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Untersuchung der Mikrostruktur des Gehirns bei Posttraumatischer Belastungsstörung und Schädelhirntrauma mittels Magnetresonanztomographie

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1 Inhaltsverzeichnis

2 Abkürzungsverzeichnis

ACC	Anterior cingulate cortex
AD	Axiale Diffusivität, axial diffusivity
ADC	Apparent diffusion coefficient
ALLO	Allopregnanolon, Allopregnanolone
BDNF	Brain-derived neurotrophic factor
CR	Corona radiata
CRH	Corticotropin-Releasing-Hormone
CSF	Cerebrospinal fluid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th version
DTI	Diffusionstensorbildgebung, Diffusion tensor imaging
EC	Capsula externa, external capsule
FA	Fraktionale Anisotropie, fractional anisotropy
FSL	FMRIB Software Library
$GABA_A$	Gamma-Aminobuttersäure A
GCS	Glasgow Coma Scale
GLM	General linear model
HCEP	Hockey Concussion Education Project
HPA-Achse	Hypothalamus-Hypophysen-Nebennierenrinden-Achse, Hypothalamic-pituitary-adrenal axis
IC	Capsula interna, internal capsule
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th version
ImPACT	Immediate Post-Concussion Assessment and Test
INTRuST	Injury and Traumatic Stress Consortium
MD	Mittlere Diffusivität, mean diffusivity
MRI	Magnetic resonance imaging
MRT	Magnetresonanztomographie
mTBI	Mild traumatic brain injury
n	Probandenzahl
PCL-C	PTSD Checklist- Civilian Version
PREGNE	Pregnenolon, Pregnenolone
PTBS	Posttraumatische Belastungsstörung
PTSD	Posttraumatic stress disorder

RD	Radiale Diffusivität, radial diffusivity
RSHI	Repetitive subconcussive head impacts
SHT	Schädelhirntrauma
SLF	Fasciculus longitudinalis superior, superior longitudinal fasciculus
SSRI	Selective Serotonine Reuptake Inhibitors
TBSS	Tract-Based Spatial Statistics
ZNS	Zentrales Nervensystem

3 Publikationsliste

Die vorliegende kumulative Dissertation beruht auf den folgenden veröffentlichten Originalarbeiten:

1. Philipp Kinzel[+], Christine E. Marx[+], Nico Sollmann, Elisabeth Hartl, Jeffrey P. Guenette, David Kaufmann, Sylvain Bouix, Ofer Pasternak, Yogesh Rathi, Michael J. Coleman, Andre van der Kouwe, Karl Helmer, Jason D. Kilts, Jennifer C. Naylor, Rajendra A. Morey, Lori Shutter, Norberto Andaluz, Raul Coimbra, Ariel J. Lang, Mark S. George, Thomas W. McAllister, Ross Zafonte, Murray B. Stein, Martha E. Shenton*, Inga K. Koerte*: **Serum Neurosteroid Levels are Associated with Cortical Thickness in Individuals Diagnosed with Posttraumatic Stress Disorder and History of Mild Traumatic Brain Injury** (Clinical EEG and Neuroscience, 2020, Impact Factor 2019: 1,765).
   +Autoren waren gleich beteiligt
   * Autoren waren gleich beteiligt

2. Nico Sollmann, Paul S. Echlin, Vivian Schultz, Petra V. Viher, Amanda E. Lyall, Yorghos Tripodis, David Kaufmann, Elisabeth Hartl, Philipp Kinzel, Lorie A. Forwell, Andrew M. Johnson, Elaine N. Skopelja, Christian Lepage, Sylvain Bouix, Ofer Pasternak, Alexander P. Lin, Martha E. Shenton, Inga K. Koerte: **Sex differences in white matter alterations following repetitive subconcussive head impacts in collegiate ice hockey players** (NeuroImage: Clinical, 2017, Impact Factor 2018: 3,943).

4 Zusammenfassung

Die vorliegende kumulative Dissertation basiert auf zwei Originalarbeiten, welche 2020 im Journal *Clinical EEG and Neuroscience* und 2017 im Journal *NeuroImage: Clinical* veröffentlicht wurden. In unseren Studien untersuchten wir mikrostrukturelle Veränderungen im Gehirn von Probanden mit Posttraumatischer Belastungsstörung (PTBS), leichtem Schädelhirntrauma (SHT) sowie nach Exposition gegenüber wiederholten subklinischen Kopferschütterungen (repetitive subconcussive head impacts, RSHI). Die gemeinsame Fragestellung beider Studien ist die Untersuchung der Auswirkung traumatischer Gehirnverletzungen auf die Struktur des Gehirnes mit Methoden der Magnetresonanztomographie (MRT). Während in **Arbeit 1** strukturelle Veränderungen der grauen Substanz untersucht werden, fokussiert sich **Arbeit 2** auf strukturelle Veränderungen der weißen Substanz. Konkret behandelt **Arbeit 1** (Erstautorenschaft) die Frage, wie sich das leichte SHT und eine zusätzliche PTBS auf die graue Substanz auswirken und ob es einen Zusammenhang zwischen der kortikalen Dicke und dem Serumspiegel neuroprotektiver Neurosteroide gibt. **Arbeit 2** (Co-Autorenschaft) behandelt die Frage, ob es geschlechtsspezifische Unterschiede bei Veränderungen in der weißen Hirnsubstanz nach Exposition gegenüber RSHI gibt.

Arbeit 1

PTBS und das leichte SHT haben in Risiko-Populationen wie beispielsweise Soldaten eine hohe Koinzidenz. Zahlreiche pathophysiologische Veränderungen führen sowohl bei der PTBS als auch bei dem leichten SHT zu einer reduzierten kortikalen Dicke. Des Weiteren kommt es bei beiden Erkrankungen zu hormonellen Dysregulationen. Insbesondere gibt es Belege für erniedrigte Serumspiegel von neuroprotektiven Neurosteroiden bei der PTBS. Es war bislang jedoch nicht bekannt, ob es bei Probanden mit PTBS und leichtem SHT einen Zusammenhang zwischen kortikaler Dicke und Neurosteroid-Serumspiegeln gibt. Durch das Injury and Traumatic Stress (INTRuST) Clinical Consortium wurden 141 Probanden in diese Studie eingeschlossen. Von diesen hatten 32 Probanden eine Anamnese eines leichten SHT (SHT-Gruppe); 41 Probanden hatten sowohl eine aktuelle PTBS als auch eine Anamnese eines leichten SHT (PTBS+SHT-Gruppe); und 68 Probanden waren gesunde Kontroll-Probanden (Kontroll-Gruppe). Bei allen Probanden erfolgte eine klinische Untersuchung, eine T1- gewichtete MRT- Bildgebung sowie die Bestimmung des Serum-Spiegels der Neurosteroide Allopregnanolon (ALLO) und Pregnenolon

(PREGNE). Die Kohorte wurde auf Gruppenunterschiede bezüglich der kortikalen Dicke und auf gruppenspezifische Assoziationen zwischen kortikaler Dicke und Neurosteroid-Spiegeln untersucht. In der PTBS+SHT-Gruppe zeigte sich eine reduzierte kortikale Dicke im Vergleich zu den beiden anderen Gruppen. Des Weiteren gab es in der PTBS+SHT-Gruppe eine positive Korrelation zwischen dem Serumspiegel von ALLO und der kortikalen Dicke im rechten superioren frontalen Cortex sowie eine positive Korrelation zwischen dem Serumspiegel von PREGNE und der kortikalen Dicke im medialen temporalen und orbitofrontalen Cortex. Diese Ergebnisse deuten darauf hin, dass Neurosteroide bei Probanden mit PTBS und leichtem SHT möglicherweise einen protektiven Effekt auf die kortikale Dicke haben. Zukünftige Studien sollten das Potenzial der Neurosteroide als prognostischer Marker und als therapeutischer Ansatz weiter untersuchen.

Arbeit 2

In der zweiten Publikation untersuchten wir mögliche Geschlechtsunterschiede bei strukturellen Veränderungen der weißen Substanz nach der Exposition gegenüber RSHI bei College-Eishockeyspielern. In bisherigen Studien konnte gezeigt werden, dass nicht nur SHT, sondern bereits wiederholte, leichte Erschütterungen des Kopfes ohne klinische Symptomatik zu strukturellen und funktionellen Veränderungen im Gehirn führen. Ferner gibt es Hinweise auf Geschlechtsunterschiede in Veränderungen der weißen Substanz nach leichtem SHT. Es gab bislang jedoch keine Studien über Geschlechtsunterschiede nach Exposition gegenüber RSHI. In diese Studie wurden 25 College-Eishockey-Spieler im Rahmen des Hockey Concussion Education Project (HCEP) eingeschlossen. Bei allen Probanden erfolgte vor und nach der Saison eine diffusionsgewichtete MRT-Bildgebung und eine kognitive Testung. Keiner der Probanden hatte während der Saison ein SHT erlitten. Mittels Tract-Based Spatial Statistics (TBSS) untersuchten wir mögliche Veränderungen der Diffusionsparameter fraktionale Anisotropie (FA), mittlere Diffusivität (MD), axiale Diffusivität (AD) und radiale Diffusivität (RD) im Verlauf der Saison für beide Geschlechter. Signifikante Geschlechtsunterschiede waren im Fasciculus longitudinalis superior (SLF), der Capsula interna (IC), der Capsula externa (EC) und der Corona radiata (CR) der rechten Hemisphäre lokalisiert. In diesen Regionen zeigte sich bei Frauen eine signifikante Veränderung aller Diffusionsparameter, bei Männern jedoch nicht. Zusammenfassend zeigen wir in unserer Studie Geschlechtsunterschiede bei strukturellen Veränderungen der weißen Substanz nach

Exposition gegenüber RSHI bei Eishockeyspielern. Möglicherweise deutet dies auf eine erhöhte Vulnerabilität bei Frauen nach wiederholten subklinischen Gehirnerschütterungen hin. In zukünftigen Studien zu RSHI sollten Geschlechtsunterschiede systematisch untersucht werden, um die zugrundeliegenden Faktoren (z.B. hormonelle Unterschiede) zu identifizieren.

5 Summary

This cumulative dissertation is based on two original articles that were published in 2017 in the Journal *NeuroImage: Clinical* and in 2020 in the Journal *Clinical EEG and Neuroscience.* In our studies we investigated structural changes in the brain in subjects with posttraumatic stress disorder (PTSD) and co-occurring mild traumatic brain injury (mTBI), and in subjects who were exposed to repetitive subconcussive head impacts (RSHI). The overall aim of our publications was to study the impact of brain trauma on brain structure using magnetic resonance imaging (MRI) methods. Paper 1 investigates structural changes in gray matter, whereas Paper 2 focuses on structural changes in white matter. Particularly, the aims of **Paper 1** were to investigate 1.) how mTBI and co-occurring PTSD affect gray matter and 2.) whether there are associations between cortical thickness and serum levels of neuroprotective neurosteroids in individuals with mTBI and PTSD. The purpose of **Paper 2** was to study sex-specific differences concerning alterations in white matter microstructure after exposure to RSHI in ice hockey players.

Paper 1

PTSD and mTBI have a high co-incidence in high-risk populations such as veterans. Several pathophysiological changes lead to reduced cortical thickness in both PTSD and mTBI. Moreover, studies have shown hormonal dysregulations in both conditions. Particularly, PTSD has been associated with reduced serum levels of neuroprotective neurosteroids. However, it is not known, whether neurosteroid serum levels are associated with cortical thickness in individuals diagnosed with both PTSD and mTBI. 141 individuals where included in the study via the Injury and Traumatic Stress (INTRuST) Clinical Consortium. The cohort was divided into the following groups: an mTBI group (individuals with a history of mTBI, n= 32), a PTSD+mTBI group (individuals with current PTSD and a history of mTBI, n= 41), and a control group (healthy control subjects, n= 68). All subjects underwent clinical assessment, T1-

weighted MRI, and serum quantifications of the neurosteroids allopregnanolone (ALLO) and pregnenolone (PREGNE). We investigated group differences in cortical thickness and group-specific associations between cortical thickness and neurosteroid serum levels. In the PTSD+mTBI group, cortical thickness was reduced compared to the other groups. Moreover, cortical thickness in the right superior frontal cortex correlated positively with serum ALLO and cortical thickness in the medial temporal and orbitofrontal cortex correlated positively with serum PREGNE in the PTSD+mTBI group. These results may indicate a possible protective effect of neurosteroids on cortical thickness in individuals with PTSD and mTBI. Future studies should further investigate the potential of neurosteroids as prognostic markers and therapeutic targets.

Paper 2

In the second study we investigated sex-specific differences in white matter alterations following RSHI in collegiate ice hockey players. Previous studies have shown that RSHI lead to structural and functional alterations in the brain. While there is evidence for sex differences concerning white matter alterations after mTBI, there are no studies investigating sex differences after repetitive RSHI. This study included 25 collegiate ice hockey players as part of the Hockey Concussion Education Project (HCEP). All subjects underwent diffusion-weighted MRI (dMRI) and cognitive testing before and after the season. None of the subjects experienced an mTBI during the season. Using Tract-Based Spatial Statistics (TBSS), we measured changes in diffusion parameters for both sexes during the course of the season. Diffusion parameters included fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). Significant sex differences were located in the superior longitudinal fasciculus (SLF), the internal capsule (IC), the external capsule (EC) and the corona radiata (CR) of the right hemisphere. In these regions, diffusion parameters significantly changed in females, but not in males. In summary, this study shows sex differences in white matter alterations after exposure to RSHI in ice hockey players. These results may indicate a higher vulnerability in females after exposure to RSHI. Future studies on RSHI need to systematically investigate sex differences to identify the underpinnings (e.g., hormonal differences).

6 Einleitung

6.1 Arbeit 1- Klinischer und wissenschaftlicher Hintergrund

6.1.1 Leichtes SHT

Epidemiologie, klinische Manifestation und Diagnostik

Das SHT ist die häufigste Todesursache bei Erwachsenen unter 45 Jahren[1] und wird meist durch Verkehrsunfälle und Stürze verursacht[2]. In Deutschland hat das SHT eine Inzidenz von ca. 200- 300 pro 100.000 Einwohner[1, 3] pro Jahr. Das SHT wird in die drei Schweregrade leicht (Grad 1), moderat (Grad 2) und schwer (Grad 3) eingeteilt. Ca. 90% der SHT sind leichte SHT[4]. In Tabelle 1 sind die diagnostischen Kriterien für die jeweiligen Schweregrade dargestellt[2, 5]. Fakultativ können beispielsweise Kopfschmerzen, Übelkeit, Schwindel, Sehstörungen oder kognitiven Störungen vorliegen[6]. Diese Akutsymptomatik vergeht in der Regel nach Tagen bis Wochen.

Schweregrad	**Glasgow Coma Scale (GCS)**	**Bewusstseinsverlust**	**Posttraumatische Amnesie**
Leicht	15-13	0-30 Minuten	≤24h
Moderat	12-9	>30 Minuten, <24h	>24h, <7d
Schwer	≤8	≥24h	≥7d

Tabelle 1: Diagnostische Kriterien der Schweregrade des SHT

Pathophysiologie des leichten SHT

Die hinter der Symptomatik stehende Pathophysiologie ist noch nicht umfassend verstanden. Jedoch wird zunehmend die neurometabolische Kaskade als grundlegender Pathomechanismus angenommen[7, 8]. So geht man davon aus, dass die auf das Gehirn einwirkenden Scherkräfte zu diffusen Verletzungen der Axone und Myelinscheiden führen. Durch diese Verletzungen kommt es zu Ionenverschiebungen und einer erhöhten Glutamatausschüttung[7, 8]. In der Folge entsteht ein erhöhter Energiebedarf in den Zellen, welcher nicht gedeckt werden kann[7, 8]. Dieses Energiedefizit ist möglicherweise eine wichtige Ursache der strukturellen und funktionellen Veränderungen nach leichtem Schädelhirntrauma.

Leichtes SHT und PTBS

Viele der Patienten mit leichtem SHT weisen eine PTBS als Komorbidität auf, da Ereignisse, welche ein leichtes SHT verursachen, häufig auch emotional traumatisierend sind. So zeigte eine Studie, dass US-Veteranen, die ein leichtes SHT erlebt hatten, in 44% der Fälle auch die Kriterien der PTBS erfüllten[9]. Bei Veteranen ohne vormaliges leichtes SHT traf dies lediglich in 9% der Fälle zu. Einerseits erhöht ein leichtes SHT also das Risiko einer PTBS, andererseits ist die PTBS der wichtigste Risikofaktor für eine Chronifizierung der Symptome eines leichten SHT[10]. Zudem gibt es Überschneidungen im klinischen Bild des leichten SHT und der PTBS[2, 11]. So können beide mit Symptomen wie Depression, Ängstlichkeit, Konzentrationsstörungen und Schlafstörungen einhergehen[2]. Die genauen Mechanismen der Wechselwirkung und einer möglichen gegenseitigen Exazerbation der beiden Erkrankungen sind jedoch noch nicht verstanden.

6.1.2 PTBS

Epidemiologie, klinische Manifestation und Diagnostik

Die PTBS ist eine Folgereaktion auf ein psychisch belastendes Ereignis. Weltweit liegt die Lebenszeitprävalenz der PTBS zwischen 1 % und 7 %, in Deutschland liegt diese bei ca. 2 %[12]. Hochrisiko-Gruppen wie Soldaten im Auslandseinsatz zeigen eine deutlich höhere Lebenszeitprävalenz. In Deutschland liegt diese bei ca. 5 %[13], in den USA zwischen 6 % und 30 %[14]. Das klinische Störungsbild umfasst affektive, kognitive und behaviorale Symptome (Tabelle 2). Die Diagnose der PTBS erfolgt in den USA nach Kriterien des Diagnostic and Statistical Manual of Mental Disorders- 5th Version (DSM-5)[15] und in Deutschland nach Kriterien der International Statistical Classification of Diseases and Related Health Conditions- 10th Version (ICD-10)[16] (Tabelle 2).

Kriterium	ICD-10	DSM-5
Traumatisches Ereignis	Ereignis von außergewöhnlicher Bedrohung oder katastrophalem Ausmaß	Konfrontation mit dem Tod, ernsthafter Verletzung oder sexueller Gewalt
Symptome	Alle Symptome müssen vorhanden sein:	
	1. Wiedererleben (wiederkehrende, belastende Gedanken, Erinnerungen, Träume) 2. Vermeidung traumaassoziierter Stimuli 3. Übererregbarkeit *oder* fehlende Erinnerung wichtiger Traumaaspekte	1. Wiedererleben (wiederkehrende, belastende Gedanken, Erinnerungen, Träume) 2. Vermeidung traumaassoziierter Stimuli 3. Übererregbarkeit (Schlafstörungen, Reizbarkeit, Konzentrationsstörungen) 4. Negative Kognition oder negativer Affekt
Weitere Kriterien	Beginn der Störung innerhalb von 6 Monaten nach Trauma	1. Dauer der Störung länger als 1 Monat 2. Hoher Leidensdruck *oder* soziale/ berufliche Beeinträchtigung 3. Die Störung wird nicht durch Substanzmissbrauch oder andere Erkrankungen verursacht

Tabelle 2: Diagnostische Kriterien der PTBS nach ICD-10 und DSM-5

Pathophysiologie der PTBS

Das biologische Verständnis der Pathogenese der PTBS ist in den letzten Jahren insbesondere von Erkenntnissen aus der Genetik, des Neuroimaging und der Neuroendokrinologie geprägt worden. Das Neuroimaging und die Neuroendokrinologie werden in eigenen Kapiteln unter 5.1.3 und 5.1.4 genauer dargestellt. Bei der Pathogenese der PTBS kommt es zu einer komplexen Interaktion von Umweltfaktoren und Genetik[17, 18]. So konnte beispielsweise gezeigt werden, dass Patienten mit Kindheitstrauma ein höheres PTBS-Risiko aufweisen, wenn bestimmte Gen-Polymorphismen vorliegen[17, 19]. Ferner zeigten Zwillingsstudien, dass genetische Einflüsse zwischen 30 % und 70 % der Varianz der Symptomschwere erklären[20, 21].

Therapie der PTBS

Therapie der Wahl bei der PTBS ist die Psychotherapie. Die Art der Psychotherapie hängt vom Patientenwunsch und von der vorherrschenden Symptomatik ab[22, 23]. Beispielsweise werden bei starken Ängsten und Vermeidungsverhalten Expositionstechniken empfohlen, bei kognitiver Verzerrung und bei Schuldgefühlen eine kognitive Verhaltenstherapie[22, 23].
Bei mangelhaftem Ansprechen auf die Psychotherapie kann augmentativ eine Pharmakotherapie z. B. mit selektiven Serotonin-Wiederaufnahmehemmern (SSRI) begonnen werden[22, 23].

6.1.3 Neuroimaging der PTBS und des leichten SHT

Neuroimaging der PTBS

Mit dem Neuroimaging und insbesondere mit Methoden der MRT konnten strukturelle Veränderungen im Gehirn bei der PTBS genauer untersucht werden. So zeigten Studien ein geringeres Volumen und eine geringere kortikale Dicke im anterioren Cingulum[24, 25], dem präfrontalen Cortex[25, 26] und dem Hippocampus[27, 28]. Zudem gibt es Hinweise auf einen inversen Zusammenhang zwischen kortikaler Dicke und der Schwere der PTBS-Symptomatik[29, 30]. Darüber hinaus konnte gezeigt werden, dass eine höhere kortikale Dicke mit einer besseren Resilienz und einem günstigeren Krankheitsverlauf bei der PTBS assoziiert ist[31, 32]. Weitere Studien belegten einen Zusammenhang zwischen der kortikalen Dicke im medialen präfrontalen Cortex und Emotionsregulation[33] sowie der Extinktion von Angst[34, 35]. Beides sind Mechanismen, die in der Pathogenese der PTBS eine wichtige Rolle spielen. Eine Hypothese zur Erklärung der strukturellen Veränderungen im zentralen Nervensystem (ZNS) bei der PTBS ist eine Stress-induzierte Reduktion der synaptischen Plastizität in limbischen Arealen, insbesondere im Hippocampus und dem anterioren Cingulum[36-40]. So ist am Mausmodell gut belegt, dass chronischer Stress zu einer Abnahme von dendritischen Spines und zu einem Verlust von Synapsen führt[38, 41, 42]. Eine weitere Studie konnte am Tiermodell zeigen, dass Stress-induzierter Verlust von grauer Substanz, der im MRT darstellbar ist, auf den Verlust von Dendriten zurückzuführen ist[43].

