Aus der Klinik für Psychiatrie und Psychotherapie
Geschäftsführender Direktor: Univ.-Prof. Dr. T. Kircher
des Fachbereichs Medizin der Philipps-Universität Marburg

Der Zusammenhang von Bindungssicherheit und einer Oxytocinrezeptor-Genvariation mit neuralen Korrelaten sozialer Kognition und Hirnmorphometrie

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Dipl. Biochem. Dipl. Psych. Henriette Schneider-Haßloff aus Berlin

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Abkürzungsverzeichnis

ACC	anteriores Cingulum
AL	Anzahl emotionaler Verlusterfahrungen
ANX	ängstlicher Bindungsstil
AV	vermeidender Bindungsstil
BLA	basolaterale Amygdala
CAS	kindliche Bindungssicherheit (childhood attachment security)
CG	Gyrus calcarinus
FG	fusiformer Gyrus
GxU	Gen-Umwelt (gene-environment, GxE)
IFG	inferiore(r) Frontalgyrus/-gyri
IoccG	inferiorer okzipitaler Gyrus
IPL	inferiore(r) Parietallappen
LG	Gyrus lingualis
MCC	mittleres Cingulum
MFG	mittlere(r) Frontalgyrus/-gyri
MRT	Magnetresonanztomographie
OXT	Oxytocin
OXTR	Oxytocinrezeptor
ParaCL	parazentrale(r) Lobulus/-i
PostCG	postzentrale(r) Gyrus/-i
PreCG	präzentrale(r) Gyrus/-i
Precun	Precuneus/-i
SFG	superiore(r) Frontalgyrus/-gyri
SPL	superiore(r) Parietallappen
SoccG	superiorer Okzipitalgyrus
ToM	Theory-of-mind, Mentalisierung
TP	Temporalpol/-e
TPJ	temporoparietale(r) Übergang/-gänge
VBM	voxelbasierte Morphometrie (voxel-based morphometry)

Zusammenfassung

Die Entwicklung des menschlichen Gehirns sowie soziokognitiver und -emotionaler Fähigkeiten wird nicht nur von genetischen Faktoren, sondern auch von frühen Fürsorgeerfahrungen beeinflusst. Die Bindungstheorie liefert ein Konzept für das Verständnis der soziokognitiven und -emotionalen Entwicklung des Menschen als Folge früher sozialer Fürsorgeerfahrungen und biologischer Veranlagungen. Individuelle Bindungsstile entwickeln sich im Laufe des Lebens zu einer relativ stabilen Persönlichkeitseigenschaft, die soziale Kognitionen, soziales Verhalten und den Umgang mit belastenden Lebensereignissen (wie z.B. emotionalen Verlusterfahrungen) beeinflusst. Auf physiologischer Ebene ist das menschliche Bindungssystem mit dem Oxytocinsystem verknüpft, das allgemein eine bedeutsame Funktion für soziales Verhalten und soziale Kognitionen hat. Das Oxytocinsystem wird durch frühe Eltern-Kind-Interaktionen in seiner Entwicklung geprägt. In humangenetischen Studien war der Genpolymorphismus rs53576 des Oxytocinrezeptors (OXTR) mit Unterschieden in der Sensitivität für soziale Reize assoziiert und interagierte mit frühen sozialen Lebenserfahrungen auf die Ausprägung sozioemotionaler Persönlichkeitseigenschaften.

Fragestellung: In dieser Arbeit sollte untersucht werden, wie neurale Korrelate der Mentalisierung und die Hirnmorphometrie a) mit der kindlichen Bindungssicherheit unter Berücksichtigung des OXTR-Genpolymorphismus rs53576 und b) mit dem Bindungsstil im Erwachsenenalter zusammenhängen.

Methoden: In einer Stichprobe von gesunden Student(inn)en wurden die neuralen Korrelate der Mentalisierung unter Anwendung der funktionellen Magnetresonanztomographie (MRT) während der Bearbeitung einer sozial interaktiven Theory-of-Mind-Aufgabe (ToM, Gefangenendilemma, n= 164) erhoben. Die Hirnmorphometrie wurde mithilfe der strukturellen MRT und dem Verfahren der voxelbasierten Morphometrie (VBM, n=196) als Volumen der grauen Substanz bestimmt. Des Weiteren wurden Fragebögen eingesetzt, um die kindliche Bindungssicherheit (CAS, „Hazan-Shaver“-Skala), den Bindungsstil im Erwachsenenalter („Relationship Scales Questionnaire“, Subskalen „ängstlicher Bindungsstil“ (ANX) und „vermeidender Bindungsstil“ (AV)), die Alexithymie im Erwachsenenalter („Toronto Alexithymia Scale 20“) und die Anzahl emotionaler Verlusterfahrungen (AL, „List of Threatening Experiences Questionnaire”,

VBM: n=192) zu erfassen. Die OXTR-Genvariation rs53576 (G/A) wurde durch Genotypisierung der DNA aus Blutproben bestimmt (ToM: n=163, VBM: n=195).

