challenges when addressing functional transitions, we will dedicate two subsections to previous functional evidence in support of either modular or smooth functional transitions.

4.2.2.1 Challenges of investigating functional transitions

When investigating the question ‘gradient versus modules’, researchers are faced with several methodological challenges. These challenges become especially relevant when reviewing previous research and will be referenced in the subsections below.

First, any given modular organization might appear smooth if the spatial resolution of the applied imaging technique is insufficient. This is especially important in fMRI studies, considering that the spatial resolution is confined by the voxel size, which to date by far exceeds the size of the underlying structural entity, namely single neurons. However, one might argue that even if fMRI does not provide a means to measure a single neuron’s firing behavior, it is still able to map the pattern of activation stemming from functional entities of voxel size. Therefore, in the concrete question of the hippocampal functional organization, fMRI studies should be able to detect functional modules if the voxel size is smaller than the estimated size of functionally meaningful hippocampal subunits (see also subsection 4.1.1). Another challenge may arise from data preprocessing, especially if it includes spatial smoothing, as smoothing may pose a potential bias towards a continuous gradient in contrast to a modular organization (details in subsection 4.1.2). Therefore, unbiased investigation of the functional pattern of the hippocampal long-axis can be problematic in fMRI studies with a spatial resolution that is lower than the size of putative subunits, which thereby become unidentifiable, especially in combination with spatial smoothing.

Second, conversely, a putative smooth organization might be detected as modular, if a parcellation scheme is artificially enforced. In other words, clustering algorithms typically cluster the input data in the attempt of grasping an underlying modular

organization, but they will always yield a parcellation even if transitions in the source data are smooth. Therefore, it is important to analyze the underlying pattern of organization and assess its 'discreteness' before clustering or to apply a data-driven approach that makes as little assumptions about the data as possible.

Third, many studies investigating functional differences along the hippocampal long-axis perform a parcellation of the hippocampal volume beforehand and analyze functional properties of the ensuing hippocampal clusters (e.g., Bast et al., 2009; Beaujoin et al., 2018; Brunec et al., 2018; Dalton et al., 2019; Kahn & Shohamy, 2013; Libby et al., 2012). Although these studies provide important insights into the functional correlates underlying a long-axis organization, it is impossible to answer the question whether these correlates follow a gradient or exhibit a modular organization. Hence, studies allowing for conclusions regarding hippocampal long-axis transitions are scarce, which underlines the necessity of entirely data-driven approaches that do not rely on a parcellation before the analyses.

4.2.2.2 Functional evidence for a discretized hippocampal organization

Although reliable insights into functional transitions of the hippocampal long-axis are scarce given the challenges outlined above, our findings are in line with several previous findings from studies addressing functional properties.

First, the concept of a modular functional organization of the human hippocampus is corroborated by several recent functional imaging studies employing so-called meta-analytic connectivity modelling (MACM) approaches (Robinson et al., 2010). The typical goal of these studies is to examine the functional connectivity of a defined region of interest. However, instead of analyzing a restricted dataset relying on a single experimental paradigm, MACM approaches analyze functional connectivity across an abundance of studies employing a variety of behavioral tasks and incorporating thousands of subjects. An example for an MACM approach applied to investigate hippocampal functional connectivity is a recent study by Robinson et al. (2015). The authors took advantage of the BrainMap database (Fox & Lancaster, 2002), which at the time comprised 2.630 papers, representing more than 12.000 experiments with roughly 52.000 subjects in total. Based on the identified functional connectivity estimates, Robinson and colleagues performed *k*-means clustering to achieve a functional connectivity-based parcellation. Interestingly, they find that the most stable

segmentation consists of three subunits in the left hippocampus and five subunits in the right hippocampus. However, the five-cluster solution in the right hippocampus is only slightly more favorable than a two-cluster solution, and the authors concede that more research is needed to determine the number of subunits in the right hippocampus. Using a similar methodology, also applied to the BrainMap database, Chase et al. (2015) tackle the same question as Robinson and colleagues, but they confine their analyses of the hippocampal long-axis to an isolated transversal subfield, the subiculum. Importantly, in contrast to most studies performing analyses in the left and right hippocampus separately, Chase and colleagues defined the subiculi of both hemispheres as one common region of interest. More precisely, they performed a parcellation based on meta-analytic functional connectivity without separating the left and right hippocampus. Their results indicate a segmentation of both subiculi into five functional clusters in total: A bilateral anterior cluster spanning the anterior parts of both subiculi, as well as two additional clusters (intermediate and posterior, respectively) in the subiculum of each hemisphere. Therefore, this study replicates Robinson's finding of a tripartite organization in the left hippocampus and furthermore demonstrates the same organization in the right subiculum. Despite restriction of the analyses to only one subfield, Chase et al. argue that the obtained parcellation is likely to reflect the organization of the entire hippocampus due to the level of effective resolution provided by the BrainMap database: Given that the traditional transversal subfields (dentate gyrus, Ammon's horn, and subiculum) closely follow and exist along the entire hippocampal long-axis (Figure 2), it is likely that due to lower spatial resolution and inference across numerous studies the detected effects not only reflect the organization of a single subfield. In contrast, the results from within one subfield may partly include other subfields and therefore describe the organization of the entire hippocampal long-axis. A third study using an MACM approach was performed by Plachti et al. (2019), who complemented previous studies by providing a parcellation based on multimodal analyses including fMRI during rest and during task as well as structural covariance. Although the authors do not make a strong claim regarding the number of subunits, they report on biological relevance for parcellations into three, five and seven subunits.

These MACM approaches lend support to the idea of a modular hippocampal organization, providing more or less explicit evidence for a tripartite organizational

pattern. MACM itself significantly propels functional imaging analyses and the mentioned studies provide fascinating insights into functional connectivity differences of the hippocampal long-axis. However, their importance regarding the concrete question of modular versus gradual transitions may be limited, as they might not entirely overcome the second problem outlined in subsection 4.2.1: Employing *k*-means clustering on functional connectivity patterns without visualizing or quantifying the underlying geometric structure may be problematic, as it cannot be entirely ruled out that a parcellation scheme was artificially enforced. Therefore, it is important to additionally consider studies pursuing a more data-driven approach.