Neuroimaging des leichten SHT

Ein wichtiger Beitrag zum Verständnis der Pathophysiologie des leichten SHT war die Weiterentwicklung bildgebender Verfahren und insbesondere der MRT. Zwar lassen sich beim leichten SHT gelegentlich neuroradiologische Befunde wie Hämorrhagien, Ödeme oder zerebrale Kontusionen nachweisen[44]. Weitaus häufiger jedoch lassen sich beim leichten SHT trotz vorhandener Symptome keine strukturellen Auffälligkeiten im konventionellen CT oder MRT darstellen. Erst mit der Entwicklung von Verfahren wie der Diffusionstensorbildgebung (DTI) konnten die diskreten mikrostrukturellen Veränderungen der weißen Substanz nach leichtem SHT ausreichend sensitiv dargestellt werden[45, 46].

Analog können mikrostrukturelle Veränderungen der grauen Substanz erst seit der Entwicklung automatisierter, präziser Methoden der Volumetrie und der Messung der kortikalen Dicke nachgewiesen werden[46, 47]. Beim leichten SHT gibt es zahlreiche Hinweise auf eine Beteiligung der grauen Substanz[46-48]. So wurde nach leichtem SHT ein erniedrigtes Volumen in subkortikalen Strukturen beschrieben, insbesondere im Thalamus, Hippocampus, der Amygdala und dem Putamen[49].

Ferner wurde gezeigt, dass beim leichten SHT das globale Volumen des Cortex erniedrigt ist und der precuneale Cortex ein reduziertes Volumen aufweist[47]. Die kortikale Dicke nach leichtem SHT wurde in unterschiedlichen Gehirnarealen als verändert beschrieben[48, 50-52]. In den bisherigen Studien wurde jedoch das akute (> 3 Monate) leichte SHT untersucht. Bislang war wenig darüber bekannt, inwiefern sich die die kortikale Dicke im Verlauf (> 1 Jahr) nach leichtem SHT verändert.

6.1.4 Endokrinologische Aspekte der PTBS und des leichten SHT: die Rolle der Neurosteroide

Endokrinologische Veränderungen bei der PTBS und dem leichten SHT

Neben den strukturellen Veränderungen im ZNS bei der PTBS und nach leichtem SHT gibt es auch zahlreiche Hinweise dafür, dass endokrinologische Veränderungen bei beiden Pathologien eine wichtige Rolle spielen[53-56].

So liegt bei ca. 10-25 % der Patienten mit leichtem SHT eine Hypophysenunterfunktion vor[57]. Zudem ist bekannt, dass chronischer Stress zu einer Dysregulation der Hypothalamus- Hypophysen- Nebennierenrinden- Achse (Hypothalamic-pituitary-adrenal axis, HPA- Achse) führt[41, 58]. In der Folge kommt es zu einer erhöhten Konzentration von Corticotropin-Releasing-Hormone (CRH)[56, 58], zu erhöhten Konzentrationen von Katecholaminen in präfrontalen Arealen[59] sowie zu erniedrigten Konzentrationen von

Neurosteroiden[60-62]. Des Weiteren kommt es bei chronischem Stress zu einer Hochregulierung der Anzahl von Glukokortikoid-Rezeptoren und einer höheren Glukokortikoid-Sensitivität[63]. Diese komplexen endokrinologischen Veränderungen führen gemeinsam mit anderen stress-induzierten Mechanismen wie Neuroinflammation[62] und einem gesteigertem Glutamat-Stoffwechsel[64] zu einer reduzierten synaptischen Plastizität[37, 41, 58] und damit zur neuronalen Atrophie, wie man sie bei der PTBS findet (siehe Abb. 1). Ferner unterliegen diese Mechanismen Geschlechtsunterschieden. Bei Frauen konnte eine stärkere Stress-Reaktivität der HPA-Achse nachgewiesen werden[65, 66]. Zudem ist bekannt, dass Estradiol eine stimulierende Wirkung auf die HPA-Achse hat[67, 68], während Androgene eine inhibitorische Wirkung auf die HPA-Achse haben[69, 70].

Die Rolle der Neurosteroide bei der PTBS und leichtem SHT

Ein wichtiger Teil dieser hormonellen Regelkreise sind Neurosteroide. Neurosteroide sind endogene Moleküle, welche an den Gamma-Aminobuttersäure-A- ($GABA_A$) Rezeptor binden[71-73] und neurotrophe[74-76], anti-inflammatorische[77, 78], anti-apoptotische[79, 80], anxiolytische[81, 82] und antidepressive[71, 83] Effekte haben. Das Neurosteroid Allopregnanolon (ALLO) und dessen Vorläufermolekül Pregnenolon (PREGNE) spielen insbesondere eine zentrale Rolle bei der Stress-Antwort. Bei akutem Stress wird die ALLO-Synthese hochreguliert und ALLO senkt die Stress-Reaktivität über eine Inhibition der HPA-Achse über $GABA_A$-Rezeptoren[84-86]. Darüber kann ALLO möglicherweise der Stress-induzierten Atrophie in limbischen Arealen entgegenwirken (siehe Abb. 1). In einer präklinischen Studie konnte ferner gezeigt werden, dass diese Stress-induzierte Synthese von ALLO und PREGNE bei weiblichen Mäusen höher ist als bei männlichen[87]. Des Weiteren gibt es Hinweise auf eine positive Korrelation zwischen kortikaler Dicke und der Dichte von $GABA_A$-Rezeptoren[61, 88]. Bei chronischer Stress-Exposition kommt es jedoch zu einer Abnahme der ALLO-Konzentration im ZNS[61, 85, 86]. Dies ist möglicherweise auf eine verringerte ALLO-Synthese oder eine vermehrte Konversion von ALLO zu anderen Metaboliten zurückzuführen[89, 90]. Ferner zeigten Studien am Mausmodell, dass es einen Zusammenhang zwischen der Stress-induzierten Abnahme der ALLO-Konzentration in limbischen Regionen und einer Abnahme der Expression von brain-derived neurotrophic factor (BDNF) in denselben Regionen gibt[91-93]. Dies unterstützt die Hypothese, dass ALLO an der Regulierung von Neurotrophinen beteiligt ist und damit das Wachstum und die Regeneration von Neuronen fördert.

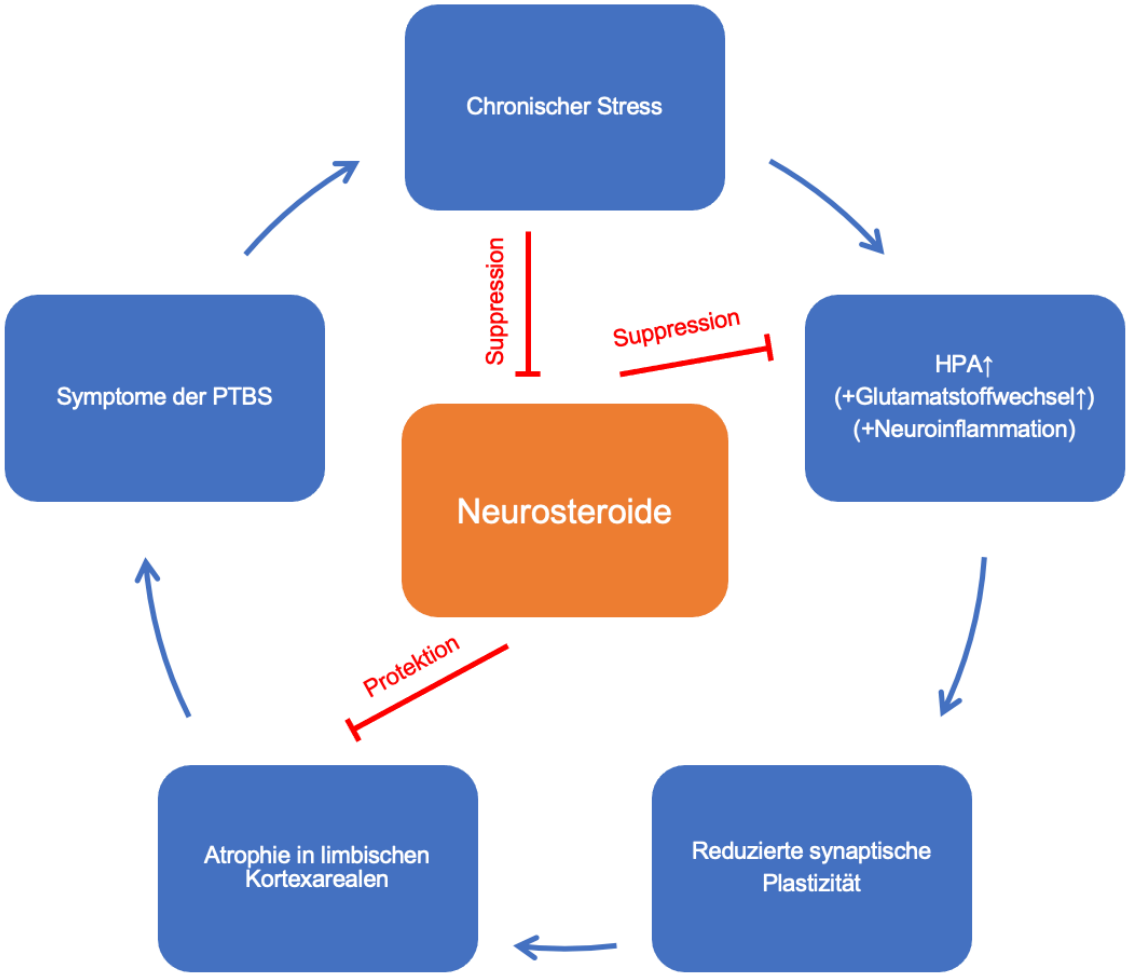

Abb. 1: Darstellung der pathophysiologischen Kaskade bei chronischem Stress sowie der Rolle der Neurosteroide (Abbildung modifiziert nach [94]).

Dass ALLO nicht nur bei Stress, sondern auch nach physischem Gehirntrauma protektiv wirkt, konnte am Tiermodel gezeigt werden. So verhindert ALLO neuronalen Zelltod[79, 95], Neuroinflammation[77] und die Bildung von Gliosen[79] nach leichtem SHT und verbessert den kognitiv-funktionellen Verlauf[79, 95] nach dem Trauma. Zudem verringert ALLO im Mausmodell das Infarktvolumen nach einem Schlaganfall[96].

Auch in der klinischen Forschung konnte gezeigt werden, dass ALLO bei der PTBS und bei leichtem SHT eine wichtige Rolle spielt. So ist bekannt, dass ALLO bei der PTBS[89, 97] und auch bei der Depression[98] im Liquor cerebrospinalis (Cerebrospinal fluid, CSF) erniedrigt ist und mit der Schwere von PTBS-Symptomen invers korreliert[99]. Auch konnte gezeigt werden, dass US-Veteranen mit leichtem SHT nach Explosionen im Vergleich zu Veteranen ohne SHT einen reduzierten Serumspiegel von Pregnanolon, einem Isomer von ALLO, aufwiesen[100]. 2019 wurde eine Pilot-Studie mit US- Veteranen veröffentlicht, welche einen Zusammenhang zwischen Neurosteroiden und kortikaler Dicke in multiplen Cortexregionen

zeigte. Es wurde jedoch nicht weiter danach differenziert, ob eine PTBS oder ein leichtes SHT vorlag[101].

6.1.5 Fragestellung

Trotz der bekannten neuroendokrinologischen Veränderungen bei der PTBS und dem leichten SHT und der belegten neuroprotektiven Eigenschaften von Neurosteroiden gibt es nach unseren Erkenntnissen bis heute keine Studien, welche den Zusammenhang zwischen Neurosteroid-Serumspiegeln und kortikaler Dicke spezifisch an Patienten mit PTBS und leichtem SHT, Patienten nur mit leichtem SHT, sowie gesunden Kontrollprobanden untersuchen. In Arbeit 1 untersuchten wir die Hypothesen, dass
1) die kortikale Dicke in der PTBS+SHT-Gruppe im Vergleich zu den anderen Gruppen reduziert ist,
2) in der PTBS+SHT-Gruppe die kortikale Dicke positiv mit Neurosteroid- Serumspiegeln korreliert und
3) die kortikale Dicke in diesen Arealen invers mit der PTBS-Symptomschwere korreliert.

6.2 Arbeit 1- Methodische Grundlagen: Analyse der kortikalen Dicke

Die kortikale Dicke wird definiert als der Abstand zwischen der Cortexoberfläche und der Grenze zwischen Cortex und weißer Substanz. Bei Gesunden beträgt die kortikale Dicke je nach Region zwischen 1,5mm und 4,5mm[102]. Eine pathologisch veränderte kortikale Dicke konnte nicht nur bei PTBS und leichtem SHT, sondern auch bei Morbus Alzheimer[103], Schizophrenie[104], Multipler Sklerose[105] sowie weiteren ZNS-Erkrankungen nachgewiesen werden. Die Messung der kortikalen Dicke in T1-gewichteten MRT-Scans erfolgte teilautomatisiert mit der Software Freesurfer[102, 106], welche eine höhere Reproduzierbarkeit hat als andere Methoden der Messung der kortikalen Dicke[107]. Die automatisierten Teilschritte, welche nach der Präprozessierung erfolgten, sollen im Folgenden kurz dargestellt werden. Zunächst erfolgte die Entfernung der Strukturen, die das Gehirn umgeben und anschließend [108] eine Segmentierung in weiße Substanz, graue Substanz und CSF anhand der Signalintensitäten (siehe Abb. 2).

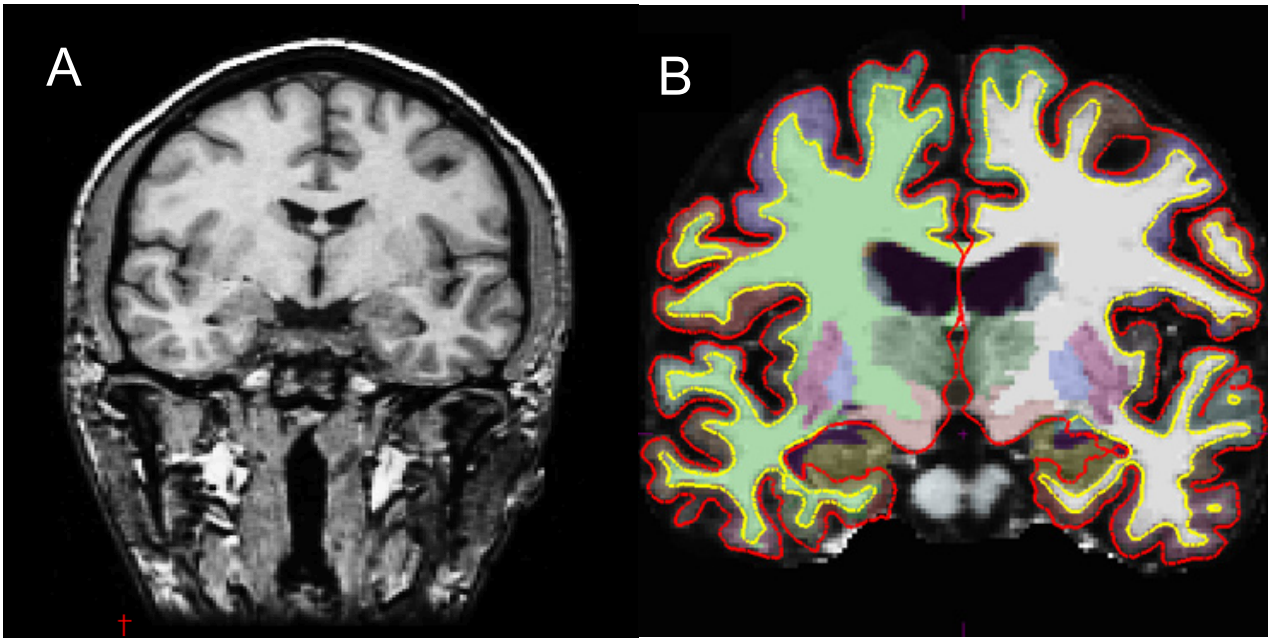

Abb. 2: Rekonstruktionsprozess mittels Freesurfer-Software. **(A)** T1-gewichtetes koronares Schnittbild. **(B)** Schnittbild nach Entfernung des Schädels und Segmentierung der grauen und weißen Substanz (Abbildung modifiziert nach [109]).

Um eine bessere Vergleichbarkeit der verschieden großen Gehirne zu erreichen, wurden die Scans in ein standardisiertes, gemeinsames Koordinatensystem projiziert[110, 111]. Die anatomische Einteilung der Gyri und Sulci erfolgte entsprechend dem Desikan-Kiliany-Atlas[112]. Die Flächen über und unter dem Cortex werden von Freesurfer als ein Netz aus Dreiecken dargestellt und die Eckpunkte zwischen den Dreiecken werden als Vertex bezeichnet. Für jeden Vertex wurde die kortikale Dicke als die kürzeste Distanz zwischen der Grenze zwischen weißer und grauer Substanz einerseits und der Grenze zwischen Pia mater und CSF andererseits berechnet (siehe Abb. 3).

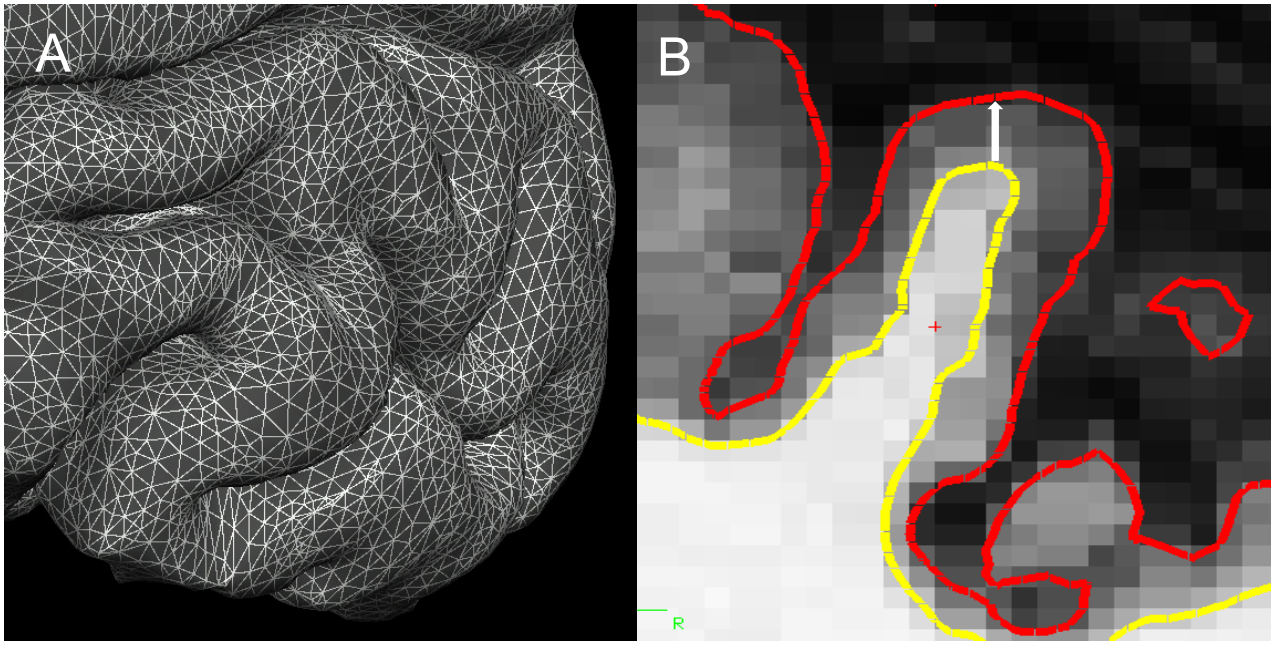

Abb. 3: Berechnung der kortikalen Dicke mit Freesurfer. **(A)** Modellierung der kortikalen Oberfläche durch Dreiecke und Vertices. (B) Berechnung der kortikalen Dicke (weißer Pfeil) an jedem Vertex (Abbildung modifiziert nach [109]).

Die statistische Auswertung der kortikalen Dicke in Bezug auf Gruppenunterschiede und Korrelationen mit Neurosteroid-Serumspiegeln erfolgte mit der Software QDEC, welche in Freesurfer integriert ist[113]. Die automatischen Berechnungen von QDEC beruhen auf dem Allgemeinen linearen Modell (General linear model, GLM). Im GLM wird die kortikale Dicke für jeden Vertex als Teil einer linearen Gleichung bzw. einer Geradengleichung dargestellt:

$$Y = \beta 0 + \beta 1 * X + \varepsilon$$

Y ist die gemessene kortikale Dicke und entspricht der abhängigen Variablen. β1 ist die unabhängige Variable bzw. der Regressor, welcher in dem Modell die abhängige Variable vorhersagen soll (also z.B. Neurosteroid-Spiegel oder Alter). β1 würde bei einer Geraden der Steigung entsprechen. β0 ist eine Konstante und derjenige Y-Achsen-Abschnitt, welcher sich ergibt, wenn X den Wert Null hat. ε ist der Vorhersagefehler, welcher sich aus der Differenz zwischen Schätzwert und tatsächlichem Messwert ergibt. Da im GLM auch mehrere unabhängige Variablen vorkommen können, werden diese durch die Regressionskoeffizienten gewichtet. So bekommen Variablen, welche den Y-Wert besser vorhersagen, einen höheren Regressionskoeffizienten und damit eine stärkere Gewichtung im Vergleich zu Variablen, welche den Wert schlechter vorhersagen. Mithilfe des GLM wird die abhängige Variable also als gewichtete Kombination von unabhängigen Variablen dargestellt. Zum Zwecke der einfacheren Berechnung wird das GLM von QDEC in Form von Matrizen und Vektoren formuliert. Zur Prüfung der verschiedenen Hypothesen wird von Freesurfer eine Kontrastmatrix erstellt, mit welcher die unabhängigen Variablen einzeln

getestet werden können, während die anderen unabhängigen Variablen als Störparameter in die Berechnung eingehen. Mit der Kontrastmatrix kann beispielsweise getestet werden, ob der Neurosteroid-Serumspiegel die kortikale Dicke im Modell voraussagt, während für Alter und Geschlecht korrigiert wird, und somit die Nullhypothese abgelehnt werden kann. Da diese Tests für jeden Vertex durchgeführt werden und somit die Wahrscheinlichkeit der Alphafehler-Kumulierung sehr groß ist, wird im Anschluss für multiples Testen korrigiert. Die Korrektur erfolgt mithilfe der Montecarlo-Clusterwise Simulation, nach welcher benachbarte Vertices nur ab einer bestimmten Clustergröße als signifikant angezeigt werden[114]. Die signifikanten Cluster werden anschließend farbkodiert auf einem standardisierten, geglätteten Gehirnmodell visualisiert.

6.3 Arbeit 1- Eigenanteil

Mein Beitrag zu Arbeit 1 setzt sich aus folgenden Anteilen zusammen:
Literaturrecherche und Aufstellung der Arbeitshypothesen, Auswahl der untersuchten Probanden mit vollständigen erhobenen Datensätzen, Zusammenfügen mehrerer Datensätze in einen Datensatz, Auswertung der T1 gewichteten Daten und anschließende statistische Analyse der Daten, Verfassen des Manuskriptes der Publikation. An Arbeit 1 beteiligte ich mich in geteilter Erstautorenschaft gemeinsam mit Frau Prof. Christine Marx. Prof. Marx war als Principal Investigator für die Erhebung und Analyse der Neurosteroid-Serum- Spiegel verantwortlich und arbeitete an der Verfassung des Manuskriptes mit.

6.4 Arbeit 2- Klinischer und wissenschaftlicher Hintergrund

Strukturelle Veränderungen im Gehirn sind nicht nur im Zusammenhang mit leichtem SHT bekannt, sondern in subtilerer Form auch nach RSHI[45]. RSHI treten am häufigsten bei militärischem Personal sowie im Kontext von Kontaktsportarten wie American Football oder Eishockey, aber auch beim Fußball auf. Bei den RSHI kommt es zu ähnlichen degenerativen Veränderungen der Neurone wie nach leichten SHT, jedoch ohne die akute klinische Symptomatik[115]. Dass RSHI dennoch die Hirnfunktion beeinträchtigen, zeigten Studien, welche kognitive Einschränkungen bei Fußball- und American Football-Spielern ohne Gehirnerschütterung in der Vorgeschichte feststellten[116, 117]. Mit der DTI stand erstmals eine Methode zur Verfügung, die sensitiv genug war, um auch strukturelle Veränderungen im Gehirn nach RSHI darzustellen[118, 119]. Somit konnten Veränderungen in der weißen

Substanz nach RSHI bei Fußballspielern[118] und bei American Football- und Hockeyspielern[119] ohne SHT in ihrer Vorgeschichte nachgewiesen werden. Weitgehend unerforscht ist die Frage, ob es Geschlechtsunterschiede in der Pathophysiologie der RSHI gibt. Beim Sport-assoziierten leichten SHT ist bereits bekannt, dass Frauen ein höheres Risiko aufweisen, ein leichtes SHT zu erleiden[120, 121] und zudem einen schlechteren klinischen Verlauf haben als Männer[122-125]. Insbesondere zeigen Frauen eine stärkere Akutsymptomatik[126], eine längere Persistenz der Symptome[126, 127] und eine stärkere kognitive Beeinträchtigung[123, 128] im Vergleich zu Männern. Eine mögliche Ursache für die Geschlechtsunterschiede im klinischen Verlauf ist der Einfluss von neuroprotektiven Neurosteroiden wir Progesteron und Östrogen[129, 130]. Diese weisen bei Männern und Frauen unterschiedliche Serum-Konzentrationen auf und fluktuieren bei Frauen in Abhängigkeit von Menstruationszyklus und Menopause. Ferner konnten auch mittels Neuroimaging Geschlechtsunterschiede nach leichtem SHT festgestellt werden. In einer ersten DTI-Studie zeigten Männer eine erniedrigte fraktionale Anisotropie (FA) im Fasciculus uncinatus im Vergleich zu Frauen[131]. Bisher gab es keine Studien über Geschlechtsunterschiede bei Veränderungen der weißen Substanz nach RSHI. Dies soll in der folgenden Arbeit untersucht werden. Wenn wir die den Geschlechtsunterschieden zugrundeliegenden Faktoren besser verstehen, können wir langfristig dazu beitragen, Risikofaktoren zu identifizieren.

6.5 Arbeit 2- Methodische Grundlagen

6.5.1 Diffusionstensorbildgebung (DTI)

Die DTI ist eine radiologische Methode zur Beurteilung mikrostruktureller Veränderungen der weißen Substanz. Physikalische Grundlage der Methode ist die Brownsche Molekularbewegung, mit welcher die zufällige, temperaturabhängige Bewegung von Teilchen in Flüssigkeiten und Gasen beschrieben wird. Das Phänomen wurde bereits 1827 von Robert Brown beobachtet, jedoch erst 1905 durch Albert Einstein und 1906 durch Marian Smoluchowski unabhängig voneinander physikalisch begründet. Die Brownsche Bewegung gilt auch bei der Diffusion von Wassermolekülen. 1986 wurde von Le Bihan et al. die diffusionsgewichtete Bildgebung (Diffusion weighed imaging, DWI) entwickelt[132]. Die Methode beruht darauf, dass mithilfe von magnetischen Feldgradienten das MR- Signal empfindlich für die Bewegung von Wasser-Molekülen wird. Damit kann die Diffusion im Gewebe nicht invasiv und in vivo gemessen werden[133-135]. Je schneller sich die Teilchen

bewegen, desto mehr nimmt das Signal ab. Im Gehirn wird in den unterschiedlichen Kompartimenten die Diffusion z.B. durch Membranen oder Makromoleküle unterschiedlich stark eingeschränkt[136]. Dies erlaubt Rückschlüsse auf die Mikrostruktur des Gewebes. So stellen sich beispielsweise Areale mit geringerer Diffusion hyperintens dar. In der klinischen Praxis hat sich die DWI in der Schlaganfalldiagnostik etabliert. Bei der Ischämie kommt es durch die Ausbildung eines zytotoxischen Ödems zu einer Diffusionsstörung. Diese kann mit der DWI wesentlich früher dargestellt werden als mit dem konventionellen MRT[137]. Bei der DWI wird die Diffusion in jedem Voxel gemittelt für alle Richtungen als Apparent diffusion coefficient (ADC) dargestellt. Eine methodische Erweiterung der DWI gelang Basser et al. 1994 mit der DTI[138]. Mit der DTI kann zusätzlich die Richtung und die Strecke der Diffusion berücksichtigt werden. Bei der DTI wird die Diffusion mithilfe von magnetischen Feldgradienten in mindestens sechs Richtungen ermittelt. Anschließend wird für jeden Voxel ein Diffusionstensor berechnet, welcher die Diffusion dreidimensional mit Vektoren darstellt. Diese drei sogenannten Eigenvektoren ε_1, ε_2 und ε_3 definieren die Richtung der Diffusion. Die Längen der Vektoren werden als Eigenwerte λ_1, λ_2 und λ_3 bezeichnet und geben das Ausmaß der Diffusion in die jeweilige Richtung an. Der längste Vektor wird als λ_1 definiert, dieser gibt die Hauptdiffusionsrichtung an. Ist die Diffusion der Wassermoleküle in alle Richtungen gleichmäßig möglich, wird dies als Isotropie bezeichnet. Bei der Anisotropie hingegen ist die Diffusion aufgrund von Begrenzungen in der Umgebung nicht in alle Richtungen möglich. Isotropie findet sich beispielsweise in den Ventrikeln, Anisotropie in der weißen Substanz. Bei anisotropier Diffusion kann der Tensor als Ellipsoid visualisiert werden (siehe Abb. 4). Bei isotroper Diffusion nimmt dieser die Form einer Kugel an.

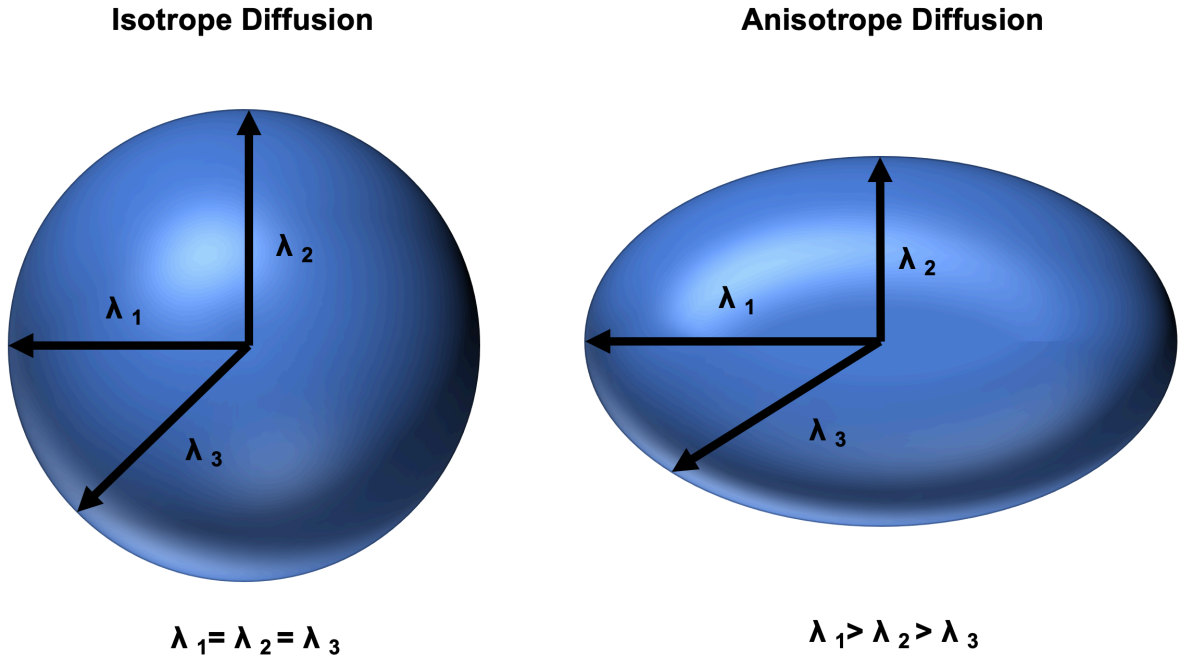

Abb. 4: Grafische Darstellung der isotropen und anisotropen Diffusion. Die Eigenvektoren ε_1, ε_2 und ε_3 geben die Richtung der Diffusion entlang von drei Achsen an. Die Eigenwerte λ_1, λ_2 und λ_3 entsprechen der Länge der Vektoren und geben das Ausmaß der Diffusion in die jeweilige Richtung an. Bei isotroper Diffusion gilt $\lambda_1 = \lambda_2 = \lambda_3$. Bei anisotroper Diffusion gilt $\lambda_1 > \lambda_2 > \lambda_3$.

Aus den Eigenwerten lassen sich weitere Paramter berechnen, welche die Diffusion genauer charakterisieren (siehe Tabelle 3). Zu diesen Parametern gehören die fraktionale Anisotropie (fractional anisotropy, FA), Trace, die mittlere Diffusivität (mean diffusivity, MD), die radiale Diffusivität (radial diffusivity, RD) und die axiale Diffusivität (axial diffusivity, AD).

AD	λ_1
RD	$(\lambda_2 + \lambda_3) / 2$
MD	$(\lambda_1 + \lambda_2 + \lambda_3) / 3$
Trace	$\lambda_1 + \lambda_2 + \lambda_3$
FA	$\sqrt{\frac{1}{2}}\frac{\sqrt{(\lambda_1-\lambda_2)^2+(\lambda_1-\lambda_3)^2+(\lambda_2-\lambda_3)^2}}{\sqrt{(\lambda_1^2+\lambda_2^2+\lambda_3^2)}}$

Tabelle 3: Berechnung der Parameter AD, RD, MD, Trace und FA.

FA ist ein Maß für die Verschiedenheit der Eigenwerte und damit für den Grad der Anisotropie[136, 139]. Damit ermöglicht die FA Rückschlüsse auf die Mikrostruktur des untersuchten Gewebes. In der weißen Substanz nähert sich die fraktionale Anisotropie dem

Wert 1 an, was einer stark anisotropen Diffusion entspricht. In den Ventrikeln liegen die Werte ungefähr bei 0, da dort isotrope Diffusionsverhältnisse herrschen. Trace und MD sind Indikatoren für das Gesamtmaß an Diffusion in einem Voxel[136, 139]. AD ist ein Maß für die Diffusion entlang dem Hauptvektor (und somit entlang der Fasertrakte) und ein Indikator für axonale Verletzungen[136, 139]. RD ist ein Maß für die Diffusion perpendikular zur Hauptdiffusionsrichtung und ein Indikator für die Integrität des Myelins[136, 139]. Die Interpretation der Parameter erlaubt Rückschlüsse auf die Mikrostruktur des Nervengewebes. Mit speziellen Softwares kann dies visualisiert werden. Beispielsweise können die einzelnen Diffusionsparameter analog zum konventionellen MRT auf Voxel-basierten Schnittbildern (sogenannten FA-, AD-, RD-, MD- Karten) dargestellt werden, in denen die Werte der Parameter bestimmten Graustufen entsprechen[134]. In weitergehenden Analysen können mit einer sogenannten Traktographie die Fasertrakte der weißen Substanz rekonstruiert werden. Bei spezifischen Fragestellungen können in sogenannten Region of Interest (ROI) -Analysen spezifische Gehirnregionen untersucht werden. Für die statistische Analyse und visuelle Darstellung von Gruppenvergleichen wird häufig die Methode der Tract-Based Spatial Statistics (TBSS) verwendet.

6.5.2 Tract-Based Spatial Statistics (TBSS)

Die TBSS ist eine statistische Methode, mit welcher Voxel-basiert Gruppenvergleiche sowie Korrelations- und Regressionsanalysen von DTI-Daten durchgeführt werden können. Die TBSS wird mit der Software FMRIB Software Library (FSL) durchgeführt[140]. Die Analysen beinhalten mehrere komplexe, teilautomatisierte Schritte, welche auf der Internetseite der FSL im Detail beschrieben werden[141]. In einem ersten Schritt werden die einzelnen FA-Karten der Probanden an einer standardisierten Muster-FA-Karte ausgerichtet und in ein gemeinsames Koordinatensystem transformiert. Dieser Prozess wird als Registrierung bezeichnet. Anschließend wird aus allen FA-Karten eine FA-Mittelwertkarte berechnet. Aus der FA-Mittelwertkarte wird ein FA-Skelett erstellt, welches die Voxel mit den höchsten FA-Werten beinhaltet. Da in den Zentren der Fasertrakte die Dichte an parallel verlaufenden Axonen am größten ist und damit die FA-Werte am höchsten sind, repräsentiert das Skelett die Zentren der großen Fasertrakte. Aufgrund der großen Variabilität der kleineren Fasertrakte werden diese durch Festlegung eines FA-Schwellenwertes aus dem FA Skelett ausgeschlossen. Die Schwellenwerte liegen in der Regel zwischen 0,2 und 0,3. Nun können die registrierten FA-Datensätze auf das FA-Skelett projiziert werden, sodass für alle Probanden jene Voxel extrahiert werden, die Teil des Skeletts sind[142]. Die entstehenden

Daten werden als skelettierte FA-Karten bezeichnet und können nun für die weitere statistische Analyse verwendet werden. Beispielsweise können für jedes Voxel der skelettierten FA-Karte Gruppenvergleiche durchgeführt werden, deren Signifikanz mit Permutationstests geprüft wird[143]. Anschließend können signifikante Cluster visualisiert werden. Analog zu den FA- Daten können basierend auf dem FA-Skelett auch skelettierte AD-, MD-, RD-Datensätze erstellt werden und ebenfalls statistisch analysiert werden. In unserer Studie beinhaltete die Analyse den Vergleich beider Geschlechter in Bezug auf die Veränderung der Diffusionsparameter im Verlauf der Eishockey-Saison.

6.6 Arbeit 2- Eigenanteil

Mein Beitrag zu Arbeit 2 bestand in der Auswertung und statistischen Analyse der Imaging-Daten und der Mitarbeit am Verfassen des Manuskriptes.

7 Zusammenfassung Arbeit 1

7.1 Methoden

In diese Studie wurden 141 Probanden eingeschlossen, die in folgende Gruppen aufgeteilt wurden: eine SHT-Gruppe mit Probanden, welche die Anamnese eines leichten SHT aufwiesen (n= 32, davon 10 Frauen und 22 Männer); eine PTBS+SHT- Gruppe mit Probanden, welche sowohl eine aktuelle PTBS als auch eine Anamnese eines leichten SHT aufwiesen (n= 41, davon 6 Frauen und 35 Männer); sowie eine Kontroll-Gruppe mit gesunden Kontroll-Probanden (n= 68, davon 35 Frauen und 33 Männer; siehe Abb. 5). Probanden, welche nur eine PTBS hatten, wurden aufgrund der geringen Anzahl (n= 6) nicht in unsere Studie eingeschlossen.

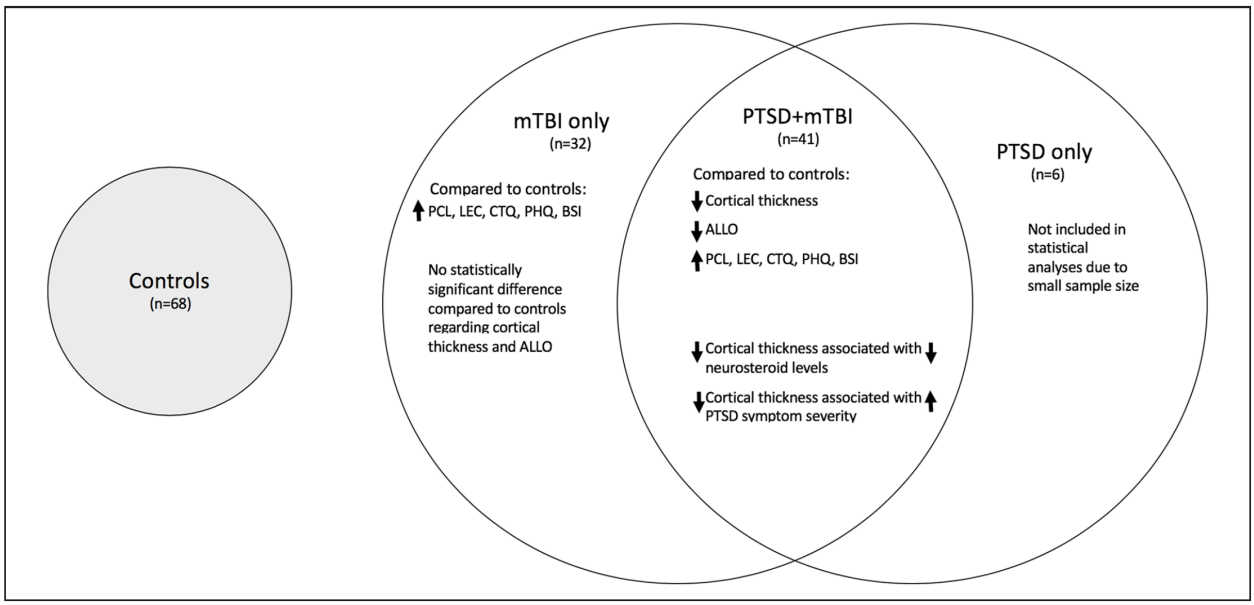

Abb. 5: Unterteilung der Probanden in vier Gruppen (Abbildung aus Arbeit 1).

Die Datenerhebung erfolgte durch das Injury and Traumatic Stress (INTRuST) Clinical Consortium zwischen 2008 und 2013 und wurde durch das US-amerikanische Verteidigungsministerium gefördert. Die sechs beteiligten Zentren waren die Dartmouth University, die Duke University, die University of South Carolina, die Harvard Medical School, die University of California, San Diego und die University of Cincinnati. Bei allen Probanden wurde eine umfassende neurokognitive Testung und eine Untersuchung der PTBS-Symptomschwere mit der PTSD-Checklist (PCL-C)[144] durchgeführt. Des Weiteren wurde bei allen Probanden eine kraniale T1- gewichtete MR-Bildgebung auf 3T-MR-Tomographen (GE 750, General Electric, Chicago, USA; Achieva, Philips Healthcare, Best, Niederlande; Tim Trio, Siemens Healthineers, Erlangen, Deutschland) durchgeführt. Zudem erfolgte eine Quantifizierung der Neurosteroide ALLO und PREGNE im Serum. Die Analyse der kortikalen Dicke erfolgte mit der Freesurfer- Software und in Post-Hoc Analysen mit SPSS.

7.2 Ergebnisse

Reduzierte kortikale Dicke in der PTBS+SHT-Gruppe

In der PTSD+SHT-Gruppe zeigte sich eine signifikant erniedrigte kortikale Dicke in sieben Clustern im Vergleich mit der Kontroll-Gruppe und in fünf Clustern im Vergleich mit der SHT-Gruppe. Außer einem okzipitalen Cluster lagen alle Cluster im frontalen und temporalen Cortex (siehe Abb. 6).