An example for a recent data-driven approach is the work of Zarei et al. (2013), who mapped the distribution of hippocampal functional connectivity to three cortical and subcortical seed regions, namely the thalamus, prefrontal cortex (PFC) and posterior cingulate cortex (PCC). Zarei and colleagues find differing connectivity profiles along the hippocampal long-axis and use these differences for a functional connectivity based parcellation. Specifically, they assign each hippocampal voxel to one of three targets regions (thalamus, PFC, or PCC) according to which target region it had the highest connectivity with. This so-called 'hard segmentation' yielded a parcellation into three distinct, longitudinally arranged subunits. Although this arguably constitutes a more data-driven approach than *k*-means clustering and of course allows for interesting conclusions regarding differential subunit involvement in cortical and subcortical functional networks, it may still not entirely overcome the second challenge outlined in subsection 4.2.1: In hard segmentation processes implementing a 'winner-takes-it-all' principle, one cannot distinguish a gradual versus discrete underlying pattern as for instance, the boundary will be drawn between two voxels regardless of whether the ratio of functional connectivity to region A versus region B is 90:10 or 51:49. Therefore, methodologically this study does not allow for conclusions on the functional transition between subunits. Besides, the study by Zarei et al. is based on fMRI data acquired on a 1.5 Tesla scanner, which may render an unbiased investigation of the transitions difficult due to lower spatial resolution (first outlined challenge in subsection 4.2.1).

Taken together, our findings, which strongly support the notion of a discretized hippocampal organization, are in line with several previous studies, including MACM based parcellation approaches as well as other more data-driven procedures applied

to functional imaging data in humans. However, most of these studies do not allow for explicit evaluation of the transitions between subunits. Thus, based on the current literature the question of gradual versus discrete functional transitions cannot be settled with certainty. Furthermore, among the mentioned studies, there is considerable ambiguity regarding the exact number of functional subunits, ranging from two to five. This ambiguity is partly reflected in our study, namely in the inconclusive outcome of the statistical tests that aimed to determine which observed peaks of functional connectivity similarity significantly exceed randomly generated peaks and thus represent biologically relevant modules (subsection 3.3). As these tests could not definitively distinguish between an organization in two and three subunits, we performed additional analyses to identify the number of hippocampal clusters, including the so-called elbow method and the quantification of the number of peaks in each participant's histogram, which both point towards three histogram peaks and therefor three functional clusters in the hippocampi of both hemispheres (subsection 3.3). However, we are aware that the implemented peak identification algorithm may not be entirely unbiased due to the introduced criterion of a minimum peak distance of four bins. This criterion was necessary to not capture every lower local peak within a higher global peak (illustration in Figure 13), but it can be criticized that the choice of explicitly four bins was arbitrary, as outlined in subsection 4.1.5. In future studies aiming to replicate or refute our findings, it may be beneficial to implement a different or even several different peak identification approaches for determining the number of clusters that optimally captures the structure of the data. In addition, *k*-means clustering may be replaced by a more data-driven parcellation algorithm to avoid the problem of specifying *k* altogether.

4.2.2.3 Functional studies suggesting a hippocampal long-axis gradient

If, as shown above, the current literature does not allow for unequivocal confirmation of a tripartite or at least discrete functional organization, one may ask themselves whether perhaps this hypothesis is simply not correct. After all, there are previous studies employing similar data-driven techniques that contradict a discretized functional long-axis organization, which shall be outlined in this subsection.

For instance, Przeździk et al. (2019) used the same connectopic mapping approach as we applied here but, interestingly, came to an opposed conclusion: The authors

report a continuous pattern of functional connectivity change, indicating gradual functional transitions along the hippocampal long-axis. Though surprising at first, this discrepancy can be comprehended considering several key differences between the two studies: First, Przeździk et al. applied connectopic mapping to resting-state fMRI data. FMRI acquisition during resting-state is fundamentally different from task-dependent fMRI, in that during the former, participants are instructed to relax, and specifically not engage in any mental activity, but keep from falling asleep. Hence, it is likely that neuronal activity, functional engagement, and therefore functional connectivity measures of a region of interest differ between task and resting-state fMRI (Di et al., 2013). This methodological difference between Przeździk's and our study is particularly interesting as it may suggest that the hippocampus exhibits different patterns of functional organization depending on whether it is functionally active or not. At first glance, the difference may manifest as a less modular pattern during resting-state fMRI, as suggested by Przeździk's findings, versus a clearly modular pattern in task data, as indicated by our work. However, this may not be a valid conclusion as one could also argue for the opposite: Engagement in a specific task, e.g., spatial navigation, may bring about a certain functional differentiation optimized for the respective task, thereby stressing putative subunits responsible for spatial processing, but possibly disregarding any modules involved in other brain functions. In contrast, resting-state fMRI may drive all putative modules, irrespective of functional specialization, as brain activity is not restricted to one functional task and might thus be able to detect an even more multifaceted differentiation. To precisely determine the difference between task and resting-state data, it would be interesting to employ different paradigms while keeping all other experimental parameters including the participants, preprocessing, and analysis pipeline constant. In fact, it is not only a remaining question how the hippocampal long-axis organization varies between fMRI without a task in comparison to fMRI with a task, but additionally how the nature of a task may affect the resulting functional organization, especially given the functional multiplicity of the hippocampus (subsections 1.3 and 4.2.1.2).

A second difference between Przeździk's and our study is that Przeździk and colleagues consider only the functional connectivity to the neocortex, disregarding any coactivation of the hippocampus with subcortical structures. Although it is difficult to make precise predictions on how that specifically alters functional connectivity profiles,

previous studies suggest that it may have an influence. For instance, Kahn and Shohamy (2013) found that the hippocampus exhibits considerable functional connectivity with the nucleus accumbens and the ventral tegmental area, which are both subcortical structures. Especially because the authors show that this connectivity is not constant along the long-axis, it can be argued that subcortical regions should also be considered for an overall analysis of hippocampal long-axis connectivity.

Third, on a technical note, the acquisition specifics are different in that Przeździk et al. analyze data from the Human Connectome Project originating from a 3 Tesla fMRI scanner and apply smoothing with a kernel of 6 mm. In contrast, in the study presented here, we used 7 Tesla fMRI with a smoothing kernel of 2.5 mm, which provides a higher spatial resolution and may potentially have pertained a putative granularity more accurately.