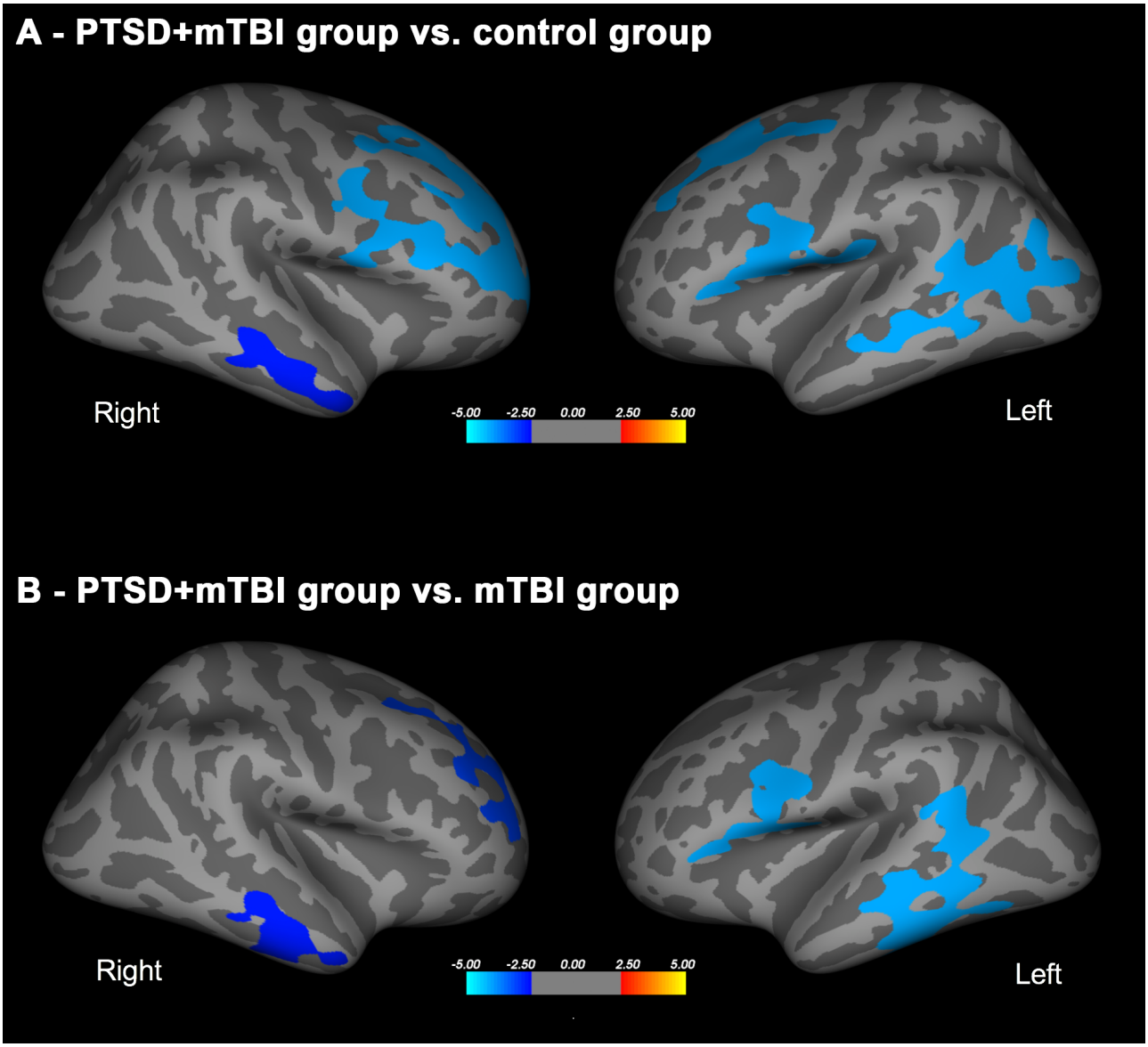

Abb. 6: (A) Erniedrigte kortikale Dicke in der PTBS+SHT-Gruppe im Vergleich zur Kontroll-Gruppe im rechten superioren frontalen Cortex, im rechten medialen temporalen Cortex, im linken medialen temporalen Cortex, im linken caudalen medialen frontalen Cortex, im linken inferioren frontalen Cortex, im linken fusiformen Cortex sowie im linken lateralen okzipitalen Cortex. **(B)** Erniedrigte kortikale Dicke in der PTBS+SHT-Gruppe im Vergleich zur SHT-Gruppe im rechten rostralen medialen frontalen Cortex, im rechten medialen temporalen Cortex, im linken inferioren frontalen Cortex, im linken lateralen okzipitalen Cortex sowie im linken inferioren temporalen Cortex. Die Farbkodierung stellt logarithmierte P-Werte dar (Abbildung adaptiert aus Arbeit 1).

Assoziation zwischen kortikaler Dicke und Neurosteroid- Serumspiegeln

Das zentrale Ergebnis dieser Studie ist eine positive Korrelation zwischen dem Serumspiegel von Neurosteroiden und der kortikalen Dicke. Signifikante Cluster lagen im rechten lateralen orbitofrontalen Cortex und im medialen temporalen Cortex für PREGNE sowie im rechten superioren frontalen Cortex für ALLO (siehe Abb. 7).

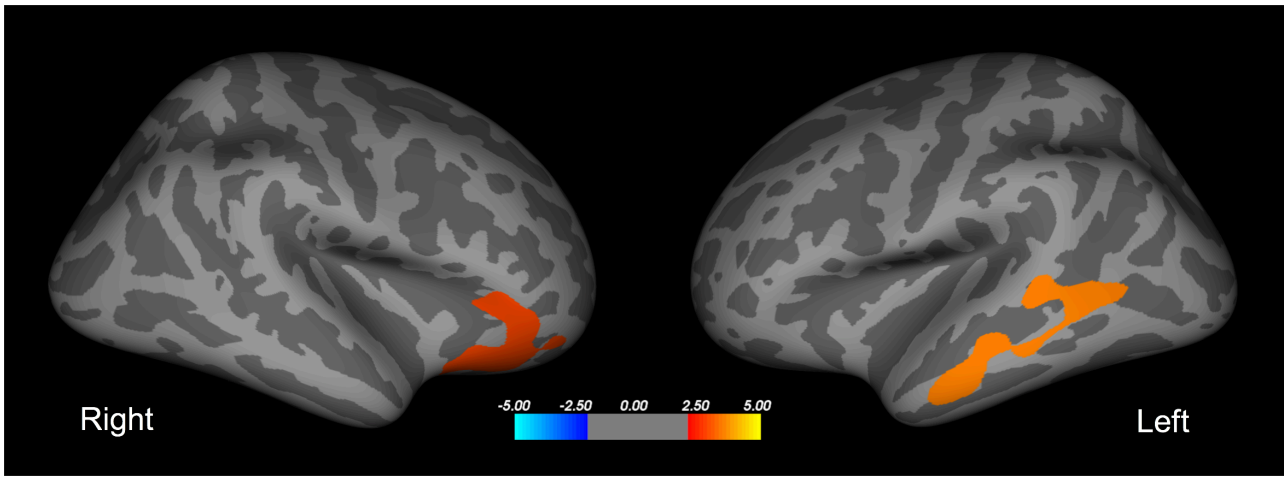

Abb. 7: (A) Signifikante positive Korrelation zwischen dem PREGN-Serumspiegel und der kortikalen Dicke im linken medialen temporalen Cortex und im rechten lateralen orbitofrontalen Cortex in der PTBS+SHT-Gruppe. Das signifikante Cluster für ALLO war zu klein, um visuell dargestellt zu werden. Die Farbkodierung stellt logarithmierte P-Werte dar (Abbildung adaptiert aus Arbeit 1).

Assoziation zwischen kortikaler Dicke und Symptomen der PTBS

In unserer letzten Analyse zeigten wir eine signifikante inverse Korrelation zwischen kortikaler Dicke und der Schwere von PTBS-Symptomen. Die signifikanten Cluster lagen im linken medialen temporalen Cortex (R= -0,368, p= 0,023) und dem rechten orbitofrontalen Cortex (R= -0,364, p= 0,025; siehe Abb. 8).

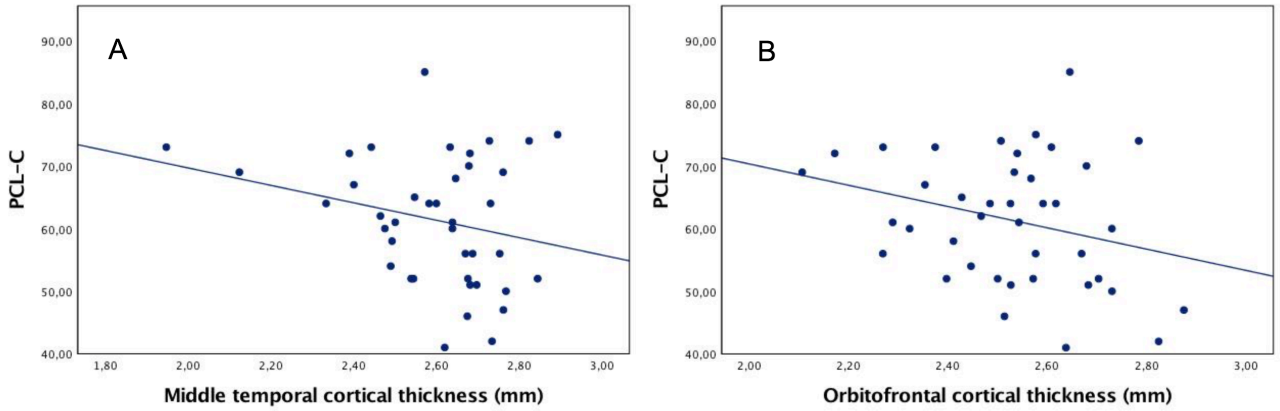

Abb. 8: Diese Streudiagramme zeigen eine inverse Korrelation zwischen PTBS-Symptomschwere nach PCL-C-Score und der kortikalen Dicke im medialen temporalen Cortex **(A)** sowie im orbitofrontalen Cortex **(B)** (Abbildung adaptiert aus Arbeit 1).

7.3 Diskussion und Schlussfolgerung

In der vorliegenden Publikation untersuchten wir die Assoziation zwischen dem Serumspiegel von Neurosteroiden und der kortikalen Dicke mit Methoden der strukturellen MRT. Die drei Hauptergebnisse dieser Studie sollen im Folgenden diskutiert werden.

Reduzierte kortikale Dicke in der PTBS+SHT- Gruppe

Die Patienten in der PTBS+SHT-Gruppe wiesen eine reduzierte kortikale Dicke im Vergleich zur Kontroll- Gruppe sowie im Vergleich zur SHT-Gruppe auf. Eine Reduktion der kortikalen Dicke ist jeweils bei der PTBS und beim leichten SHT im Vergleich zu Gesunden bereits gut belegt[25, 48, 52, 145]. Der Vergleich der kortikalen Dicke zwischen PTBS+SHT und SHT wurde zwar in der bisherigen Forschung kaum untersucht, jedoch gibt es auch hierfür mehrere Erklärungsansätze. Möglicherweise entsteht eine PTBS insbesondere bei jenen Patienten mit leichten SHT, bei denen das SHT den frontalen Cortex betrifft, denn der frontale Cortex spielt eine zentrale Rolle in der Pathogenese der PTBS. Ferner gibt es Studien, die zeigen, dass insbesondere leichte SHT nach Exposition gegenüber Explosionen mit einer reduzierten kortikalen Dicke im frontalen Cortex assoziiert sind[50, 146, 147]. Weiterhin konnte ein Zusammenhang zwischen der kortikalen Dicke im frontalen Cortex und der PTBS-Symptomschwere nachgewiesen werden[50]. Dennoch ist es nach aktuellem Stand unklar, ob biomechanische Verletzungen des frontalen Cortex zu PTBS-Symptomen führen können. Eine wahrscheinlichere Erklärung für die Unterschiede zwischen der PTBS+SHT-Gruppe und der SHT-Gruppe sind additive Effekte in der PTBS+SHT-Gruppe, da beide Pathologien die kortikale Dicke betreffen. Studien zeigten, dass Patienten mit PTBS und leichtem SHT einen stärkeren Zusammenhang zwischen kortikaler Dicke und PTBS-Symptomen zeigten als Patienten, die nur eine PTBS aufwiesen[29]. Dies zeigt, dass das Gehirn nach einem leichten SHT möglicherweise empfindlicher auf Stress reagiert. Die PTBS wiederum gilt als wichtigster Risikofaktor für persistierende Symptome nach einem leichten SHT[10]. Diese Studien deuten darauf hin, dass der Unterschied zwischen der PTBS+SHT-Gruppe und der SHT-Gruppe möglicherweise auf additive Effekte zurückzuführen ist.

Assoziation zwischen kortikaler Dicke und Neurosteroid-Serumspiegeln

Hauptergebnis dieser Studie ist die positive Korrelation zwischen dem Serumspiegel von Neurosteroiden und der kortikalen Dicke bei Patienten mit PTBS+SHT. Die signifikanten Cluster lagen im rechten lateralen orbitofrontalen Cortex und im linken medialen temporalen Cortex für PREGNE sowie im rechten superioren frontalen Cortex für ALLO. In früheren Studien wurde bereits gezeigt, dass in diesen Regionen die kortikale Dicke und das kortikale Volumen bei der PTBS verändert sind. Diese Veränderungen der grauen Substanz wurden insbesondere für das anteriore Cingulum beschrieben (Anterior cingulate cortex, ACC)[24, 25], welches ein Teil des superioren frontalen Cortex ist. Das ACC kontrolliert zudem unter Stressbedingungen die Aktivität der HPA-Achse[148]. Aktuell wird angenommen, dass die Stress-induzierten Veränderungen in limbischen Regionen wie dem ACC oder Hippocampus insbesondere durch eine reduzierte synaptische Plastizität verursacht werden[37, 39, 43]. Neurosteroide haben neurotrophe[74, 91], neuroprotektive[74, 75] und antiinflammatorische[74, 77] Eigenschaften und hemmen zudem die Aktivität der HPA-Achse[56, 149]. Über diese Effekte wirken sich Neurosteroide positiv auf synaptische Plastizität und auf die Integrität der grauen Substanz aus. Dies könnte eine mögliche Erklärung für den Zusammenhang zwischen dem Neurosteroid- Serumspiegel und der Dicke des Cortex sein. Der protektive Effekt von Neurosteroiden wurde auch in funktionellen MRT-Studien belegt. So hemmt ALLO die Aktivität in limbischen Regionen, welche an der Entstehung negativer Emotionen beteiligt sind[150, 151]. Dadurch verbessert ALLO die Emotionsregulation, welche auch bei der PTBS beeinträchtigt ist.

Assoziation zwischen kortikaler Dicke und Symptomen der PTBS

Unser drittes Ergebnis ist eine inverse Korrelation zwischen der PTBS-Symptomschwere nach PCL-C-Score und der kortikalen Dicke im medialen temporalen Cortex und im rechten orbitofrontalen Cortex. Diese Korrelation ist bereits in früheren Studien belegt worden[26, 29, 152]. Weitere Studien zeigten, dass der mediale temporale Cortex eine zentrale Rolle bei der Entstehung kognitiver Defizite bei der PTBS spielt[153, 154].

Schlussfolgerung

Zusammenfassend zeigt diese Studie eine Assoziation zwischen dem Serumspiegel von Neurosteroiden und der kortikalen Dicke bei Patienten mit PTBS und leichtem SHT. Möglicherweise deutet dies auf einen protektiven Effekt von Neurosteroiden bei der PTBS und beim leichten SHT hin, dennoch müssen in Zukunft longitudinale Studien durchgeführt werden, um diese Frage genauer beantworten zu können.

7.4 Originalarbeit 1:

Serum Neurosteroid Levels are Associated with Cortical Thickness in Individuals Diagnosed with Posttraumatic Stress Disorder and History of Mild Traumatic Brain Injury

Special Issue: The Legacy of Robert W McCarley for Multimodal Neuroimaging in Psychiatry

Clinical EEG and Neuroscience
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Serum Neurosteroid Levels Are Associated With Cortical Thickness in Individuals Diagnosed With Posttraumatic Stress Disorder and History of Mild Traumatic Brain Injury

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Abstract

Posttraumatic stress disorder (PTSD) co-occurring with mild traumatic brain injury (mTBI) is common in veterans. Worse clinical outcome in those with PTSD has been associated with decreased serum neurosteroid levels. Furthermore, decreased cortical thickness has been associated with both PTSD and mTBI. However, it is not known whether decreased neurosteroids are associated with decreased cortical thickness in PTSD co-occurring with mTBI. This study included 141 individuals divided into the following groups: (*a*) mTBI group (n = 32 [10 female, 22 male] veterans with a history of mTBI); (*b*) PTSD + mTBI group (n = 41 [6 female, 35 male] veterans with current PTSD with a history of mTBI); and (*c*) control group (n = 68 [35 female, 33 male] control participants), which were acquired through the Injury and Traumatic Stress (INTRuST) Clinical Consortium. Subjects underwent clinical assessment, magnetic resonance imaging at 3 T, and serum neurosteroid quantifications of allopregnanolone (ALLO) and pregnenolone (PREGN). Group differences in cortical thickness and associations between serum neurosteroid levels and cortical thickness were investigated. Cortical thickness was decreased in the PTSD + mTBI group compared with the other groups. In the PTSD + mTBI group, decreased cortical thickness was also associated with lower serum ALLO (right superior frontal cortex) and lower serum PREGN (left middle temporal and right orbitofrontal cortex). Cortical thickness in the middle temporal and orbitofrontal cortex was associated with PTSD symptom severity. There were no significant associations between neurosteroids and cortical thickness in the mTBI or control groups. Decreased cortical thickness in individuals with PTSD + mTBI is associated with decreased serum neurosteroid levels and greater PTSD symptom severity. Causality is unclear. However, future studies might investigate whether treatment with neurosteroids could counteract stress-induced neural atrophy in PTSD + mTBI by potentially preserving cortical thickness.

Keywords

cortical thickness, mild traumatic brain injury, neurosteroids, posttraumatic stress disorder

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Introduction

Posttraumatic stress disorder (PTSD) and mild traumatic brain injury (mTBI) are among the most common injuries in US military personnel involved in the most recent conflicts.[1,2] PTSD has an estimated prevalence of 7% in the general population[3] but shows a prevalence of up to 20% in military populations.[4] Similarly, mTBI has an estimated incidence of about 1% in the general population,[5] but up to 15% in military personnel.[6] Importantly, there is a high co-occurrence of PTSD and mTBI in military populations.[6-10] This may be due to a shared etiology as the same incident can lead to both emotional trauma and physical brain injury. Of further note, mTBI has been shown to increase the risk for

developing PTSD.[6] In turn, PTSD has also been shown to be the strongest risk factor associated with persistent symptoms following mTBI,[11] suggesting additive effects of PTSD and mTBI on brain structure and function. Nonetheless, despite the known high co-occurrence, the high likelihood of a shared etiology, as well as the potential additive effects of PTSD and mTBI particularly in veterans, the underlying pathological mechanisms remain unknown.

Investigations of brain structure using advanced neuroimaging techniques have demonstrated alterations in brain structure in both PTSD and mTBI. For example, gray matter (GM) has been investigated using both volume and cortical measures in both patients with PTSD and mTBI. In PTSD, lower GM volume and smaller cortical thickness have been consistently reported in the anterior cingulate cortex (ACC),[12-16] prefrontal cortex,[12,16,17] and hippocampus.[18-20] Furthermore, there is evidence that cortical thickness is correlated with PTSD symptom severity.[12,21-23] Additionally, the thicker the cortex, the better resilience and recovery from PTSD symptoms in those diagnosed with PTSD.[24-26]

In mTBI, there is also evidence of widespread GM alterations[27] as well as global brain volume and precuneal GM volume decreases at 1-year postinjury follow-up.[28] Furthermore, decreased volume of subcortical GM structures, including the thalamus, hippocampus, amygdala, and putamen, has been reported following mTBI.[29] Alterations in cortical thickness have also been observed following mTBI.[30-32] However, to date, most studies have focused on patients with either mTBI or PTSD. Studies on subjects suffering from co-occurrence of both conditions are still lacking.

In addition to alterations in brain structure observed in PTSD or mTBI, recent evidence highlights the role of endocrine changes in both disorders.[33,34] Chronic stress is known to lead to a dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis, which includes altered neurosteroid and glucocorticoid levels[35-38] and increased levels of catecholamines, particularly in the prefrontal cortex.[37,39] Neurosteroids are endogenous molecules that are known to have neurotrophic,[40,41] neuroprotective,[41,42] anti-inflammatory,[41,43,44] anxiolytic,[45,46] and antidepressant effects.[47,48] These effects may be mediated through a modulation of the gamma-aminobutyric acid-A ($GABA_A$) receptor.[49-51] Particularly allopregnanolone (ALLO), derived from its precursor pregnenolone (PREGN) via progesterone, is pivotal to the stress response. ALLO is upregulated following exposure to acute stress and it reduces acute stress reactivity by suppressing the HPA axis via $GABA_A$ receptors.[52-55] In contrast, ALLO decreases with chronic exposure to stress,[52-54,56] including cases of chronic PTSD.[57-59] This decrease in ALLO may represent an imbalance between inhibitory and excitatory neurosteroids in PTSD, possibly due to either an increased conversion of ALLO to inactive metabolites or a decreased conversion of precursors to ALLO.[57,58] Taken

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together, these findings suggest an important role of disrupted neuroendocrine circuits in the recovery from both, PTSD and mTBI, and, of note, there is evidence of an association between cortical thickness and GABA receptor density.[60,61] Although a neuroprotective role of neurosteroids in the context of both PTSD and mTBI and associations with cortical thickness seem likely, to date there have been no studies investigating the relationship between neurosteroid levels and the brain's GM in patients with PTSD and mTBI.

The aim of this study was to investigate the association between cortical thickness and serum neurosteroid levels of ALLO and PREGN in individuals with both PTSD and a history of mTBI, mTBI only, and healthy controls. We hypothesize that (1) serum levels of neurosteroids will be positively associated with cortical thickness in individuals with PTSD + mTBI and (2) cortical thickness will be inversely associated with PTSD symptom severity in individuals with PTSD + mTBI. Our goal is to understand the impact of neurosteroid dysregulation on cortical thickness in PTSD comorbid with mTBI.

Materials and Methods

Study Design and Participants

Study participants were part of the Injury and Traumatic Stress (INTRuST) Clinical Consortium, funded by the Department of Defense, with open enrollment from 2008 to 2013. The INTRuST Clinical Consortium consisted of 10 sites across the United States with the main goal to improve the understanding and treatment of PTSD and mTBI in military service personnel. All study procedures were carried out in accordance with the Declaration of Helsinki, and each site's respective institutional review board approved study procedures. Written informed consent was obtained from all study participants.

Out of the 771 participants enrolled in INTRuST, 426 underwent magnetic resonance imaging (MRI) in 6 out of the 10 INTRuST sites; 380 of these participants had scans that passed quality assessment (see section "Acquisition of Magnetic Resonance Imaging and Image Processing"). Of these, 147 subjects were included in the current study. The smaller number is accounted for by including only those with MRI scans who also had serum neurosteroid quantifications available and were also diagnosed with PTSD and/or history of mTBI, or were considered as healthy controls.

For subjects diagnosed with PTSD and/or history of mTBI, exclusion criteria were (1) lifetime bipolar I, psychotic, or dementia disorders, delirium, current alcohol or substance dependence (within 30 days); (2) serious disorders of the central nervous system (eg, aneurysms, anoxic events, brain tumors); (3) pregnancy/lactating; (4) current medications (other than psychotropic medications) that affect brain function; (5) English as a second language after the age of 5 years; (6) history of a learning disability; and (7) weight >300 pounds or other MRI-incompatible conditions (eg, metal in body). Due to the high prevalence of psychotropic medication in INTRuST patient populations, psychotropic medications were permitted, as well as a history of alcohol or substance abuse if in remission for the last 30 days before enrollment. Specific exclusion criteria for healthy controls were (1) serious disorders of the central nervous system; (2) medication exclusions, including more than 1 antihypertensive drug, psychotropic drugs within the past 90 days, herbal psychoactive substance use, or steroid use in the past 4 months; (3) pregnancy/lactating; (4) history of mood, anxiety, psychotic, dementia, delirium, or substance dependence in the past 12 months; (5) history of probable traumatic brain injury (TBI); and (6) MRI incompatibility.

The 147 subjects included in the current study were composed of 32 subjects diagnosed with a lifetime history of mTBI but no current diagnosis of PTSD (84.4% of subjects were veterans; mTBI group), 41 subjects diagnosed with current PTSD with a history of mTBI (100% of subjects were veterans, PTSD + mTBI group), and 68 healthy subjects (control group). Six subjects were diagnosed with PTSD only, and due to the small number, this group was not analyzed with respect to this study's hypotheses.

Diagnostic and Clinical Assessments

Subjects recruited for INTRuST were part of different cohorts and therefore PTSD diagnosis was determined using different tools. PTSD diagnosis was based on the Mini-International Neuropsychiatric Interview (MINI)[62] (n = 27), the PTSD Checklist–Civilian Version (PCL-C)[63] (n = 10), the Clinician Administered PTSD Scale (CAPS) for Diagnostic and Statistical Manual of Mental Disorders–5 (DSM-5)[64] (n = 9), or the Structured Clinical Interview for DSM-IV[65] (n = 1). History of mTBI was determined using the INTRuST TBI Screening Instrument, which is a 3-item, self-report questionnaire establishing mTBI on the basis of past head or brain injury with either (1) immediate loss of consciousness or alteration of consciousness or unawareness of the event or (2) amnesia before or after the event.

In addition, all study participants completed a set of questionnaires to acquire demographic information and to assess psychosocial, neurocognitive, and clinical measures. The questionnaires included the Rivermead Post-Concussion Questionnaire (from which the RPQ-3 and RPQ-13 were calculated),[66] the Alcohol Use Disorders Identification Test (AUDIT-10),[67] the Life Events Checklist (LEC),[68] the Childhood Trauma Questionnaire (CTQ),[69] the Patient Health Questionnaire (PHQ-9),[70] the Brief Symptom Inventory (BSI-18),[71] the Insomnia Severity Index (ISI),[72] and the Wide Range Achievement Test (WRAT-4).[73] PTSD symptom severity was measured using the PCL-C, which assesses the presence and severity of key PTSD symptoms with respect to the previous month.[63,74]

Neurosteroid Quantifications

Serum neurosteroid quantifications were performed by highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) preceded by high-performance liquid chromatography (HPLC) purification, as previously described.[75-78] Serum samples were frozen at −80°C for 6 to 42 months prior to

neurosteroid quantifications. One milliliter of serum was extracted 3 times in ethyl acetate prior to HPLC purification using tetrahydrofuran, ethanol, and hexane in the mobile phase on an 1100 Series Agilent instrument. Standards and samples were derivatized utilizing heptafluorobutyric acid anhydride (HFBA) and injected onto an Agilent 5973 MS coupled to an Agilent 6890 N GC equipped with an Agilent HP-5MS 30 m × 0.250 mm × 0.25 μm capillary column. Positive ion electron impact ionization was utilized in the GC/MS component with helium as the carrier gas. In addition to the GC/MS retention time characteristic of each neurosteroid, the definitive structural identification of each neurosteroid was provided by its unique mass fragmentation pattern. MS single ion monitoring was used to focus on the most abundant ion fragment for each HFBA derivative (ALLO 496.2, PREGN 298.2). A subset of serum samples (20%) was run in duplicate. Intra-assay coefficients of variation were 4.4% for ALLO and 2.0% for PREGN. Appropriate deuterated internal standards were utilized, specifically D4-allopregnanolone for ALLO and D4-pregnenolone for PREGN. For neurosteroid quantifications, the standard curve for the steroid of interest was prepared by combining varying known quantities of steroids (Steraloids) with a constant amount of deuterated internal standard. Identical to the experimental samples, each standard curve sample was extracted 3 times in ethyl acetate prior to HPLC purification and GC/MS injection; standard curve $r^2 = 0.99$ for each neurosteroid. The area under the peak of a known quantity of each steroid was divided by the area under the peak of the internal standard. This ratio was then plotted on the *y*-axis against known quantities of each steroid to generate the standard curve. Only peaks with a signal-to-noise ratio greater or equal to 5:1 were integrated. The limit of neurosteroid quantification with this methodology is 1 pg for ALLO and PREGN (femtomolar sensitivity).

Acquisition of Magnetic Resonance Imaging and Image Processing

The MRI scans were collected in 6 of the 10 INTRuST sites and were acquired on 3-T scanners: 2 GE 750 scanners (General Electric, Chicago, IL, USA), 2 Tim Trio scanners (Siemens Healthineers, Erlangen, Germany), and 2 Achieva scanners (Philips Healthcare, Best, The Netherlands). Among other sequences, a cranial 3-dimensional (3D) T1-weighted sequence was acquired with the sequence parameters shown in Table 1. Sequence parameters (specifically, flip angle and inversion time) were carefully tuned across vendors to provide the same GM/white matter (WM) contrast and were tested on agar phantoms and on 1 subject who was scanned at all sites.

The imaging data were preprocessed using scripts that were part of the in-house pipeline of the Psychiatry Neuroimaging Laboratory, Brigham and Women's Hospital, Harvard Medical School (https://github.com/pnlbwh/pnlutil/blob/master/pipeline/README.md). Data quality was visually checked using 3D Slicer (http://www.slicer.org; version 4.5, Surgical Planning Laboratory, Brigham and Women's Hospital, Boston, MA, USA).[79] Image acquisition parameters were automatically assessed for uniformity. Out of the 426 structural scans acquired for the INTRuST Clinical Consortium, 46 were excluded due to severe motion artifacts or the use of acquisition parameters that were not prescribed for INTRuST. All scans included in this study had no severe motion artifacts and all were completed using the prescribed acquisition parameters (Table 1).

Table 1. Acquisition Parameters of the T1-Weighted Sequences.[a]

	GE	Siemens	Philips
Sequence type	3D IR-SPGR	3D MPRAGE	3D TFE
Orientation	Sagittal	Sagittal	Sagittal
Flip angle (deg)	10	7	7
FOV (mm)	256	256	256
Bandwidth (kHz)	25.0	25.6	24.5
TE (ms)	3.7	3.3	3.5
TR (ms)	9150	2530	7600
Inversion time (ms)	600	1100	1100
Resolution matrix	256 × 256	256 × 256	256 × 256
Voxel size (mm^3)	1 × 1 × 1	1 × 1 × 1	1 × 1 × 1
Slices (n)	176	176	176
Time (min:s)	5:15	6:03	5:13

Abbreviations: 3D, 3-dimensional; FOV, field of view; TE, echo time; TR, repetition time.
[a]This table lists the sequence parameters for each of the 3-T scanners used at the different sites (GE 750, General Electric, Buckinghamshire, UK; Tim Trio, Siemens Healthineers, Erlangen, Germany; Achieva, Philips Healthcare, Best, The Netherlands).

Cortical Thickness Analysis

Brain segmentations and cortical thickness analyses were performed using FreeSurfer (http://surfer.nmr.mgh.harvard.edu; version 5.3, Laboratory for Computational Neuroimaging, Charlestown, MA, USA).[80] Preprocessing of imaging data included removal of non-brain tissue, automated Talairach transformation, grayscale intensity normalization, and correction for any inhomogeneities in the magnetic field, automated topology correction, and surface deformation correction according to intensity gradients in order to optimally delineate the GM/WM and GM/cerebrospinal fluid boundary.[81,82] Subsequent steps included surface inflation, registration to a common spherical atlas, and parcellation of the cortex with regard to sulcal and gyral patterns according to the Desikan-Killiany atlas.[83] Cortical thickness was computed as the closest distance between the GM/WM boundary and the pial surface at each vertex of the cortical mantle. Morphometric analyses in FreeSurfer have been shown to have good reliability across scanner manufacturers.[84] For improved contrast, cortical thickness measurements were smoothed using a default Gaussian kernel.

Statistical Analyses

SPSS (version 25.0, IBM SPSS Statistics for Windows, Armonk, NY, USA) was used to calculate descriptive statistics and to test for group differences in sociodemographic, psychosocial,

Table 2. Cohort Characteristics.[a]

	mTBI	mTBI + PTSD	Controls	PTSD	*P* (mTBI vs mTBI + PTSD vs Controls vs PTSD)	*P* (mTBI vs Controls)	*P* (mTBI + PTSD vs Controls)
Number of subjects	32	41	68	6	—	—	—
Military personnel (% of subjects)	84.4	100	0	50	**<.001**	**<.001**	**<.001**
Age (mean ± SD, in years)	39.7 ± 13.1	37.3 ± 10.1	32.5 ± 12.8	32.8 ± 8.2	**.001**	**.010**	**.031**
Sex (% male)	68.8	85.4	48.5	66.7	**.001**	.058	**<.001**
History of mTBI (% of subjects)	100	100	0	0	**<.001**	**<.001**	**<.001**
Education (mean ± SD, in years)	14.8 ± 2.0	13.9 ± 3.5	14.6 ± 2.1	13.4 ± 1.1	.326	.663	.267
Handedness (% of subjects)							
Right	59.3	82.9	70.5	83.3	.369	.285	.592
Left	18.8	9.8	11.8	0			
Both	3.1	2.4	1.5	0			
Unknown	18.8	4.9	16.2	16.7			
Race (% of subjects)							
Native	3.1	2.4	0	0	.481	.122	.191
Asian	0.0	2.4	7.4	0			
African American	9.4	17.1	14.6	33.3			
White	84.4	70.7	72.1	66.7			
Unknown	3.1	7.3	5.9	0			
Scores of questionnaires							
RPQ-3	1.7 ± 1.9	4.5 ± 3.1	—	0.0	—	—	—
RPQ-13	12.7 ± 12.0	30.9 ± 11.0	—	18.3 ± 15.9	—	—	—
AUDIT-10	3.0 ± 2.4	2.8 ± 2.9	2.3 ± 2.1	3.8 ± 4.4	.373	.165	.361
LEC	4.8 ± 2.8	7.5 ± 2.9	2.2 ± 2.2	4.7 ± 3.9	**<.001**	**<.001**	**<.001**
CTQ	44.2 ± 24.6	49.7 ± 21.8	29.7 ± 12.7	49.2 ± 27.1	**<.001**	**.003**	**<.001**
PHQ-9	5.3 ± 4.3	13.2 ± 5.7	0.6 ± 1.0	12.2 ± 6.6	**<.001**	**<.001**	**<.001**
BSI-18	28.7 ± 11.0	44.5 ± 14.0	19.0 ± 1.6	41.8 ± 9.5	**<.001**	**<.001**	**<.001**
ISI	10.2 ± 8.5	10.9 ± 9.5	8.6 ± 7.8	12.4 ± 10.4	.499	.393	.207
WRAT-4	63.2 ± 5.3	63.3 ± 4.4	63.9 ± 4.1	65.2 ± 3.1	.698	.501	.503
PCL-C	31.7 ± 11.4	61.4 ± 10.2	18.1 ± 2.4	62.3 ± 15.5	**<.001**	**<.001**	**<.001**

Abbreviations: mTBI, mild traumatic brain injury; PTSD, posttraumatic stress disorder; RPQ, Rivermead Post-Concussion Questionnaire; AUDIT, Alcohol Use Disorders Identification Test; LEC, Life Events Checklist; CTQ, Childhood Trauma Questionnaire; PHQ, Patient Health Questionnaire; BSI, Brief Symptom Inventory; ISI, Insomnia Severity Index; WRAT, Wide Range Achievement Test; PCL-C, PTSD Checklist–Civilian Version; SD, standard deviation.
[a]This table lists the sociodemographic, psychosocial, neurocognitive, and clinical measures for the mTBI group, PTSD with history of mTBI (PTSD + mTBI) group, the PTSD group, and the group of healthy controls.
Significant *P* values are marked in bold

neurocognitive, clinical, and neurosteroid quantifications between the mTBI, PTSD + mTBI, and control groups. Chi-squared tests were used for categorical variables, and analysis of variance (ANOVA) or *t*-tests were performed for continuous variables. The level of statistical significance was set at $P < .05$.

For cortical thickness analyses, a whole-brain general linear model was computed using the QDEC package in FreeSurfer (http://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/QdecGroupAnalysis_freeview). As noted previously, due to the very small number of subjects in the PTSD group (n = 6), this group was not included in analyses of cortical thickness. In QDEC, we first assessed group differences in cortical thickness between the mTBI, PTSD + mTBI, and control groups. We then investigated associations between cortical thickness and ALLO/PREGN serum levels within each group while correcting for age and sex.

A cluster-wise *P* value (CWP) and a maximum negative decadic logarithm of the *P* value, $\max(-\log_{10}(P))$, were obtained for each statistically significant cluster. This was followed by extraction of the mean cortical thickness from statistically significant clusters for each subject to obtain information on cortical thickness on single-subject level for post-hoc analyses. A cluster-wise Monte Carlo simulation was applied using a threshold of 0.05 to adjust for multiple comparisons.

In post-hoc analyses performed in SPSS, we investigated in the PTSD + mTBI group the relationship between cortical thickness in regions that were significantly associated with serum neurosteroid levels and severity of PTSD symptoms as measured by the PCL-C. For this purpose, we conducted a partial correlation analysis for scores of the PCL-C and each of the significant clusters while correcting for age and sex.

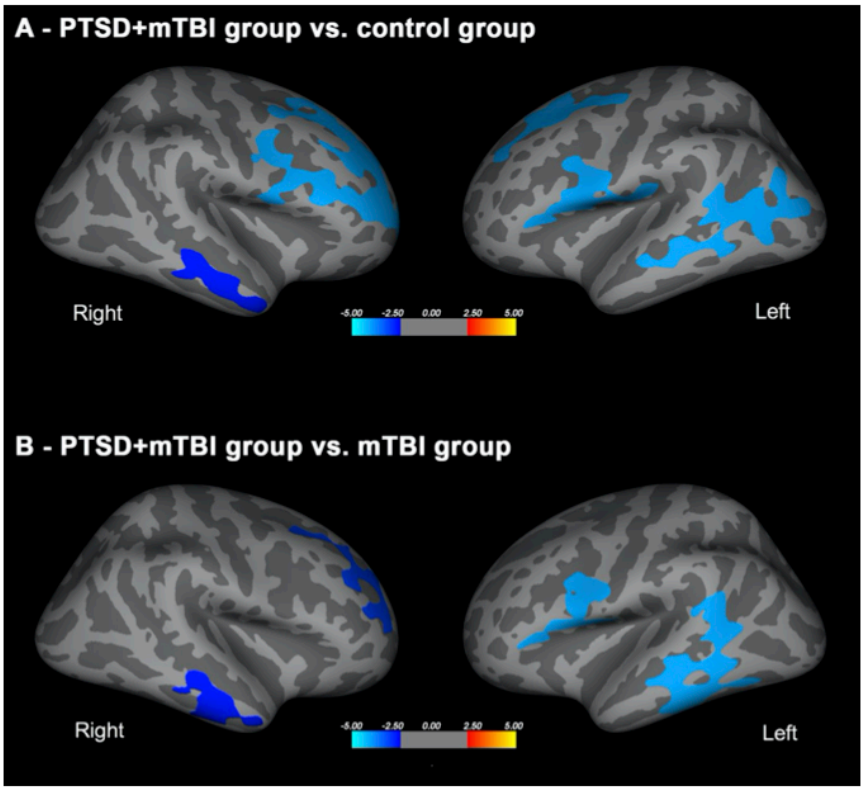

Figure 1. Cortical thinning in the PTSD + mTBI group compared with the control group and in the PTSD + mTBI group compared with the mTBI group. Part A depicts clusters with significantly thinner cortex in the PTSD + mTBI group when compared to the control group, which were located in the left middle temporal gyrus, left caudal middle frontal gyrus, left lateral occipital cortex, left inferior frontal gyrus, right superior frontal gyrus, and right middle temporal gyrus. The cluster in the left fusiform gyrus was too small to be displayed visually using the default smoothing factor. Part B depicts clusters with significantly thinner cortex in the PTSD + mTBI group when compared with the mTBI group, including the left inferior frontal gyrus, left lateral occipital cortex, left inferior temporal gyrus, right middle temporal gyrus, and right rostral middle frontal gyrus. The color coding represents logarithmic *P* values after correction for age, sex, and multiple comparisons. PTSD, posttraumatic stress disorder; mTBI, mild traumatic brain injury.

Results

Cohort Characteristics

Cohort characteristics are shown in Table 2. Regarding demographics, there was a statistically significant difference between the mTBI and the control group for age ($P = .01$) and between the PTSD + mTBI group and the control group for age ($P = .031$) and sex ($P < .001$).

Group Comparisons of Cortical Thickness

Whole-brain group comparisons revealed a significantly thinner cortex in the PTSD + mTBI group compared to the control group (Figure 1A) in 7 cortical clusters, including the left middle temporal gyrus (PTSD + mTBI group, 3.0 ± 0.2 mm; control group, 3.2 ± 0.2 mm; CWP, .017; max $-\log_{10}(P) = -5.024$); the left caudal middle frontal gyrus (PTSD + mTBI group, 2.3 ± 0.1 mm; control group, 2.4 ± 0.1 mm; CWP, .001; max $-\log_{10}(P) = -4.697$); the left lateral occipital cortex (PTSD + mTBI group, 2.5 ± 0.1 mm; control group, 2.6 ± 0.2 mm; CWP, .001; max $-\log_{10}(P) = -2.834$); the left inferior frontal gyrus, pars opercularis (PTSD + mTBI group, 2.5 ± 0.2 mm; control group, 2.6 ± 0.2 mm; CWP, .001; max $-\log_{10}(P) = -4.64$); the left fusiform gyrus (PTSD + mTBI group, 2.3 ± 0.2 mm; control group, 2.4 ± 0.2 mm; CWP, .0393; max $-\log_{10}(P) = -3.144$); the right superior frontal gyrus (PTSD + mTBI group, 2.3 ± 0.1 mm; control group, 2.4 ± 0.1 mm; CWP, .0001; max $-\log_{10}(P) = -5.411$); and the right middle temporal gyrus (PTSD + mTBI group, 2.7 ± 0.2 mm; control group, 2.8 ± 0.2 mm; CWP, .0019; max $-\log_{10}(P) = -4.038$).

In addition, there were 5 clusters with reduced cortical thickness in the PTSD + mTBI group compared with the mTBI group (Figure 1B), including the left inferior frontal gyrus, pars opercularis (PTSD + mTBI group, 2.5 ± 0.1 mm; mTBI group, 2.6 ± 0.2 mm; CWP, .001; max $-\log_{10}(P) = -3.643$); the left lateral occipital cortex (PTSD + mTBI group, 2.2 ± 0.2 mm; mTBI group, 2.2 ± 0.1 mm; CWP, .0119; max $-\log_{10}(P) = -3.512$); the left inferior temporal gyrus (PTSD + mTBI group, 2.5 ± 0.2 mm; mTBI group, 2.5 ± 0.2 mm; CWP, .0001; max $-\log_{10}(P) = -3.4$); the right middle temporal gyrus (PTSD + mTBI group, 2.7 ± 0.2 mm; mTBI group, 2.8 ± 0.2 mm; CWP,

Table 3. Serum Neurosteroid Levels.[a]

	mTBI	mTBI + PTSD	Controls	PTSD	P (mTBI vs mTBI + PTSD vs Controls vs PTSD)	P (mTBI vs Controls)	P (mTBI + PTSD vs Controls)
Pregnenolone (PREGN) (mean ± SD, in pg/mL)	437.5 ± 291.2	482.8 ± 251.8	582.0 ± 532.9	654.7 ± 547.9	.317	.155	.266
Allopregnanolone (ALLO) (mean ± SD, in pg/mL)	64.6 ± 64.3	48.5 ± 31.0	98.9 ± 127.3	65.0 ± 73.6	.050	.154	**.003**

Abbreviations: mTBI, mild traumatic brain injury; PTSD, posttraumatic stress disorder; SD, standard deviation..
[a]Serum levels of allopregnanolone (ALLO) and its precursor pregnenolone (PREGN) are listed for the mTBI group, PTSD with history of mTBI (PTSD + mTBI) group, the PTSD group, and the group of healthy controls.
Significant *P* values are marked in bold.

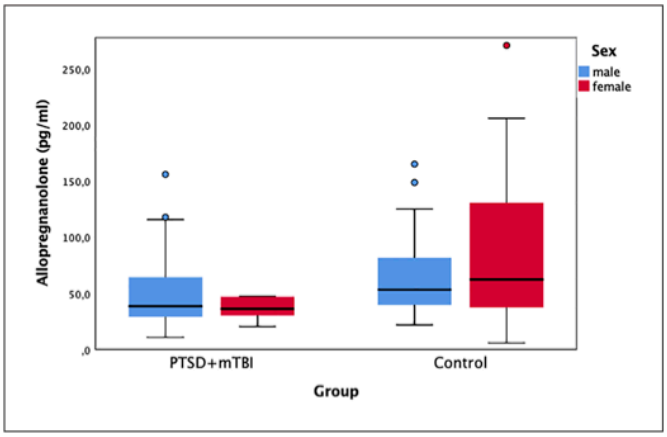

Figure 2. Serum ALLO levels in the PTSD + mTBI group compared with the control group. Each group is clustered into male and female participants. ALLO, allopregnanolone; PTSD, posttraumatic stress disorder; mTBI, mild traumatic brain injury.

.0019; max $-\log_{10}(P) = -3.995$); and the right rostral middle frontal gyrus (PTSD + mTBI group, 2.2 ± 0.1 mm; mTBI group, 2.2 ± 0.1 mm; CWP, .0007; max $-\log_{10}(P) = -2.456$).

There were no statistically significant differences in cortical thickness between the mTBI and the control group ($P > .05$).

Serum Neurosteroid Levels

Serum levels of PREGN and ALLO for all 3 groups (mTBI, PTSD + mTBI, and controls) are shown in Table 3. The PTSD + mTBI group showed significantly reduced ALLO levels compared with the control group ($P = .003$) (Figure 2). There was no statistically significant difference in PREGN or ALLO levels between the mTBI group and the control group ($P > .05$).

Associations Between Cortical Thickness and Serum Neurosteroid Levels

Cortical thickness was positively correlated with both ALLO and PREGN in the PTSD + mTBI group in frontal and temporal regions, including the left middle temporal cortex (CWP, .003; max $-\log_{10}(P) = 5.534$) and the right lateral orbitofrontal cortex (CWP, .005, max $-\log_{10}(P) = 2.878$) for PREGN, and the right superior frontal cortex (CWP, .03; max $-\log_{10}(P) = 3.801$) for ALLO (Figures 3 and 4A-C).

There were no significant associations between serum neurosteroid levels and cortical thickness in the mTBI group or in the control group ($P > .05$).