Lastly, Przeździk et al. corroborate the biological significance of the identified gradient by showing a correlation of the gradients with behavioral data from the same participants, including performance in a memory task. Specifically, the authors compare the gradient model to a dichotomy model with two subunits regarding how well each model explains the differences between task performance and find that the gradient model is superior. However, although a gradient may predict behavioral data better than a two-partite model, it is unclear as to whether this holds true when comparing a gradient to a tripartite model.

Therefore, Przeździk's and our findings do not contradict or invalidate each other, but in contrast work together to open interesting perspectives for future research, especially regarding functional differences during rest versus task engagement and the importance of connectivity with cortical versus subcortical regions.

However, Przeździk and colleagues were not the only ones suggesting a functional long-axis gradient in the hippocampus. For instance, a very recent functional imaging study by Masouleh et al. (2020) demonstrates a smooth transition of structural covariance along the longitudinal axis. Per definition, structural covariance is based on the similarity of macrostructural variations between two brain regions (Alexander-Bloch et al., 2013; Mechelli et al., 2005). Hence, it is thought to be primarily determined by structural aspects including direct monosynaptic connections (Yee et al., 2018) or common genetic cues during early development (Raznahan et al.,

2011). However, structural covariance may not only reflect purely structural information but to some extent also accommodate the underlying functional organization, which is supported by the finding that high structural covariance between two regions correlates to high functional connectivity (Kotkowski et al., 2018). Like Przeździk et al., Masouleh and colleagues apply the structural covariance approach to a large cohort from the Human Connectome Project acquired at 3 Tesla, but additionally replicate their findings in an independent dataset from the enhanced Rockland cohort. Additional parallels to the methodology in Przeździk's study include the use of resting-state fMRI data and spatial smoothing with a similar kernel size of 5 mm. Thus, the same potential limitations as discussed above regarding the lower spatial resolution and differences between task and resting-state fMRI data may be present. Besides, Masouleh et al. base their conclusion of a smooth long-axis transition on the group-level results, which demonstrate a continuous change of structural covariance. At this point, it may be relevant to revisit an observation from our study, namely that individual modular patterns were merged into an overall smooth looking gradient on the group-level (subsection 3.2). Clearly, with a sample size of 377 individuals it is unfeasible to thoroughly evaluate single-subject patterns, however it would be very interesting to view a random sample of single-subject patterns from Masouleh's study.

In sum, although considerable differences between studies providing evidence for a modular organization and studies demonstrating a smooth gradient can be discussed, one can currently only speculate to what extent these differences may explain the opposing findings. To accurately determine the role of these differences, more data-driven approaches investigating functional transitions along the hippocampal long-axis are needed, perhaps with an emphasis on the difference between task-dependent and resting-state fMRI.

4.2.3 Functional correlates of a putative tripartite organization

Although more studies are needed to settle the matter regarding a modular or smooth hippocampal long-axis, one can already discuss the potential roles of putative longitudinal subunits. As our results are in line with a tripartite functional long-axis organization, this subsection will focus on the potential roles of an anterior, intermediate, and posterior functional parcel. Within the frameworks provided by the

above-mentioned models of anterior-posterior distinction (subsection 4.2.1), there is a substantial body of evidence regarding the roles of an anterior and posterior parcel (for review see e.g., Poppenk et al., 2013). For a brief recapitulation, the anterior hippocampus may be implicated in the processing of emotional, coarse, and self-centered information, whereas the posterior hippocampus may engage in spatial, fine-grained, and world-related processing. However, few publications broach the issue regarding the specific role of an intermediate subunit. One of the first studies to explicitly address the intermediate hippocampus was conducted by Bast et al. (2009), who examined the effects of partial hippocampal damage on rats' performance in a rapid, one-trial spatial learning task. Interestingly, dorsal or ventral lesions sparing the intermediate hippocampus left performance largely intact, whereas lesions compromising the integrity of the intermediate hippocampus severely impaired performance. Furthermore, using electrophysiological models Bast and colleagues demonstrate that despite the lack of successful behavioral performance following damage to the intermediate hippocampus, the dorsal hippocampal residue is still able to encode spatial information rapidly and accurately. Hence, the authors suggest that the intermediate hippocampus is essential for translating rapid spatial learning into the appropriate behavior and acts as an integrative interface between the dorsal and ventral portions. However, this finding yet remains to be replicated in humans.

Evidence for a specific role of the human intermediate hippocampus has emerged from functional imaging studies demonstrating significant differences in functional connectivity profiles between the three longitudinal subunits (e.g., Cheng & Fan, 2014; Robinson et al., 2015; Zarei et al., 2013). Strikingly, Kahn and Shohamy (2013) demonstrate that the resting-state functional connectivity of the hippocampus with two subcortical structures prominently involved in motivation and reward processing, namely ventral tegmental area and nucleus accumbens, is localized to a rather circumscribed region in the middle of the hippocampus. The authors propose that the involvement of the intermediate hippocampus in a motivation and reward network could provide a pathway for the enhancing effect of motivation on memory formation (Miendlarzewska et al., 2016). Like in the model suggested by Bast et al., this may regard the intermediate hippocampus as an interface between the anterior and posterior portion. Additionally, the suggested role may be compatible with the 'emotional versus cognitive' view of an anterior-posterior distinction (subsection 4.2.1).

After all, motivation typically reflects personal goals which are based on intrinsic emotional processes and therefore can be seen as pertaining to emotional processing: Perhaps the intermediate hippocampus mediates the influences of emotional content, namely motivation and goal-orientation, on memory encoding. Of course, this speculation requires future studies for evaluation and validation.

An entirely different role of the intermediate hippocampus in humans was suggested in a recent study by Robinson et al. (2015), who performed an MACM based hippocampal parcellation and yielded a division into three subunits in the left and five subunits in the right hippocampus (detailed in subsection 4.2.2). In addition to the parcellation, Robinson and colleagues aimed for a functional characterization of the obtained subdivisions using the behavioral taxonomy provided by the employed BrainMap database (http://brainmap.org/taxonomy/paradigms.html). Interestingly, within the proposed three-cluster solution for the left hippocampus, Robinson et al. report an association of the anterior cluster with emotional processes, an association of the intermediate cluster with cognition-based processes including more specific tasks like recall of a previously learnt association of items and more general processes like encoding of stimuli, and lastly an association of the posterior cluster with perception-based processing. Although these findings roughly confirm the model of 'emotional versus cognitive processing' along the hippocampal long-axis and furthermore suggest a distinct role for the intermediate hippocampus, it is surprising that no association of memory tasks and especially spatial memory was found for the posterior parcel. This may be partly because navigation tasks are scarce in fMRI research due to arising challenges for data acquisition and therefore also not contained in considerable number in the BrainMap taxonomy. In addition, the functional distinction emotion (anterior) – encoding (intermediate) – perception (posterior) is rather inconclusive, especially given the current controversy regarding the role of the hippocampus in perception (Suzuki & Baxter, 2009), and poses more questions for future research.