Associations Between Cortical Thickness and PTSD Symptom Severity

In the PTSD + mTBI group, PTSD symptom severity (higher scores in the PCL-C) was associated with decreased cortical thickness in a cluster in the left middle temporal gyrus ($R = -0.368$, $P = .023$; Figure 5A) and a cluster in the right orbitofrontal cortex ($R = -0.364$, $P = .025$; Figure 5B).

Discussion

This study investigated the association between cortical thickness and serum levels of ALLO and PREGN in individuals with a history of mTBI but no PTSD, in individuals with PTSD and a history of mTBI (PTSD + mTBI), and in healthy controls (Figure 6). There are 3 main findings regarding the PTSD + mTBI group. First, individuals with PTSD + mTBI show decreased cortical thickness in several brain areas when compared with both individuals with mTBI only and controls, including temporal and frontal cortex regions. Second, the thinner the cortex in these brain areas, the higher the PTSD symptom severity. Third, cortical thickness in these brain areas is positively associated with serum levels of ALLO and PREGN. Taken together, these findings demonstrate a connection between neurosteroids, which are assumed to have neuroprotective effects, cortical thickness in key regions often associated with anxiety disorders, and PTSD symptoms.

Reduced Cortical Thickness in the PTSD + mTBI Group

Individuals diagnosed with PTSD + mTBI show reduced cortical thickness in several brain regions when compared

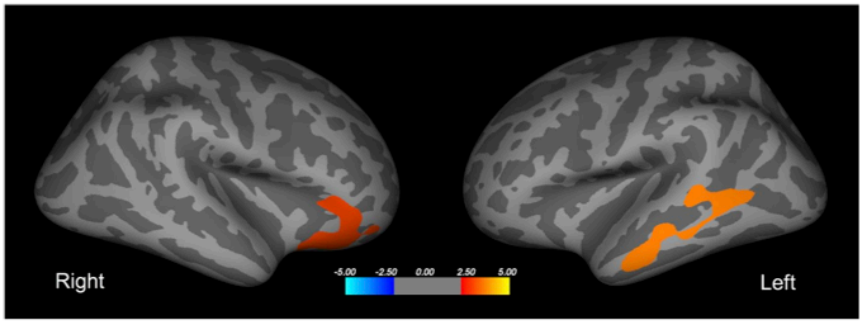

Figure 3. Regions of associations between cortical thickness and serum neurosteroid levels in the PTSD + mTBI group. This figure shows clusters of significant positive correlations between cortical thickness and PREGN (left middle temporal cortex and right lateral orbitofrontal cortex) in the PTSD + mTBI group. The cluster for ALLO in the right superior frontal cortex was too small to be displayed visually using the default smoothing factor. The color coding represents logarithmic *P*-values after correction for age, sex, and multiple comparisons. ALLO, allopregnanolone; PREGN, pregnenolone; PTSD, posttraumatic stress disorder; mTBI, mild traumatic brain injury.

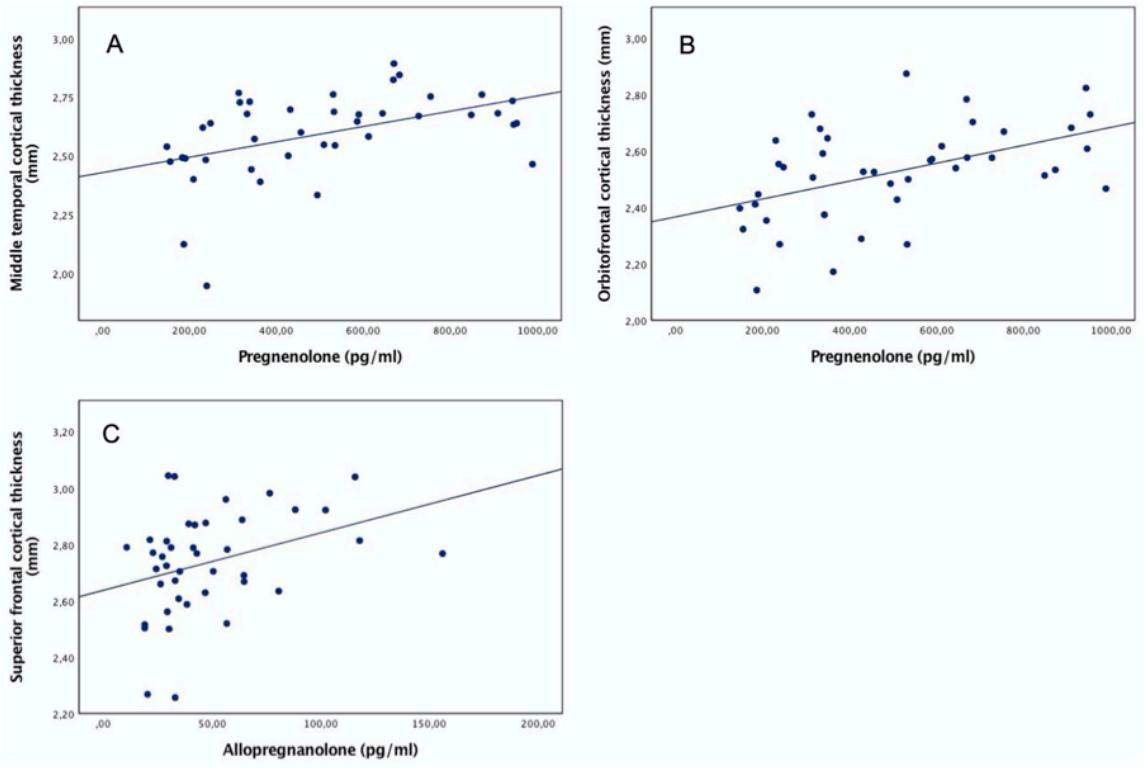

Figure 4. Correlations between cortical thickness and serum neurosteroid levels in the PTSD + mTBI group. Scatter plots showing positive correlations between cortical thickness in extracted clusters and pregnenolone (PREGN; parts A and B) or allopregnanolone (ALLO; part C) in the PTSD + mTBI group. PTSD, posttraumatic stress disorder; mTBI, mild traumatic brain injury.

with controls or individuals with mTBI only. One potential explanation could be that PTSD symptoms develop following mTBI in those individuals with injury to the frontal lobe. There are studies indicating that particularly blast-related mTBI is associated with reduced cortical thickness in the frontal lobe.[30,85,86] One of these studies further reports an association between decrease in frontal cortical thickness and PTSD symptom severity. To our knowledge, it remains, however, unknown if biomechanical injury to the frontal lobe can lead to PTSD symptoms.

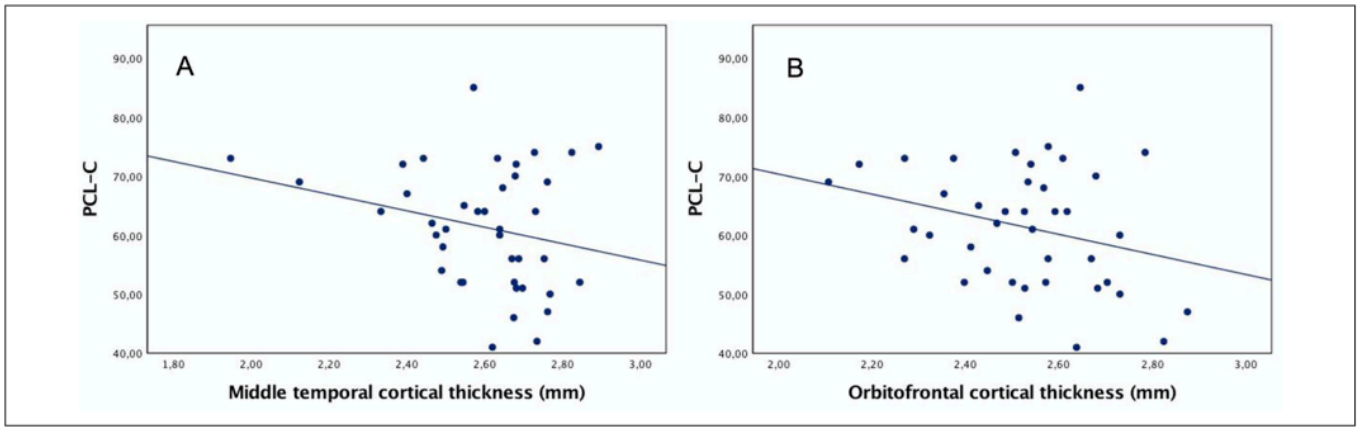

Figure 5. Associations between cortical thickness and PTSD symptom severity in the PTSD + mTBI group. Scatter plots showing that the thinner the cortex in the left middle temporal cortex (part A) and the right orbitofrontal cortex (part B), the higher PTSD symptom severity in the PTSD + mTBI group. PTSD, posttraumatic stress disorder; mTBI, mild traumatic brain injury.

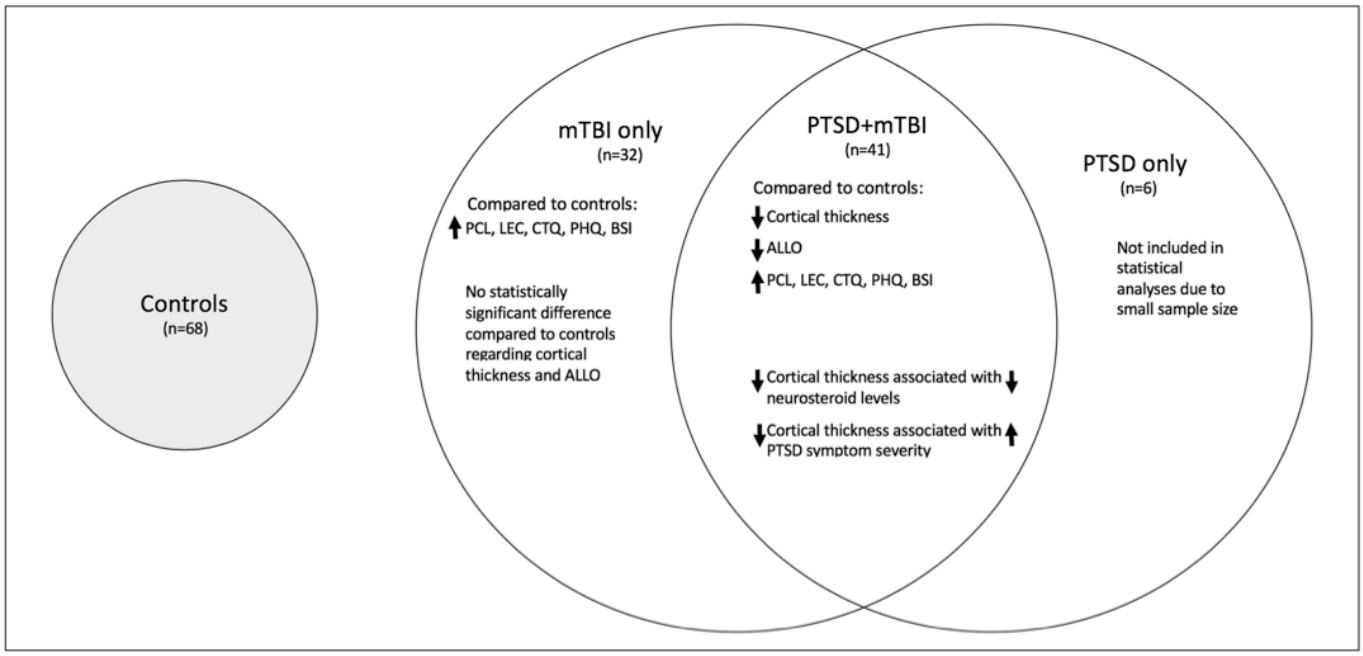

Figure 6. Summary of main findings. This figure highlights the main findings for the mild traumatic brain injury (mTBI) group, posttraumatic stress disorder (PTSD) with history of mTBI (PTSD + mTBI) group, and the group of healthy controls.

A more likely explanation for the differences in cortical thickness between the mTBI and PTSD + mTBI groups are additive effects of both conditions. First, there are studies showing that PTSD itself has an effect on cortical thickness in the frontal and temporal lobes.[12-14,16,17,85] Individuals with co-occurring PTSD and mTBI also show stronger associations between stress symptoms and cortical thickness when compared to subjects with PTSD only.[22] This indicates that mTBI possibly contributes to an environment in which brain tissue is more susceptible to damage induced by stress. PTSD in turn has been shown to be the strongest risk factor associated with persistent symptoms following mTBI.[11] These findings suggest additive effects between mTBI and PTSD, which could explain reduced cortical thickness in the PTSD + mTBI group compared with the mTBI group.

Association Between Cortical Thickness and Serum Neurosteroid Levels

Our study reveals a positive association between cortical thickness and serum neurosteroid levels, such that the higher the serum neurosteroid concentration, the thicker the cortex in individuals with PTSD + mTBI. The statistically significant cortical clusters were located in the right lateral orbitofrontal cortex and the left middle temporal cortex for PREGN

and in the right superior frontal cortex for ALLO. Although, to date, to our knowledge no other studies have been conducted on the association between serum neurosteroid levels and cortical thickness, the brain regions identified in our study are in accordance with previous reports of reduced GM volume and cortical thickness in PTSD. More specifically, decreased GM volume has been consistently reported for the ACC, which is part of the superior frontal cortex.[12-16] Neurosteroids have neurotrophic,[40,41] neuroprotective,[41,42] anti-inflammatory,[41,43,44] anxiolytic,[45,46] and antidepressant[47,48] effects. These effects preserve synaptic plasticity and counteract stress-induced GM atrophy, which has been shown to affect corticolimbic areas such as the ACC and the hippocampus. This is also supported by a recent study in mice, which reported stress-induced GM volume loss due to a loss of synaptic spine density and dendritic length in the ACC and hippocampus.[87] In humans, functional MRI studies have shown that PREGN and ALLO may affect corticolimbic connections, which are known to be important in emotion regulation.[77,78] Specifically, higher levels of PREGN and ALLO were associated with decreased connectivity between the amygdala and the prefrontal cortex, the precuneus, and the hippocampus.[77] Higher neurosteroid levels were associated with reduced activity in the amygdala and insula.[78]

Stress is one mechanism that likely triggers a cascade of responses including dysregulation of the HPA axis, including alterations of neurosteroid and glucocorticoid levels.[35-38] By suppressing the HPA axis via $GABA_A$ receptors,[49-51] neurosteroids may counteract stress-induced responses, support synaptic plasticity, and thereby preserve GM volume. Of note, there was no significant association between serum neurosteroid levels and cortical thickness in the mTBI group. This may be due to time since injury in this group, which was 10.9 years. Neurosteroids may have beneficial effects particularly acutely following such injury as ALLO has been shown to be upregulated in acute stress but decreased in chronic stress.[52-56]

Associations Between Cortical Thickness and PTSD Symptom Severity

In the PTSD + mTBI group, cortical thickness correlated with PTSD symptom severity as measured by the PCL-C, which assesses the presence and severity of key PTSD symptoms with regard to the previous month.[74] More specifically, the thinner the cortex in the left middle temporal cortex and the right orbitofrontal cortex, the higher the PTSD symptoms. Since decreased neurosteroids in the left middle temporal cortex were also associated with decreased cortical thickness, it is possible that treatment with neurosteroids in proximity to the traumatic event could potentially prevent cortical thinning; however, this should be confirmed by future longitudinal studies investigating cortical thickness in patients before and after treatment with neurosteroids.

Furthermore, previous research has already shown predominantly inverse associations between cortical thickness and PTSD symptom severity.[12,21-23] In one study, CAPS correlated negatively with cortical thickness in various clusters, including the cingulate cortex and superior frontal cortical areas.[12] Another study reported a negative relationship between current PTSD symptom severity and thickness in postcentral gyri and middle temporal gyri[22]. The present study also reported negative associations between PTSD severity and cortical thickness in clusters located in the temporal and frontal cortices. Of note, impairments in the temporal lobe, and most often the medial temporal lobe, have been frequently observed in individuals with PTSD.[88,89] Impairments in the temporal lobe have previously been proposed to underlie cognitive deficits in PTSD.[90-92]

Limitations

There are limitations of this study that need to be taken into account. The cross-sectional study design does not allow for the interpretation of causal relationships of cortical thickness and serum neurosteroid levels following PTSD and mTBI. Future studies need to address this question using a longitudinal study design. Further, there is evidence for sex differences in HPA axis activity both at baseline condition and after exposure to stress.[93,94] More specifically, females show a stronger stress responsivity as reflected by a greater adrenocorticotropic hormone and corticosterone synthesis and corticotropin-releasing hormone gene transcription following stress exposure.[95,96] Furthermore, gonadal steroids have been suggested as a major factor behind sex differences in stress reactivity, as estradiol has a stimulatory effect on the HPA axis,[97,98] whereas androgens inhibit HPA function.[99,100] Moreover, a recent study in mice showed that the stress-induced upregulation of ALLO and PREGN measured in the brain is higher in female mice.[101]

Sex ratio was unequal across the 4 study groups. As women are known to have a greater range of ALLO levels due to elevated ALLO levels in the luteal phase of the menstrual cycle, the group difference between the PTSD + mTBI group and controls may have been in part driven by the higher percentage of women in the control group. However, Figure 2 demonstrates that male participants with PTSD + mTBI also have lower ALLO levels, although the effect size is smaller in males (Cohen's d is 0.428 for males compared with 0.787 for females). Further, when we randomly reduced the percentage of women in the control group to 15%, the group differences remained significant. This suggests that lower levels of ALLO in those with PTSD + mTBI are unlikely only the result of sex differences. Furthermore, in this study there was no information available regarding the time of the blood draw, which is important considering the circadian fluctuation of neurosteroid levels that may have influenced the results. As mentioned above, ALLO levels are dependent on menstrual cycle phase with an increase in the luteal phase.[102-104] Future studies should collect information on menstrual cycle phase as well as hormonal contraception. Moreover, the group of individuals with PTSD only was too small to perform meaningful statistical comparisons and therefore was not further considered. The cohort of the present study, due to the small number of participants with PTSD only, did not allow us to differentiate further the effects due to PTSD and/or mTBI, although previous research suggests possible additive effects that need to be investigated in future studies.

Conclusion

Taken together, results from this study demonstrate an association between cortical thickness and serum neurosteroid levels in individuals with PTSD + mTBI. These findings suggest that neurosteroids could be potentially therapeutic in the context of PTSD and mTBI and preserve cortical thickness via their neurotrophic and neuroprotective effects, among other actions. Longitudinal studies are, however, needed to elucidate the time course of changes in cortical thickness in PTSD + mTBI and to investigate further possible sex-specific differences in the neuroprotective effects of neurosteroids in PTSD + mTBI.

Author Contributions

PK contributed to conception and design; contributed to analysis and interpretation; drafted manuscript; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy. CEM contributed to conception and design; contributed to acquisition, analysis and interpretation; drafted manuscript; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy. NS contributed to conception and design; contributed to analysis and interpretation; drafted manuscript; critically revised manuscript; gave final approval. EH contributed to analysis and interpretation; critically revised manuscript; gave final approval. JPG contributed to analysis and interpretation; critically revised manuscript; gave final approval. DK contributed to interpretation; critically revised manuscript; gave final approval. SB contributed to conception and design; contributed to acquisition and analysis; critically revised manuscript; gave final approval. OP contributed to conception and design; contributed to acquisition and analysis; critically revised manuscript; gave final approval. YR contributed to conception and design; contributed to acquisition and analysis; critically revised manuscript; gave final approval. MJC contributed to conception and design; contributed to acquisition and analysis; critically revised manuscript; gave final approval. AVDK contributed to conception and design, contributed to acquisition; critically revised manuscript; gave final approval. KH contributed to conception and design, contributed to acquisition; critically revised manuscript; gave final approval. JDK contributed to acquisition and analysis; critically revised manuscript; gave final approval. JCN contributed to acquisition and analysis; critically revised manuscript; gave final approval. RAM contributed to acquisition and interpretation; critically revised manuscript; gave final approval. LS contributed to acquisition; critically revised manuscript; gave final approval. NA contributed conception and design; contributed to acquisition; critically revised manuscript; gave final approval. RC contributed to conception and design; contributed to acquisition; critically revised manuscript; gave final approval. AJL contributed to conception and design; contributed to acquisition; critically revised manuscript; gave final approval. MSG contributed conception and design; contributed to acquisition; critically revised manuscript; gave final approval. TWM contributed conception and design; contributed to acquisition and interpretation; critically revised manuscript; gave final approval. RZ contributed to conception and design; contributed to acquisition and interpretation; critically revised manuscript; gave final approval. MBS contributed to conception and design; contributed to acquisition; critically revised manuscript; gave final approval. MES contributed to conception and design; contributed to acquisition, analysis and interpretation; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy. IKK contributed to conception and design; contributed to analysis and interpretation; drafted manuscript; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy. This manuscript is part of the dissertation of PK.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr Marx is an applicant on pending patent applications focusing on the use of neurosteroids and derivatives for CNS disorders; no patents have been issued; no licensing in place; VA 208 waiver in place. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

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8 Zusammenfassung Arbeit 2

8.1 Methoden

In diese Studie wurden 25 College-Eishockey-Spieler (14 Männer, 11 Frauen) im Rahmen des Hockey Concussion Education Project (HCEP) zwischen 2011 und 2012 eingeschlossen. Bei allen Probanden erfolgte vor und nach der Saison eine kraniale diffusionsgewichtete MR- Bildgebung in einem 3T-MR-Tomographen (Achieva, Philips Healthcare, Best, Niederlande) mit einer Voxel-Größe von 2,2mm x 2,2mm x 2,2mm und 60 Diffusionsrichtungen. Des Weiteren erfolgte vor und nach der Saison eine Testung mit dem Immediate Post-Concussion Assessment and Test (ImPACT)[155], welcher Symptome eines leichten SHT abfragt und die allgemeine neurokognitive Funktion prüft. Keiner der Probanden hatte während der Saison ein SHT erlitten. Mittels TBSS untersuchten wir mögliche Veränderungen der Diffusionsparameter fraktionale Anisotropie (FA), mittlere Diffusivität (MD), axiale Diffusivität (AD) und radiale Diffusivität (RD) im Verlauf der Saison für beide Geschlechter sowie mögliche Zusammenhänge zwischen der Veränderung der Diffusionsparameter und dem ImPACT-Score.