Interestingly, Robinson and colleagues were not the only ones applying an MACM based parcellation and characterizing subunits regarding their behavioral relevance. A study by Chase et al. (2015), introduced in subsection 4.2.2, suggests that activation in the right intermediate hippocampus is consistently related to the imagination of objects or scenes. Although this insight requires replication and further testing, it may

fit into the above-mentioned ‘self versus world’ model, in which the anterior hippocampus takes part in self-centered processing, whereas the posterior pole is involved in world-related contents (Plachti et al., 2019): Imagination of objects or scenes is on the one hand partly self-related in that imagination evidently takes place inside one’s mind and is influenced by one’s emotional experiences, but on the other hand also relates to world-centered processing in that it requires the mental modelling of external stimuli. Therefore, it may be speculated that the intermediate parcel takes on a role within the ‘self versus world’ framework by dealing with content that is not purely attributed to a single of these two modalities but rather takes place in between.

All in all, current studies propose diverging evidence regarding the functional role of a distinct intermediate hippocampal subunit and clearly more research is needed to corroborate a common view. Especially the investigation of functional connectivity in task-based fMRI may provide further insights, as most previous functional connectivity quantifications stem from resting-state data. Although hitherto a clear framework incorporating the mentioned evidence into a common view is lacking, existing studies lend support to the idea of a modular hippocampal organization with a distinct intermediate subunit exerting a specific function.

4.2.4 Structural underpinning of a functional discretization

Having outlined previous studies addressing the functional long-axis organization and having discussed potential functional correlates of a modular, tripartite organization, so far only evidence from functional studies has been presented. Although the correlation of structure to function is a matter of current debate and requires more studies to draw definitive conclusions, it is widely assumed that generally functional anatomy to some extent reflects an underlying structural anatomy (Eickhoff & Grefkes, 2011). Therefore, it is an interesting and unresolved question as to which structural aspects may underpin the hippocampal long-axis organization and correlate to a modular or gradual change of function.

An obvious, yet important structural property that may sustain a hippocampal long-axis differentiation is anatomical connectivity. In contrast to functional connectivity, which is quantified computationally and reflects the occurrence of functionally coactive brain

regions, anatomical or neuronal connectivity is a more tangible measurement as it refers to direct axonal projections of neurons. These projections can be divided into extrinsic connections, including projections to and from cortical and subcortical structures, versus intrinsic connections, reflecting the inherent wiring of neurons within the hippocampus itself. Regarding extrinsic connectivity, most projections originating from various brain regions reach the hippocampus via the entorhinal cortex (Duvernoy, 2005). As has first been shown more than three decades ago, these entorhinal-hippocampal connections are topographically organized and were initially thought to follow a smooth gradient (Amaral & Witter, 1989; Witter et al., 1989). Specifically, neurons from more lateral parts of the entorhinal cortex reach more dorsal parts of the hippocampus, while neurons from more medial parts of the entorhinal cortex project to more ventral hippocampal neurons. However, subsequent studies demonstrate that despite being topographically organized, these projections can be divided into three segregated domains that show relatively little overlap (Dolorfo & Amaral, 1998; Witter et al., 2000). This tripartite pattern of extrinsic connectivity may even extend to connections with other subcortical brain regions, as hippocampal projections to the lateral septal nucleus can be divided into three broad domains (Risold & Swanson, 1996). In addition, projections from the amygdala to the hippocampus were shown to be restricted to the ventral portion of the hippocampal long-axis (Fudge et al., 2012; Krettek & Price, 1977), which further supports the hypothesis of discrete rather than gradual changes in anatomical connectivity. As opposed to extrinsic projections, studies regarding intrinsic hippocampal connectivity are also consistent with discrete demarcations: Both major associational fiber systems of the hippocampus are organized in two hardly overlapping divisions (Li et al., 1994; Ishizuka et al., 1990; Amaral & Witter, 1989; Fricke & Cowan, 1978; Swanson et al., 1978): Although few fibers cross between the two divisions, there is a remarkable divergence of axons within either the ventral one-third or the dorsal two-thirds of the hippocampus, respectively, pointing towards a discrete segregation of intrinsic connectivity (Kondo et al., 2008; 2009). These latter findings appear to suggest a rather dichotomic distinction between ventral one-third and dorsal two-thirds, yet they might still be in line with a tripartite model of functional specialization considering the putative functional role of an intermediate hippocampal subunit, as discussed in subsection 4.2.2: If one speculates that the intermediate hippocampus combines aspects of the anterior and posterior portions into a new functional entity and if each

module comprises approximately a third of the hippocampal mass along the longitudinal axis, the intermediate third might share certain properties, like its intrinsic wiring, with the dorsal or ventral third.

In addition to anatomical connectivity, which may provide a structural foundation for a tripartite functional organization, another structural property that was studied along the hippocampal long-axis is synaptic plasticity. Generally, synaptic plasticity is understood as the ability of synapses to strengthen or weaken over time and can occur on a shorter (short-term plasticity) or longer (long-term plasticity) timescale. Terminologically, the long-term strengthening of synapses is mediated by effects of so-called long-term potentiation, and conversely synaptic weakening is mediated by long-term depression. As this flexible means of synaptic rearrangement is thought to pivotally govern mechanisms of memory formation (Bliss & Collingridge, 1993), synaptic plasticity is a much-investigated structural feature, especially in regions that are essential for mnemonic processing, like the hippocampus (Martin et al., 2000; Morris et al., 1990). One way of studying synaptic plasticity in a brain region is by applying electrical stimulation, which induces long-term potentiation or depression depending on the applied stimulation frequency, and subsequently recording neuronal responses, which allows for measuring specific characteristics of synaptic plasticity, including for instance frequency-dependency and persistence over time. Using such an electrophysiological approach in alive rats, Kenney and Manahan-Vaughan (2013) compared synaptic plasticity in the adorsal and intermediate portion of a transversal hippocampal subfield, namely the dentate gyrus (transversal subfields illustrated in Figure 2). Importantly, the authors find major differences in the mechanisms of long-term potentiation and long-term depression between the dorsal and intermediate hippocampal subunits. Briefly, the intermediate dentate gyrus is more susceptible to expression of long-term potentiation and less able to exhibit long-term depression than the dorsal portion. Kenney and Manahan-Vaughan conclude that the intermediate hippocampus is more than just a transitional zone within a smooth dorsal-ventral hippocampal gradient and may constitute a distinct functional entity.

In addition to anatomical connectivity and synaptic plasticity, the investigation of genomic data has received increased attention in recent years. The implementation of comprehensive genome-wide gene expression libraries paved the way for studies examining in-depth genomic specificities in different brain regions across various

species (Lein et al., 2007; Shen et al., 2012). A prominent line of evidence emerges from work in rodents by Thompson et al. (2008), who mapped gene expression patterns of hippocampal subfield cornu Ammonis (CA) 3 and reveal a molecular organization in several discrete, sharply demarcated gene expression domains. Specifically, Thompson and colleagues demonstrate that the hippocampal long-axis can be divided into nine distinct genetic subdivisions. Building upon these findings, Dong et al. (2009) suggested that these nine subdomains can be grouped into three major genetic domains. The authors corroborate this conclusion by revealing three molecular subunits in hippocampal subfield CA1 and argue that both CA1 and CA3, and presumably cornu Ammonis as a whole are divided into three molecular subunits. A very recent study investigated the genomic anatomy of the hippocampal long-axis for the first time in humans: Vogel and colleagues (2020) employed an extensive methodology based on gene expression data from several databases in the endeavor to bridge the gaps between brain structure and function, behavior, and moreover vulnerability to disease. Among other analyses, the authors demonstrate that the position of a tissue sample on the anterior-posterior hippocampal axis can be accurately predicted using expression patterns of a restricted set of genes. Although this supports the notion of a smooth long-axis organization in a genomic gradient, there may still be a chance that these findings are in line with a discretized organization: The employed prediction model is based on merged datasets incorporating tissue samples from six deceased individuals, hence group-level averaging may have potentially obscured single-subject organizations. Furthermore, the authors argue that their conclusions may be limited by the fact that the gene expression data originated from a restricted number of samples. However, despite the limitation in terms of generalizability from six individuals to larger populations, Vogel et al. used 188 tissue samples in total and examined at least 14 (to a maximum of 31) samples along the hippocampal long-axis of each individual, which theoretically seems sufficient for an unbiased detection of either gradual or discrete transitions. All in all, Vogel and colleagues' analyses can hardly be interpreted as coinciding with previous suggestions of discrete genetic domains, although it would be interesting to scrutinize their findings on a single-subject level.

It should be noted that the notion of a long-axis gradient in terms of structural properties is also supported by previous research, including a study in rodents by Kjelstrup et al.

(2008). As mentioned in subsection 4.2.1, Kjelstrup and colleagues used in-vivo electrophysiological recordings to map the receptive fields of hippocampal place cells, which are responsible for creating a cognitive map of space in the hippocampus. They find that not only the size of place fields is considerably larger in the anterior than in the posterior hippocampus, but also this change in scale occurs linearly in a gradient-like manner from the ventral to the dorsal hippocampal pole. However, there may be a possibility to reunite this conclusion with a modular organization: First, it should be noted that although the general inference of a linear change in place field size is based on the results pooled across 21 animals, the authors show striking recordings from individual animals, which clearly reveal that the firing patterns of individual place cells differ between ventral and dorsal locations (Kjelstrup et al. (2008), Figure 1 and Supplementary Figure 5). However, it is unclear how many recordings stem from each single animal, thus it is possible that although ventral place fields are significantly larger than dorsal ones, the amount of recorded cells per an individual animal's hippocampus may not have been sufficient to reliably deduce information regarding the transition of place field size.

4.2.5 Bottom line: Reconciling modular and gradual transition models

As hopefully conveyed throughout the subsections above, the hippocampal long-axis is an intriguing topic of much debate in the neuroscientific community and diverging findings regarding the long-axis organization of functional and structural characteristics open up different perspectives. Specifically, an abundance of studies across different modalities and species points towards a long-axis specialization with different functional implications of the anterior and posterior hippocampal poles (subsection 4.2.1), whereas there is a plurality of theories regarding the functional roles of these portions and even more ambiguity regarding the transition between the anterior and posterior hippocampal poles (subsection 4.2.2). Two lines of evidence point towards on the one hand a smooth gradient of functional specialization with the intermediate hippocampus solely acting as a transition zone between two functional entities and on the other hand a modular organization of function with presumably three sharply demarcated, distinct subunits, in which the intermediate hippocampus takes on an additional specific function.

To reconcile these seemingly opposed findings, namely smooth versus discrete transitions, Strange et al. (2014) proposed that there may not be a single, universal model of functional organization but instead different organizational patterns may be superimposed on the hippocampal long-axis. As Strange and colleagues outlined in their review, future studies are required to disambiguate the relative contributions of differing structural characteristics, namely discrete modules, i.e., gene expression domains, and smooth gradients, i.e., continuous transitions of place field size, to given behaviors. Moreover, Strange and colleagues' unified model provides a starting point for further contemplations, for instance regarding the way in which different models may be superimposed. As mentioned in subsection 4.2.2, the hippocampus may exhibit different organizational patterns during rest as opposed to during engagement in a task. This may imply that differential task engagement may bring about different functional organizations which are optimized for the respective, specific task. Another possibility is that the detection of different organizational patterns stems from differences between transversal hippocampal subfields: In fact, many studies assume that the transversal hippocampal subfields exhibit an analogous wiring and generalize inferences from individual subfields to the entire hippocampus (e.g., Dong et al., 2009). Nonetheless, it is possible that transversal subfields exhibit different functional patterns of organization, which may explain differences between findings originating from studies each investigating a single subfield. To substantiate this idea and potentially disentangle different organizations across transversal subfields, future studies with high anatomical precision are needed. Lastly, sharp and smooth transitions may be superimposed in the sense that different modalities are organized differently. This may relate to structure – function differences in that some structural properties may be reflected to a bigger extent as a gradient, whereas function may be predominantly organized in modules.