8.2 Ergebnisse

Es zeigten sich Cluster mit signifikanten Geschlechtsunterschieden bezüglich der Veränderung von FA, MD, RD und AD im Verlauf der Saison (Siehe Abb. 9, Abb. 10). Die signifikanten FA- Cluster lagen im Fasciculus longitudinalis superior (superior longitudinal fasciculus, SLF), der Capsula interna (internal capsule, IC) und der Corona radiata (CR) der rechten Hemisphäre. In diesen Clustern zeigte sich eine signifikante Reduktion der FA bei Frauen im Verlauf der Saison. Bei Männern gab es keine signifikante Veränderung der FA-Werte im Verlauf. Die signifikanten Cluster von MD, RD und AD lagen ebenfalls im rechten SLF, der rechten IC und der rechten CR sowie zusätzlich in der Capsula externa (external capsule, EC). In diesen Clustern kam es zu einem signifikanten Anstieg der Werte bei Frauen, jedoch nicht bei Männern. Es konnte kein Zusammenhang zwischen einer Änderung der Diffusionsparameter und dem ImPACT-Score gezeigt werden.

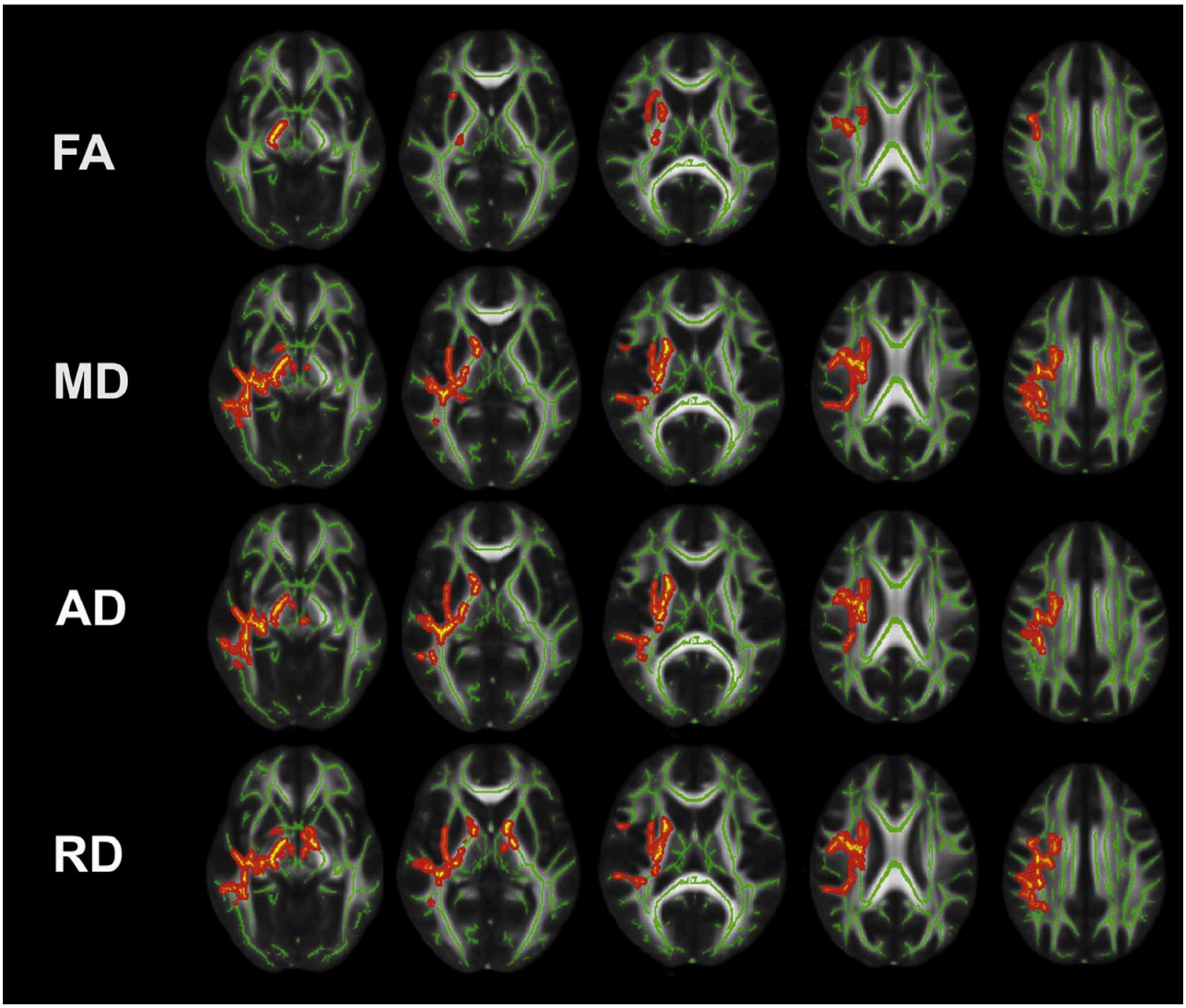

Abb. 9: Signifikante Cluster der TBSS-Analyse für FA, MD, AD, RD. Diese axialen Ansichten zeigen Cluster mit signifikanten Geschlechtsunterschieden ($p < 0,05$). In den Clustern kam es bei Frauen zu einem signifikanten Absinken der FA-Werte bzw. signifikantem Ansteigen der MD-, AD- und RD-Werte im Verlauf der Saison. Bei Männern gab es keine Unterschiede im Verlauf. Zur besseren Darstellung wurden die signifikanten Voxel vergrößert und rot-gelb markiert auf das FA-Skelett projiziert. Die linke Seite auf der Darstellung entspricht der rechten Hemisphäre (Abbildung adaptiert aus Arbeit 2).

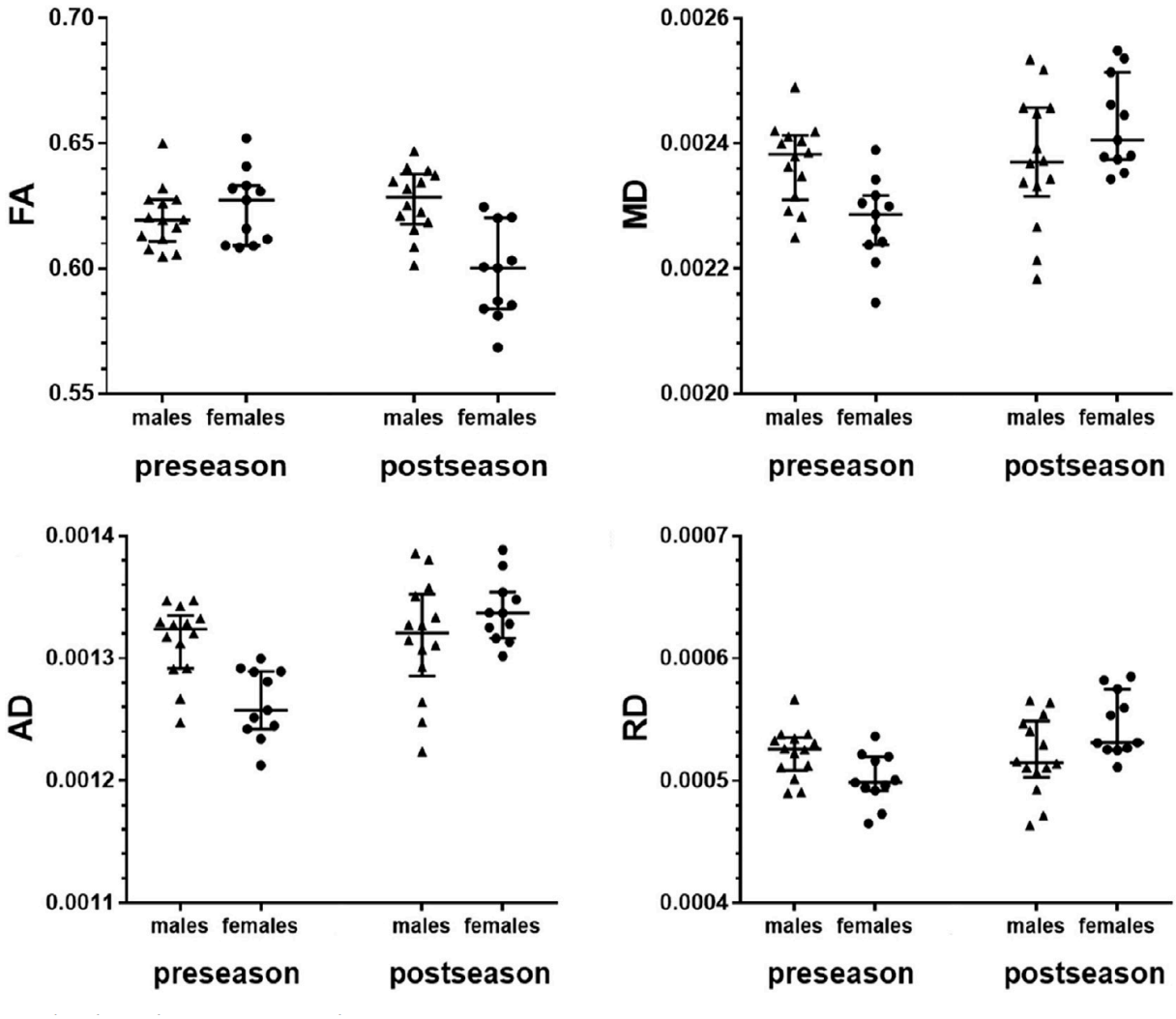

Abb. 10: Scatterplots der signifikanten Cluster der TBSS- Analyse. Die Scatterplots zeigen die Mittelwerte für FA, MD, AD und RD in den signifikanten Clustern. Die Werte werden separat für Frauen und Männer jeweils für den Zeitpunkt vor und nach der Saison angezeigt (Abbildung adaptiert aus Arbeit 2).

8.3 Diskussion und Schlussfolgerung

In der vorliegenden Studie zeigten wir signifikante Geschlechtsunterschiede in der Veränderung von Diffusionsparametern nach RSHI im Verlauf einer Eishockey-Saison. Bei Frauen zeigte sich ein signifikantes Absinken der FA-Werte in Clustern im SLF, der IC und der CR im Vergleich zu Männern. Zudem zeigte sich bei Frauen ein signifikantes Ansteigen der MD-, RD- und AD- Werte im SLF, der IC, der EC und der CR im Unterschied zu Männern. In bisherigen Studien konnte bereits ein Zusammenhang zwischen RSHI und erniedrigten FA-Werten sowie erhöhten AD- und RD-Werten bei Fußballspielern ohne SHT in der Vorgeschichte gezeigt werden[118, 156]. Ferner konnte in einer Kohorte von American Football-

und Eishockeyspielern ohne SHT in der Vorgeschichte ein Zusammenhang zwischen RSHI und einem Anstieg der MD-Werte gezeigt werden[157].

Für die von uns dargestellten Geschlechtsunterschiede gibt es zwei mögliche Erklärungen. Zum einen könnten die Veränderungen in der weißen Substanz bei Frauen auf eine erhöhte Inzidenz und erhöhte Intensität von RSHI zurückzuführen sein. Es konnte gezeigt werden, dass Frauen beim Sport ein höheres Risiko haben, ein leichtes SHT zu erleiden als Männer[158-160]. Dies wurde auf anatomische Geschlechtsunterschiede wie beispielsweise eine geringer ausgeprägte Nackenmuskulatur bei Frauen zurückgeführt[161]. Möglicherweise ist durch einen ähnlichen Mechanismus auch bei RSHI das Verletzungsrisiko bei Frauen höher. Eine zweite mögliche Erklärung sind hormonelle und physiologische Geschlechtsunterschiede. Frauen und Männer weisen unterschiedliche Konzentrationen von Östrogen, Progesteron und deren Metaboliten auf und zudem zeigen sich bei Frauen Fluktuationen im Rahmen des Menstruationszyklus. In einigen Studien konnten protektive Effekte von Östrogen und Progesteron nach leichtem SHT gezeigt werden[79, 129, 162]. Ferner konnte in einem Mausmodell des Schlaganfalls gezeigt werden, dass es bei männlichen Mäusen nach der Ischämie zu einer reaktiven Progesteron-Erhöhung kommt[163]. Bei weiblichen Mäusen blieb diese Erhöhung aus. Möglicherweise gibt es ähnliche Mechanismen auch beim leichten SHT und nach RSHI. Des Weiteren gibt es Hinweise dafür, dass Frauen einen erhöhten basalen Glukosebedarf[164] und einen erhöhten basalen zerebralen Blutfluss[165] haben. Dies führt möglicherweise zu einer Exazerbation der neuro-metabolischen Kaskade nach Gehirnverletzung bei Frauen[122]. Die meisten der oben zitierten Studien untersuchten nicht RSHI, sondern das SHT und sind damit nur eingeschränkt auf unsere Publikation übertragbar. Die Pathophysiologie der Geschlechtsunterschiede bei RSHI sollte in weiteren Studien genauer untersucht werden.

8.4 Originalarbeit 2:

Sex differences in white matter alterations following repetitive subconcussive head impacts in collegiate ice hockey players

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Sex differences in white matter alterations following repetitive subconcussive head impacts in collegiate ice hockey players☆

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ABSTRACT

Objective: Repetitive subconcussive head impacts (RSHI) may lead to structural, functional, and metabolic alterations of the brain. While differences between males and females have already been suggested following a concussion, whether there are sex differences following exposure to RSHI remains unknown. The aim of this study was to identify and to characterize sex differences following exposure to RSHI.

Methods: Twenty-five collegiate ice hockey players (14 males and 11 females, 20.6 ± 2.0 years), all part of the Hockey Concussion Education Project (HCEP), underwent diffusion-weighted magnetic resonance imaging (dMRI) before and after the Canadian Interuniversity Sports (CIS) ice hockey season 2011–2012 and did not experience a concussion during the season. Whole-brain tract-based spatial statistics (TBSS) were used to compare pre- and postseason imaging in both sexes for fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Pre- and postseason neurocognitive performance were assessed by

Abbreviations: AD, axial diffusivity; CIS, Canadian Interuniversity Sports; CR, corona radiata; dMRI, diffusion magnetic resonance imaging; EC, external capsule; FA, fractional anisotropy; HCEP, Hockey Concussion Education Project; IC, internal capsule; ImPACT, Immediate Post-Concussion Assessment and Cognitive Test; LH, left hemisphere; MD, mean diffusivity; MRI, magnetic resonance imaging; NCAA, National Collegiate Athletic Association; r_s, Spearman's rank correlation coefficient; RD, radial diffusivity; RH, right hemisphere; RSHI, repetitive subconcussive head impacts; SD, standard deviation; SLF, superior longitudinal fasciculus; TBI, traumatic brain injury; TBSS, tract-based spatial statistics; WM, white matter

☆ Previous presentation of data:
Portions of this work were presented in poster form at the 12th World Congress on Brain Injury, New Orleans, LA, USA, March 29–April 1, 2017.

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the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT).
Results: Significant differences between the sexes were primarily located within the superior longitudinal fasciculus (SLF), the internal capsule (IC), and the corona radiata (CR) of the right hemisphere (RH). In significant voxel clusters ($p < 0.05$), decreases in FA (absolute difference pre- vs. postseason: 0.0268) and increases in MD (0.0002), AD (0.00008), and RD (0.00005) were observed in females whereas males showed no significant changes. There was no significant correlation between the change in diffusion scalar measures over the course of the season and neurocognitive performance as evidenced from postseason ImPACT scores.
Conclusions: The results of this study suggest sex differences in structural alterations following exposure to RSHI. Future studies need to investigate further the underlying mechanisms and association with exposure and clinical outcomes.

1. Introduction

Concussion is a common injury in contact sports, with an incidence ranging between 1.6 and 3.1 per 1000 athlete exposures (Agel et al., 2007a, 2007b; Flik et al., 2005). Women are at higher risk than men for sustaining a sports-related concussion and they represent a large proportion of the athletic community in organized sports (Abrahams et al., 2014; Black et al., 2017; Covassin et al., 2003; Gessel et al., 2007). In fact, female participation in National Collegiate Athletic Association (NCAA) sanctioned sports is currently at an all-time high, where an estimated 43% (~210,000) of all collegiate student-athletes are women (Irick, 2015). However, despite the high number of female athletes, females remain an understudied population, as only a small number of studies have focused on female athletes. Moreover, evidence from these studies suggests that females have worse outcomes following concussion compared with males (Baker et al., 2016; Broshek et al., 2005; Colvin et al., 2009; Covassin et al., 2013, 2012, 2007; Majerske et al., 2008; Miller et al., 2016; Zuckerman et al., 2014). Specifically, women reported more post-concussive symptoms with greater symptom severity (Zuckerman et al., 2014), performed worse on neurocognitive tests (Broshek et al., 2005; Colvin et al., 2009; Covassin et al., 2013, 2012, 2007; Majerske et al., 2008), and demonstrated longer periods of recovery compared to males (Baker et al., 2016; Miller et al., 2016; Zuckerman et al., 2014).

Following a concussion, brain alterations have been detected using advanced neuroimaging techniques (for review see Shenton et al., 2012). One of these advanced techniques is diffusion magnetic resonance imaging (dMRI), which has been repeatedly used to detect and to characterize white matter (WM) alterations related to brain injury (Koerte et al., 2015; Shenton et al., 2012). However, to date, there is only one study using dMRI that has investigated sex differences in structural brain alterations following a concussion (Fakhran et al., 2014). This study included 47 male and 22 female individuals after a confirmed concussion (Fakhran et al., 2014). In this study, findings indicated that male concussed individuals demonstrated decreased fractional anisotropy (FA) in the uncinate fasciculus compared to concussed females or controls (Fakhran et al., 2014).

Even more common than concussions are subconcussive head impacts in contact sports. Evidence here suggests that repetitive subconcussive head impacts (RSHI) may also result in structural, functional, and metabolic alterations of the brain (for review see Koerte et al., 2015). Of note, dMRI has shown sensitivity to detect even subtle WM alterations related to RSHI (Koerte et al., 2015). Furthermore, dMRI parameters have predicted impairments in executive function, attention, memory, speed of processing, and learning following traumatic brain injury (TBI) (Caeyenberghs et al., 2011a, 2011b, 2014). Detection of sex-specific WM changes related to RSHI could facilitate an individualized clinical management at an early stage of potential brain injury. However, to date, there are no studies investigating sex differences in brain alterations following exposure to RSHI. Thus, the aim of this study is to evaluate potential sex differences in the brain's WM following exposure to RSHI in a sample of collegiate ice hockey players using dMRI.

2. Materials and methods

2.1. Participants and procedures

All study participants were part of the Hockey Concussion Education Project (HCEP), which was conducted during the Canadian Interuniversity Sports (CIS) ice hockey seasons of 2009–2010 and 2011–2012. The present study analyzed participants of the 2011–2012 HCEP, which used clinical examination, neurocognitive assessment, and pre- and postseason magnetic resonance imaging (MRI) as well as sequential testing and imaging at three time points after any concussion among ice hockey players (Echlin, 2012). Data from the HCEP have already been analyzed with respect to other specific research questions (Chamard et al., 2012; Echlin, 2010, 2012; Echlin et al., 2014, 2010a,

Table 1
Participant-related characteristics.

		Males	Females	p-Value
Number of players		14	11	–
Age (in years) (mean ± SD)		21.7 ± 1.3	19.2 ± 1.8	0.0005
Handedness (right/left/ambidextrous)		10/3/1	10/1/0	0.6040
ImPACT score (preseason testing) (mean ± SD)	Verbal memory	90.9 ± 4.5	91.0 ± 8.6	0.3615
	Visual memory	83.7 ± 8.6	85.4 ± 10.0	0.5358
	Visual motor speed	44.1 ± 4.2	42.7 ± 3.7	0.3712
	Reaction time	0.5 ± 0.1	0.6 ± 0.1	0.0862
ImPACT score (postseason testing) (mean ± SD)	Verbal memory	89.4 ± 7.7	94.7 ± 4.1	0.0608
	Visual memory	81.8 ± 11.9	79.2 ± 9.9	0.4623
	Visual motor speed	47.4 ± 5.3	42.9 ± 5.4	0.0344
	Reaction time	0.5 ± 0.1	0.5 ± 0.1	0.4613

This table gives an overview of participant-related characteristics, including the number of male and female participants, age, handedness, and pre- and postseason scores according to the four composite scores (verbal memory, visual memory, visual motor speed, and reaction time) derived from the results of the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT). One female participant did not undergo neurocognitive assessment by the ImPACT.

2012, 2010b, 2010c; Helmer et al., 2014; Koerte et al., 2012b; Pasternak et al., 2014; Sasaki et al., 2014). The study was approved by ethics committees at each CIS university, and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the investigations.

For the 2011–2012 HCEP, exclusion criteria were general MRI exclusion criteria (e.g., metallic or electronic implants), structural MRI abnormalities, previous eye surgery, severe cognitive impairment, and/or a history of any psychiatric or neurological diseases. The team physician conducted the pre- and postseason clinical examinations. Moreover, concussions during the season were diagnosed and reported by an independent designated specialist physician who attended the games. In this context, concussion was defined with respect to the Zürich consensus statement, which met the criteria by a later consensus conference (McCrory et al., 2009, 2013). For the present study, only HCEP participants that (1) did not experience a concussion during the course of the 2011–2012 CIS ice hockey season, and (2) completed pre- and postseason dMRI were included in the analyses.

In total, 45 ice hockey players (25 males and 20 females) were enrolled in the 2011–2012 HCEP. Among this cohort, 5 males and 6 females sustained a concussion during the season and were therefore excluded from the analysis. An additional 9 participants were excluded due to the following reasons: missing pre- or postseason dMRI (4 males), poor scan quality in either pre- or postseason dMRI sequences (2 males and 1 female), incidental finding of a large arachnoidal cyst (1 female), or age more than eight standard deviations (SDs) above the cohort's mean age (1 female). Thus, 25 participants (14 males and 11 females) were included in the analyses (Table 1).

2.2. Cognitive testing

Neurocognitive function was assessed using the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT) before the beginning of the season and at the end of the season (ImPACT Applications Inc., San Diego, CA, USA; https://www.impacttest.com). The ImPACT is a computer-based assessment composed of a concussion symptom inventory as well as 6 modules for assessment of neurocognitive function. Although it is primarily applied in subjects with reported concussion, it can also be used to evaluate neurocognitive function in general. Based on the results obtained from the 6 neurocognitive test modules, 4 composite scores were generated (verbal memory, visual memory, visual motor speed, and reaction time). ImPACT composite scores have already been used in previous investigations among the 2011–2012 HCEP participants (Echlin et al., 2012; Sasaki et al., 2014). The ImPACT results were independently evaluated by a neuropsychologist.

2.3. Acquisition of dMRI

All imaging was performed on a 3T MRI scanner with an eight-channel head coil array (Achieva, Philips Medical Systems). A sequence with two averages and 60 non-colinear diffusion directions (TR/TE: 7015 ms/60 ms, b: 0 and 0.7 ms/mm^2, 70 slices) was acquired using a 2.2 mm isotropic voxel size and a 100 × 100 matrix reconstructed into a 112 × 112 matrix with a resolution of 2 × 2 × 2.2 mm^3.