An especially intriguing aspect about Strange and colleagues' reconciling view regarding hippocampal functional organization is its potential generalizability. Specifically, when reviewing literature regarding the organization of the neocortex as a whole, one similarly stumbles across diverging hypotheses: On the one hand, it has been established more than a century ago that the cortical surface can be divided into numerous sharply demarcated units, which until now remains an accepted view (Brodmann, 1909; Kaas, 1987). These so-called Brodmann areas have first been

described using microstructural properties but are moreover thought to represent distinct functional entities (Schaefer et al., 2018; Strotzer, 2009). Other evidence for a modular neocortical organization emerges from more recent functional imaging studies that describe the cortex as a compilation of several modules exerting distinct functions (Bertolero et al., 2015), therefore stressing the importance of functional connectivity based parcellations (Eickhoff et al., 2018). However, not all researchers view the cortical organization as purely modular (Amunts & Zilles, 2015). Given the complexity and multiplicity of organizational axes, others have asked the question whether a discrete nomenclature corresponding to functionally distinct cortical areas is just an erroneous attempt to force a finite taxonomy on in truth continuous distributions (Goldberg, 1989). Contrasting a modular view, studies have investigated the continuous nature of the cerebral cortex and found gradients with respect to microstructural properties like gene expression (Hawrylycz et al., 2015) as well as functional characteristics like functional connectivity (Fornito et al., 2019; Margulies et al., 2016). Based on microstructural, genomic, and connectivity data, Huntenburg et al. (2018) suggested a large-scale cortical gradient spanning from sensorimotor areas on the one end to transmodal cortices on the other. Therefore, similarly to the hippocampal reconciliation model suggested by Strange et al., the entire cerebral cortex may be viewed as a system in which on the one hand distinct and on the other hand smooth transitions of functional organization are superimposed. In addition, one may speculate that this analogy between the hippocampus and the entirety of the cerebral cortex is in fact not a coincidence but hints at an evolutionary mechanism: According to the dual origin hypothesis, the cerebral cortex may have developed radially from specific phylogenetically conserved structures of the limbic system over the course of evolution. Specifically, a ventral/anterior system may have emanated from the perirhinal and amygdalar cortex, whereas a dorsal/posterior system may have evolved from the hippocampus and parahippocampal gyri (Giaccio, 2006). Therefore, intriguingly, the hippocampal long-axis may serve as a microcosm to study large-scale organizational patterns of the cerebral cortex, which may have evolved from and therefore are mirrored by the hippocampal organization (Vogel et al., 2020).

Although these contemplations serve as fascinating matter of debate in the current literature, clearly more evidence is required to eventually accept or refute these speculations.

4.3 Clinical implications

Despite the unequivocally fascinating nature of fundamental research questions regarding the hippocampal long-axis organization, readers with a clinical background may ask themselves whether these findings are relevant for them and how they may someday be implemented in the clinical practice. To provide potential answers, this final subsection highlights the potential of both our methodology as well as our results for possible clinical applications. Hence, this subsection provides an outlook, in which some of the outlined possibilities are already quite tangible, whereas currently some aspects rather remain hopes for the farther future.

4.3.1 Automated segmentation tool for neuroscientific research questions

As outlined in the Introduction, hippocampal integrity is critically impaired in a variety of neuropsychiatric diseases, for instance neurodegenerative illnesses, like most prominently Alzheimer's disease (La Joie et al., 2014) and frontotemporal dementia (Vogel et al., 2020), but also schizophrenia (Harrison, 2004), bipolar disease (Altshuler et al., 2000), major depressive disorder (Kemmotsu et al., 2013), anxiety (Cha et al., 2016), and post-traumatic stress disorder (Karl et al., 2006). Importantly, similar to the functional differentiation of brain functions along the hippocampal long-axis, the vulnerability to disease has also been shown to exhibit longitudinal differences (Lladó et al., 2018; Ranganath & Ritchey, 2012; Vogel et al., 2020). Therefore, fundamental as well as clinical studies addressing the organization of the hippocampal long-axis and its role in pathophysiological processes are important tools to advance our understanding of the underlying diseases and promote treatment options. However, to date comparison between such studies is majorly impaired by methodological inconsistencies, especially regarding the delineation and segmentation of the hippocampus and its subunits. For example, the current literature shows considerable disagreement across existing protocols for the delineation of transversal subfields (Yushkevich et al., 2015), which led to the formation of a dedicated scientific consortium, the Hippocampal Subfields Group (www.hippocampalsubfields.com), in the endeavor to develop a harmonized protocol for transversal subfield segmentation (Wisse et al., 2017). Analogous, previous studies examining long-axis differences have

also used a variety of methods for the definition of boundaries between anterior, (intermediate,) and posterior parcels: Some authors divide the hippocampus by volume percentage (Bast et al., 2009), some use anatomical landmarks (Poppenk et al., 2013) or the position along the longitudinal length (Fernández et al., 1998), whereas others forego a delineation altogether by showing all transversal slices along the entire anterior-posterior axis (Small et al., 2001). Given this heterogeneity, the scientific community in fundamental as well as clinical hippocampal research would strongly benefit from a unified method for segmenting longitudinal subunits, that may ideally be data-driven and automated. In contrast to the endeavors of the Hippocampal Subfield Group aiming to harmonize transversal subfield segmentation protocols, there is not yet a joint effort to unify segmentation protocols for long-axis subunits, which is most likely due to the relatively recent discovery of a functionally meaningful long-axis organization. Providing a solution to the problem of heterogeneity between protocols, we believe that the methodology applied here, namely connectopic mapping in combination with *k*-means clustering, has the potential to serve as a unified approach for automated hippocampal long-axis segmentation. Of course, *k*-means clustering does not provide an entirely data-driven clustering tool, which is discussed in depth above, therefore such a protocol would not allow for conclusions regarding the number of subunits or the nature of long-axis transitions. However, regarding the bulk of studies solely aiming to quantify differences along the hippocampal long-axis, our proposed method may alleviate the burden of a pre-analysis parcellation for the individual experimenter, and furthermore facilitate comparison between findings on a global level if universally applied across studies.

Furthermore, the possible benefit of the proposed method is not necessarily restricted to application on the hippocampus. In fact, the connectopic mapping algorithm as itself (i.e., not combined with *k*-means clustering) has already been successfully applied to other brain areas including primary motor and visual cortices (Haak et al., 2018), entorhinal cortex (Navarro Schröder et al., 2015), striatum (Marquand et al., 2017), as well as the cerebral cortex as a whole (Margulies et al., 2016). Thus, connectopic mapping combined with *k*-means clustering may serve as a useful tool to determine the functional organization of any given brain region and produce a parcellation based on the topography of functional connectivity similarity. Such a parcellation can provide interesting insights for itself but can also constitute a methodological basis for limitless

further analyses, for instance examining functional or structural differences along the identified main axis of organization in healthy versus diseased individuals.

4.3.2 Clinical predictive tool for neurodegenerative diseases

In addition to the possible benefits of our methodology for (clinical) neuroscientific research throughout a wide spectrum of applications, our findings may provide an avenue for advanced diagnostics and progression tracing in neurodegenerative diseases. This shall be elaborated using Alzheimer's disease as an example. Diagnostic standards for this condition are set in the current S3 guideline for dementia, jointly issued by the German Association for Psychiatry, Psychotherapy and Psychosomatics (DGPPN) and the German Society of Neurology (DGN). Regarding the initial diagnosis of Alzheimer's disease, the guideline mentions structural imaging as a complementary tool in that it shall be used to exclude other causes of dementia, like vascular dementia, and to assess the extent of overall cerebral and specifically temporal lobe atrophy (DGPPN & DGN, 2016). Apart from this suggestion of an initial structural MRI scan, imaging techniques are not recommended for follow-up controls or progression monitoring unless the disease shows an atypical progress in individual patients. According to the guideline, the rather minor importance of structural imaging is due to the low specificity of MRI regarding the discrimination between different forms of dementia. In contrast to structural MRI, functional MRI is not yet included in the guideline at all. However, previous fMRI studies in patients with Alzheimer's disease have demonstrated several parameters that may come into question as a diagnostic or disease progression marker, including for instance the resting-state functional connectivity of certain brain networks (Greicius et al., 2004) and of the hippocampus (Wang et al., 2006). A problem of some of these suggested parameters is the expected low specificity for a single disease, as for example the overall hippocampal functional connectivity is disrupted not only in Alzheimer's disease but in numerous other neuropsychiatric conditions as well (Garrity et al., 2007). This problem of insufficient specificity may potentially be overcome using a more sophisticated approach to quantifying functional connectivity, for instance using an accurate mapping of the functional connectivity topography or even a functional connectivity-based parcellation, which we used here. This proposition is based upon a line of evidence suggesting that

longitudinal hippocampal subunits exhibit selective changes in functional connectivity patterns in early stages of Alzheimer's disease (Zarei et al., 2013; Zhang et al., 2010). It is likely that functional connectivity differences between long-axis subunits are more specific for a disease than the overall hippocampal connectivity, as it has already been shown that diseases differentially target the hippocampal long-axis (Vogel et al., 2020) and that for example in schizophrenia the functional connectivity appears to be selectively altered in the anterior hippocampus (Zhou et al., 2008). Therefore, the accurate topography of functional connectivity similarity along the hippocampal long-axis is likely to display differentially impaired patterns in Alzheimer's and other diseases, which may each be specific for the respective condition. To test this, future studies are needed to examine whether hippocampal connectopic maps are indeed significantly different between healthy and diseased individuals, and across different illnesses. Furthermore, it is an open question whether size, shape, or arrangement of functional subunits change depending on whether the subject is healthy, aged, or affected by disease in different stages. If so, our approach, combining connectopic mapping and *k*-means clustering, would become even more interesting as a potential clinical predictive tool. Generalizing from the described example of Alzheimer's disease to other neurodegenerative diseases, potential applications are limitless given the multiplicity of conditions that the hippocampus is involved in: The use of fMRI as a clinical tool may not be restricted to diagnostics or progression tracing but could also be expanded to monitoring a patient's treatment response, thereby advancing our understanding of the mechanisms underlying neuropharmacological manipulations (Wise & Preston, 2010). To further explore these clinical applications of fMRI analyses, more research is needed to bridge the gap between the hippocampal long-axis organization in health to specific alterations in various diseases. However, it is far from unrealistic to imagine that the hippocampal long-axis organization, or parcellation, may serve as a clinical predictive tool for neuropsychiatric diseases one day.

However, critics may argue that fMRI requires the patient to lie still for a longer time than for instance a structural MRI scan, which may be difficult for cognitively affected individuals. Other disadvantages refer to high cost and lower availability of fMRI scanners and protocols, although of course collective financial reasoning should not replace considerations prioritizing an individual patient's wellbeing. On the other hand, fMRI is a noninvasive, painless, and virtually riskless examination, therefore posing

fewer risks for the patient than more invasive methods including for instance a lumbar puncture, which is also part of the current S3 guideline for dementias including Alzheimer's disease. In addition, fMRI is more independent of the patient's intrinsic motivation than for example neuropsychological testing of a patient suffering from major psychological impairment: In many tasks currently used for the assessment of a patient's cognitive status, noncompliance cannot be reliably be differentiated from malperformance. Of course, however, it would be similarly challenging to convince a noncompliant patient to perform a task in the MRI scanner. Therefore, in patients exhibiting problematic noncompliance, resting-state fMRI would be more feasible than task-based fMRI, although one may argue that even noncompliant patients could more easily be convinced to perform a game-like task in the scanner than to for instance perform mathematical operations (which is part of the Mini Mental State Test widely used for neuropsychological testing in dementias (Tombaugh & MyIntyre, 1992)). Nonetheless, as mentioned in subsection 4.2.2 and 4.2.5, the hippocampal organization and therefore connectopic maps derived from resting-state fMRI data may differ from task-based results. Thus, before connectopic mapping can be applied in a clinical setting and tested as a diagnostic biomarker, future studies should investigate the difference between resting-state and task-based results.

In conclusion, state-of-the-art fundamental neuroscientific research has already provided and is working to solidify the foundation for potential applications of fMRI in a clinical setting. Although fMRI may someday outperform previous clinical methods, its current implementation in the clinical routine is hindered by impracticability and dubiety regarding its additional benefit. However, at least the latter doubts may be resolved by further research in the coming years so that fMRI, and specifically mapping the functional topography of the hippocampal long-axis, proceeds towards being used as a cutting-edge clinical predictive tool.