Between preseason and postseason imaging, a scanner update took place (gradient coil change). A hardware update could potentially affect diffusion measures. However, since the present study compares the change in dMRI over the course of one season for each individual (postseason minus preseason) and the update would have affected all included data sets in the same way, this should not have confounded our longitudinal results.

2.4. Analysis of dMRI

2.4.1. Data processing

First, quality checks were performed by visually inspecting diffusion-weighted data sets using 3D Slicer (http://www.slicer.org; version 4.5.0-1, Surgical Planning Laboratory, Brigham and Women's Hospital, Boston, MA, USA) (Fedorov et al., 2012). To remove misalignments, an affine registration with the baseline volume was conducted for the data sets of each participant, and eddy current corrections were carried out using the MCFLIRT and eddy tools of the FMRIB Software Library (FSL, version 5.0.9; The Oxford Centre for Functional MRI of the Brain, Oxford, UK). Then, automated OTSU masks covering the entire brain were generated for each participant, excluding non-brain areas and background noise (3D Slicer, version 4.5.0-1). The resulting brain masks were again visually assessed for quality, and were manually edited where necessary (e.g., incorrect overlap of the mask with brain volume, missing voxels within the brain volume). A diffusion tensor was estimated for each voxel using a multivariate linear fitting algorithm, and three pairs of eigenvalues and eigenvectors were obtained. Diffusion scalar measures, which included FA, mean diffusivity (MD, also known as trace), axial diffusivity (AD), and radial diffusivity (RD), were then calculated for each voxel based on these values, as described previously (Koerte et al., 2012b; Sasaki et al., 2014).

2.4.2. White matter analysis

For analysis of WM diffusion properties, tract-based spatial statistics (TBSS) were carried out (Smith et al., 2006). All analysis protocols and detailed descriptions of the TBSS approach, which is part of FSL, are freely available (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS) (Jenkinson et al., 2012).

TBSS was conducted separately for FA, MD, AD, and RD, whereas the WM skeleton was generated based on FA maps. The individual maps were aligned and registered to the FMRIB58_FA template, which is in the same space as the MNI152 standard space image (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FMRIB58_FA). The mean FA map was projected to the FMRIB58_skeleton to create a mean FA skeleton. The FA threshold was set at > 0.3 to exclude peripheral tracts where there was considerable inter-subject variability or partial volume effects (Koerte et al., 2012b; Sasaki et al., 2014). MD, AD, and RD maps were registered to the FMRIB58_FA template by applying the nonlinear transformation obtained from the FA registration.

The voxels that formed the skeletons were extracted for each individual scan using the fslsplit command. This step was a prerequisite for subsequent subtraction of the participant-specific data sets obtained during pre- and postseason scanning using the fslmaths command. To depict the change in diffusion scalar measures over the course of the ice hockey season, the preseason data sets were subtracted from the postseason data sets, which generated skeletonized delta maps for each participant for FA, MD, AD, and RD, respectively. The delta maps were then merged across participants into a single file using the fslmerge command.

2.5. Statistical analyses

To identify voxel clusters with statistically significant group differences between females and males in the change in diffusion scalar measurements over the course of the play season, unpaired *t*-tests were performed applying the randomise command (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM), adjusted for age and handedness. The random permutation number was set at 5000, and a p-value of < 0.05 was considered statistically significant, following threshold-free cluster enhancement and correction for multiple comparisons. The resulting statistical maps for FA, MD, AD, and RD were visualized in FSLView (version 3.2.0). Then, using the FSL cluster tool, we extracted the size of the statistically significant voxel clusters for FA, MD, AD, and RD, respectively. For improved illustration, the statistically significant voxel

clusters were enlarged using the tbss_fill command.

Then, the statistical map for each of the diffusion scalar measures, thresholded at $p < 0.05$, was transformed into a binary map using fslmaths. These binary maps distinguished between statistically significant and non-significant voxels. Then, average diffusion scalar measures were extracted from the statistically significant voxel clusters for each participant and visualized by scatter plots using GraphPad Prism (version 7.0; GraphPad Software Inc., La Jolla, CA, USA). The spatial location of the significant voxel clusters was determined in relation to WM anatomy using the atlasquery command in combination with the ICBM-DTI-81 white-matter labels atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) (Mori et al., 2008).

Additionally, means ± SD were calculated for the participants' four composite scores derived from the results of the ImPACT evaluations. Mann-Whitney and Fisher exact tests were performed to assess differences between male and female participants. The individuals' change in FA, MD, AD, and RD values derived from the statistically significant voxel clusters as identified using TBBS were correlated with the postseason ImPACT composite scores using Spearman's rank correlation coefficient (r_s). To adjust for multiple comparisons, we controlled the false discovery rate using the Benjamini & Hochberg procedure (Benjamini and Hochberg, 1995). GraphPad Prism (version 7.0) was used for these statistical tests, with the significance level set at $p < 0.05$.

3. Results

3.1. Participant characteristics

Table 1 shows participant-related characteristics and pre- and postseason scores of the four composite scores derived from the ImPACT assessments. There was a statistically significant difference in age between female and male participants (21.7 ± 1.3 vs. 19.2 ± 1.8 years, $p = 0.0005$; Table 1).

3.2. White matter diffusion

Voxel clusters with statistically significant differences between male and female participants in change over time (postseason minus preseason) are shown for FA, MD, AD, and RD in Fig. 1.

The statistically significant FA cluster primarily includes the superior longitudinal fasciculus (SLF), internal capsule (IC), and corona radiata (CR) of the right hemisphere (RH; Fig. 1). There was no statistically significant FA cluster detected in the left hemisphere (LH; Fig. 1). In the statistically significant cluster, FA values did not change significantly in male participants over the course of one season (pre- vs. postseason: 0.6202 ± 0.0121 vs. 0.6270 ± 0.0131, $p > 0.05$), whereas a decrease in FA in female participants was observed (pre- vs. postseason: 0.6247 ± 0.0147 vs. 0.5978 ± 0.0184, $p < 0.05$; Figs. 1 & 2). The statistically significant FA cluster had a size of 1494 voxels.

The statistically significant MD cluster mainly includes the SLF, IC,

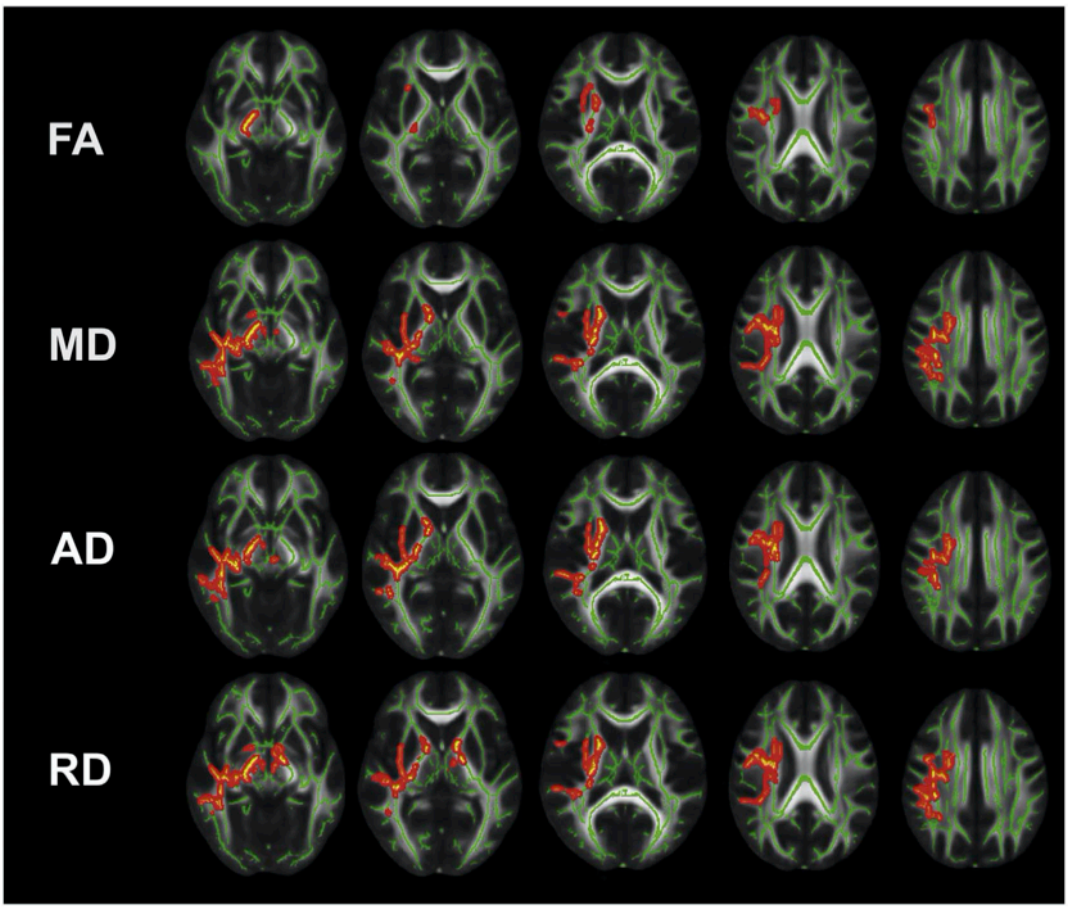

Fig. 1. Results of the tract-based spatial statistics (TBSS) analysis I.
This figure illustrates the results of the TBSS analysis (axial view). Voxel clusters with statistically significant differences ($p < 0.05$) in change over time (postseason minus preseason data sets) between male and female participants are highlighted in red to yellow. The TBSS analysis was carried out for fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Voxels of the statistically significant clusters are thickened into local tracts on a standardized FA skeleton (FMRIB58_FA-skeleton; green) and a standardized diffusion-weighted image (FMRIB58_FA). The left side in each image corresponds to the right hemisphere (RH). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

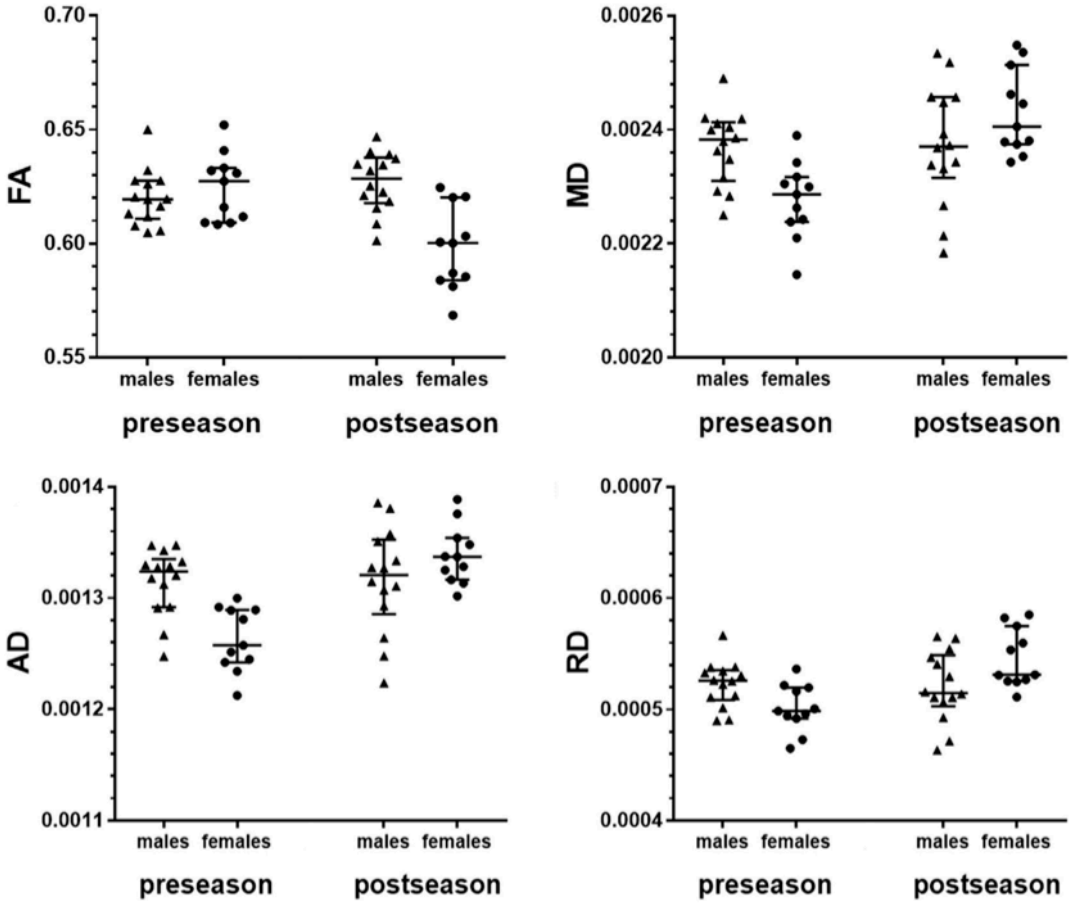

Fig. 2. Results of the tract-based spatial statistics (TBSS) analysis II.
This figure depicts scatter plots of average values in the voxel clusters with statistically significant group differences ($p < 0.05$; Fig. 1) for fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). The values are shown for males vs. females and pre- vs. postseason data, respectively. Circles or triangles represent individual values, whereas horizontal bars represent the median and interquartile range. There was a statistically significant difference between pre- and postseason FA, MD, AD, and RD in female participants ($p < 0.05$). In contrast, no statistically significant changes were observed in males over the course of one season with respect to FA, MD, AD, and RD ($p > 0.05$).

CR, and the external capsule (EC) of the RH, whereas the LH again showed no statistically significant cluster (Fig. 1). In the significant voxel cluster, MD did not change significantly in male participants (pre- vs. postseason: 0.002369 ± 0.00007 vs. 0.002373 ± 0.00011, $p > 0.05$), whereas female participants demonstrated an increase in MD (pre- vs. postseason: 0.002276 ± 0.00007 vs. 0.002431 ± 0.00008, $p < 0.05$; Figs. 1 & 2). The statistically significant MD cluster was composed of 7481 voxels.

Regarding both AD and RD, values increased in female participants over the course of one season (AD: pre- vs. postseason: 0.001263 ± 0.00003 vs. 0.001339 ± 0.00003, $p < 0.05$; RD: pre- vs. postseason: 0.000501 ± 0.00002 vs. 0.000546 ± 0.00003, $p < 0.05$), whereas they did not in male participants (AD: pre- vs. postseason: 0.001315 ± 0.00003 vs. 0.001316 ± 0.00005, $p > 0.05$; RD: pre- vs. postseason: 0.000523 ± 0.00002 vs. 0.000520 ± 0.00003, $p > 0.05$; Figs. 1 & 2). Again, statistically significant clusters primarily involved the SLF, IC, CR, and EC of the RH (Fig. 1). The statistically significant AD cluster had a size of 6110 voxels, and the statistically significant RD cluster included 7355 voxels.

3.3. Correlation of diffusion scalar measures with ImPACT scores

Table 1 shows the results of pre- and postseason ImPACT assessments regarding the four composite scores (verbal memory, visual memory, visual motor speed, and reaction time). There were no statistically significant differences between female and male participants except for visual motor speed at postseason assessment, where male athletes demonstrated significantly improved function in visual motor speed compared to females ($p = 0.0344$; Table 1).

Furthermore, there were no statistically significant correlations of postseason ImPACT composite scores with individuals' change in FA, MD, AD, or RD over the season of play derived from significant voxel clusters.

4. Discussion

This study revealed sex-specific differences of change in diffusion measures over the course of one ice hockey season (Figs. 1 & 2). Statistically significant voxel clusters were observed in several brain regions, including the SLF, IC, CR, and EC (Fig. 1). More specifically, in these voxel clusters female athletes demonstrated a decrease in FA and an increase in MD, AD, and RD whereas, in contrast, diffusion measures did not change significantly over the course of the season in male athletes (Fig. 2).

Changes in WM diffusivity over time can be observed during aging but have also been associated with a variety of psychiatric or neurological diseases such as mild TBI (Assaf and Pasternak, 2008; Westlye et al., 2010). Evidence suggests that RSHI may also lead to detectable WM alterations (Koerte et al., 2012a, 2012b; Lipton et al., 2013; McAllister et al., 2014). In this context, decreased FA and increased AD and RD have been shown to be associated with heading the ball in soccer (Koerte et al., 2012a; Lipton et al., 2013), whereas increased MD has been reported in contact-sports athletes compared to non-contact

sports athletes after one season (McAllister et al., 2014). However, although these studies included male and female athletes in their study cohorts, sex-specific differences in WM diffusivity were not reported. To the best of our knowledge, we here demonstrate for the first time widespread statistically significant differences between female and male athletes following RSHI for changes in diffusion measures.

Sex differences in the change of WM diffusivity were predominantly located within the RH. The underlying mechanisms may potentially include differences in vulnerability, developmental characteristics, or differences in exposure to head impacts. Future studies will need to elucidate reasons for asymmetric changes due to RSHI and the underlying mechanisms for sex-specific differences in the change of WM diffusivity following RSHI. There are two main components that may play a role regarding sex-specific WM diffusivity changes over time. First, sex differences following exposure to RSHI could be associated with differences in RSHI incidences and intensities. Studies have reported that female athletes are at greater risk for concussions when compared to males (Covassin et al., 2003; Forward et al., 2014; Marar et al., 2012), which has been associated with smaller neck girth and weaker neck muscles compared to males (Tierney et al., 2005). This increased risk for brain trauma may also be the case when exposed to RSHI and could explain why differences in change in diffusion measures occurred over the course of one ice hockey season between male and female participants (Figs. 1 & 2). Second, sex differences in the change of WM diffusivity following RSHI could be due to physiological or hormonal differences between males and females, as suggested by investigations among patients suffering from TBI (Djebaili et al., 2005; Emerson et al., 1993; Kupina et al., 2003; Roof and Hall, 2000). Both estrogen and progesterone, which exist in different concentrations in males and females, may have neuroprotective effects after TBI, with previous data suggesting that females may profit from a higher neuroprotective effect (Djebaili et al., 2005; Kupina et al., 2003; Roof and Hall, 2000). However, an opposite situation has also been observed in a study where estrogen was administered to rats prior to inducing a TBI, leading to the observation that estrogen exacerbated injury in female rats but not in males (Emerson et al., 1993). Furthermore, greater rates of basal glucose metabolism and cerebral blood flow in females have been suggested as contributing to differences between the sexes in response to concussion (Andreason et al., 1994; Esposito et al., 1996). In females increased demands for glucose and increased blood flow may lead to an exacerbation of the neuro-metabolic cascade after injury (Broshek et al., 2005). However, it is important to note that most of the previous study results have been restricted to moderate to severe TBI rather than to RSHI, or they have been conducted in animal models, thus leaving open the question of whether such results are directly translatable to human RSHI.

In concussion, sex differences in neurocognitive and clinical outcome have been shown, with the number of symptoms and symptom severity being higher among concussed females (Zuckerman et al., 2014). Furthermore, worse verbal, visual, and motor speed deficits have been reported in females (Covassin et al., 2012; Covassin et al., 2007; Majerske et al., 2008), and symptom duration was prolonged when compared to males (Baker et al., 2016; Miller et al., 2016; Zuckerman et al., 2014). The present study used the ImPACT assessment to test pre- and postseason neurocognitive performance. Although no statistically significant differences were found between female and male athletes at preseason evaluation, at postseason assessment, male athletes demonstrated significantly improved function in visual motor speed compared to their female counterparts (Table 1). However, there was no statistically significant correlation of change in diffusion scalar measures over the course of the season of play and postseason ImPACT composite scores. In this context, it is important to note that the ImPACT assessment, which has been designed for the detection of concussion-related symptoms, may not be sufficiently sensitive for the detection of subtle neurocognitive alterations following RSHI. It is therefore not surprising that we did not find significant correlations with postseason ImPACT scores in our study that focused on RSHI. More sensitive methods to assess the effects of RSHI are currently being developed (Echemendia et al., 2016; Koerte et al., 2017; Zhang et al., 2013). However, it could also be the case that major cognitive changes due to RSHI occur later and, thus, may not have been detectable by postseason ImPACT assessments. Thus, further studies are needed to explore the relationship between sex-specific changes in WM diffusivity and potential subtle neurocognitive changes, using more sensitive neurocognitive measures. Furthermore, additional complementary techniques such as electrophysiological measurements, analyses of functional connectivity, and evaluation of cerebral blood flow may help to investigate further and to enhance our understanding of the underlying mechanisms following RSHI, and particularly to explore WM diffusivity differences between males and females related to RSHI. Regarding concussion, different modalities have already been applied to study sex differences. In contrast, approaches using different techniques or even multi-modal setups in RSHI are just emerging (Covassin and Elbin, 2011; Koerte et al., 2015; Resch et al., 2017).

There are limitations to this study that need to be taken into account when interpreting the data. First, without a control group, the difference between pre- and postseason dMRI cannot be attributed to RSHI only and other factors such as training might play a role. However, the changes found confirm the existing literature on WM alterations following exposure to RSHI. Second, results from this study may not be generalizable to other sports and thus need to be followed-up by further studies in larger cohorts and including other sports. Third, head impact forces and frequencies were not measured in our present study. Future studies should include quantitative assessments of head impact exposure to understand better the underlying mechanisms of sex-specific differences in alterations in WM diffusivity, and we need to determine whether or not the observed sex differences can distinctly be attributed to RSHI exposure. Fourth, there was a statistically significant difference in age between female and male participants (Table 1). Although the analyses performed in this study were adjusted for age, we cannot categorically rule out any potential effect of age on WM diffusivity changes following RSHI. Fifth, group-wise analysis using TBSS may not be sensitive to the spatial location of changes in diffusion properties in heterogeneous conditions such as exposure to RSHI. However, results of this study provide an overview of several regions involved that should be investigated further regarding subject-specific changes. Finally, despite these limitations, we think that the present study demonstrates for the first time sex differences in WM alterations following exposure to RSHI, which, importantly, may pave the way for future research on sex-specific alterations.

5. Conclusions

Previous research has shown that exposure to RSHI during a single varsity ice hockey season can result in significant alterations in WM diffusivity. The results of this study further suggest sex differences in WM diffusivity following exposure to RSHI. The underlying mechanisms remain to be elucidated but may include an increased vulnerability of the female brain to RSHI. Future studies are also needed to investigate the association between neurocognitive and clinical outcome with brain alterations in more detail.

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