4. Summary and outlook

In this thesis, we set out to investigate the functional organization of the human hippocampus in healthy adults. To this end, we analyzed an ultra-high-resolution fMRI dataset that had been acquired on a 7 Tesla fMRI scanner. During scanning, the 22 participants performed a self-paced object-location memory task, which was facilitated by a 3D virtual reality setup. The fMRI data was processed with a novel, data-driven analysis algorithm, connectopic mapping (Haak et al., 2018), to determine the dominant topography of functional connectivity similarity within the hippocampus, based on its functional connectivity to the rest of the brain.

The obtained connectopic maps reveal a pattern of functional connectivity similarity that clearly follows the hippocampal long-axis, suggesting a functional anterior-posterior differentiation. Interestingly, this distinction exhibits a discretized pattern with several peaks of functional connectivity similarity, pointing towards a modular hippocampal long-axis organization. To determine the number of functional subunits, we performed a validation approach based on a simulated null distribution of functional connectivity similarity peaks. Comparison of the observed peaks to the 95th percentile of the null distribution suggests that the first and second highest peaks likely correspond to biologically meaningful modules, whereas the forth through tenth highest peaks could equally be caused by chance. However, the validation approach yielded inconsistent results regarding the significance of the third order peak between the left and right hippocampus, pointing towards either two or three longitudinal modules. Hence, to further specify the number of functional subunits, we quantified the number of peaks across participants, which indicated an overall rounded mean of three modules. Additionally, we applied the so-called elbow method (Kodinariya & Makwana, 2013) to determine the optimal number of clusters, providing further evidence for three functional subunits. Thus, we performed functional parcellations of participant-specific connectopic maps into three clusters using *k*-means clustering. Critically, 15 out of 22 participants exhibited consistent, longitudinally arranged subunits, whereas seven parcellations yielded rather irregularly arranged clusters.

Taken together, our findings provide evidence for a discretized functional organization of the hippocampal long-axis in healthy humans and lend support to the notion of a tripartite parcellation into an anterior, intermediate, and posterior functional subunit.

These results coincide with an abundance of previous literature indicating a tripartition of the hippocampal long-axis, including insights from recent functional imaging and behavioral studies. In contrast, there is a second line of research, supported by other functional imaging approaches, pointing towards a smooth functional long-axis gradient. Due to different methodologies and potential limitations, contradicting studies are often not entirely comparable and to date, controversial findings have not yet been fully conciliated. The analyses presented in this thesis are not able to definitively settle the matter, as certain shortcomings of our methodology cannot be ruled out, foremost including some ambiguity regarding the number of subunits and a potential lack of spatial precision due to the applied spatial smoothing during data preprocessing.

Despite the mentioned controversy in the literature and potential inconsistencies of our methodology, intriguing questions arise from the possibility of a tripartite hippocampal organization, including for instance the precise functional roles of three putative longitudinal modules. Previous studies have corroborated the general view that the anterior hippocampus takes part in emotional processing, whereas the posterior hippocampus is involved in spatial and cognitive processes. Within this anterior-posterior distinction, the role of an intermediate module is more ambiguous. Recent work suggests that the intermediate hippocampus may on the one hand mediate emotional influences on memory encoding and on the other hand take part in translating spatial learning into behavior, hence potentially providing an integrative interface between the anterior and posterior cluster. However, the analyses presented in this thesis rely on data acquired during an experimental task that involved spatial memory and navigation behavior but did not distinguish between different aspects of hippocampal function. Hence, our ability to assess the full functional spectrum of the hippocampus may be limited.

Moreover, it is an open question to what extent a functional tripartition may be reflected in structural aspects along the hippocampal long-axis. On the one hand, extrinsic and intrinsic neuronal projections of the hippocampus can be segregated into three scarcely overlapping divisions. Coincidingly, electrophysiological and genetic studies indicate that mechanisms of synaptic plasticity as well as gene expression are organized in three distinct domains. On the other hand, however, especially in terms of gene expression, a contradicting view is currently discussed, as other recent studies suggest a smooth genomic gradient.

In sum, previous research on the functional and structural organization of the hippocampal long-axis produced diverging hypotheses, namely modular versus gradual transitions of functional specialization. However, the two opposing theories could be reconciled by a recently suggested view that different patterns of long-axis organization may be superimposed. Within this framework, different modalities, i.e., different structural or functional aspects, may be organized in different ways, potentially even depending on if the hippocampus is functionally active or at rest.

Future studies will be able to corroborate or refute this proposed conciliation model. Specifically, ultra-high-resolution fMRI studies may be combined with fully data-driven analysis algorithms to fairly assess the data with as little assumptions as possible. Special care may be taken regarding the preprocessing pipeline to rule out that any applied procedure limits the spatial precision to detect the precise pattern of organization. Moreover, a sophisticated experimental setup involving various tasks may be considered to determine potential differences in the hippocampal long-axis organization during different task modalities and to tackle the question of functional correlates pertaining to potential modules. In addition, multimodal approaches will have the potential to bridge the gap between functional and structural insights.

In addition to these contemplations relating our findings to the unresolved question of the hippocampal long-axis organization, the analyses presented here may provide a methodological basis for numerous future applications. Not only could our data-driven approach, specifically connectopic mapping combined with *k*-means clustering, serve as a unified approach for hippocampal segmentation, which could benefit future hippocampal research and harmonize hitherto heterogeneous segmentation protocols. Moreover, the presented approach can be applied to any given region of interest and thereby be of use for limitless other neuroscientific questions.

Besides, our approach could not only be applied in the fundamental neurosciences, but has the potential to be used in a clinical setting: Since hippocampal integrity is disrupted in various conditions, including Alzheimer's disease, schizophrenia, and major depressive disorder, mapping the functional connectivity topography of the hippocampus comes into question as a clinical predictive tool for diagnostic purposes, for tracing a disease's progression, or quantifying treatment responses.

All in all, the thesis at hand provides novel insights into the functional organization of the human hippocampus, based on data-driven analyses of a 7 Tesla fMRI dataset. Despite potential shortcomings and the need for future research to fully elucidate the structural and functional anatomy of the hippocampus, our findings complement previous research and lend support to the discretized, tripartite view of hippocampal long-axis organization. Not only given numerous open questions arising from our analyses but also regarding future clinical applications of our methodology, it will be thrilling to observe what future approaches in fundamental and clinical hippocampal research may bring with regards to these and additional questions.

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