Ergebnisse: Signifikante Interaktionseffekte von rs53576 und CAS (d.h. eine GxU-Interaktion) zeigten sich für ANX, Alexithymie, Hirnstruktur und -funktion: Strukturelle GxU-Interaktionseffekte wurden in einem bilateralen fronto-parietalen und links-temporalen Netzwerk (einschließlich Hippokampus und Amygdala) beobachtet. Funktionelle GxU-Interaktionseffekte fanden sich in einem rechtsfrontalen und bilateralen parieto-temporo-okzipitalen ToM-assoziierten Netzwerk. GG-Homozygote waren im Vergleich zu A-Allel-Trägern empfänglicher für CAS in Bezug auf das Volumen grauer Substanz, ANX und Alexithymie. Einige der beobachteten GxU-Interaktionseffekte waren sexuell dimorph. Strukturelle und funktionelle GxU-Interaktionseffekte überlappten zum Teil regional und waren, wie exploratorische Regressionsanalysen zeigten, untereinander und mit der Ausprägung von ANX und Alexithymie assoziiert. Des Weiteren wurde bei GG-Homozygoten ein signifikant höheres Volumen der grauen Substanz im Temporalpol und Hippokampus beobachtet. Die Bindungsstile des Erwachsenenalters AV und ANX unterschieden sich signifikant in ihrem Zusammenhang mit ToM-assoziierten neuralen Aktivierungen (u.a. in den bilateralen inferioren Frontalgyri (IFG), dem rechten mittleren Cingulum und der Amygdala) und dem Volumen der grauen Substanz im Pars opercularis des linken IFG.

Diskussion: Interaktionseffekte von CAS und rs53576 wurden insbesondere für das Volumen und die Aktivierung von Hirnregionen beobachtet, die in soziale Kognitionen wie ToM und das Spiegelneuronensystem involviert sind. Des Weiteren zeigten sich strukturelle GxU-Interaktionseffekte und genetische Haupteffekte in Hirnarealen mit Funktionen für die Gedächtnisbildung. Genetische Effekte auf das Gedächtnis und/oder epigenetische Mechanismen könnten den beobachteten GxU-Interaktionseffekten auf Hirnstruktur, -funktion und Persönlichkeitseigenschaften zugrunde liegen. Die Bindungsstile ANX und AV waren signifikant unterschiedlich mit ToM-assoziierten neuralen Aktivierungen und mit dem Volumen von Hirnregionen assoziiert, die in die Emotionsregulation involviert sind. Die Ergebnisse dieser Arbeit tragen zu einem besseren Verständnis der biologischen Aspekte von Bindung bei. Sie liefern weitere Hinweise, wie sich die neurobiologischen Grundlagen der sozialen Kognition im Zusammenspiel von Bindungssicherheit und Genetik möglicherweise entwickeln.

Abstract

Human socio-cognitive, -emotional and neural brain development is not only shaped by genes, but also by early caregiving experiences. A framework for the understanding of the socio-cognitive and -emotional development as a consequence of early caregiving experiences and biological dispositions is offered by attachment theory. Individual attachment styles evolve over time into a moderately stable personality trait that affect social cognition, social behavior and coping with stressful life events (like e.g. affective loss experiences). The attachment system is physiologically connected to the oxytocin system. The oxytocin system plays a significant role in human social behavior and cognition and its development is shaped by early parent-infant interactions. In human genetic approaches the polymorphism of the oxytocin receptor (OXTR) gene rs53576 has been linked to varying sensitivity to social stimuli. Rs53576 also interacted with early social experiences to modulate socioemotional personality traits.

Questions: This research project addressed the question how neural correlates of mentalizing and brain morphometry are associated a) with childhood attachment security taking the OXTR gene polymorphism rs53576 into account and b) with adult attachment style.

Methods: In a sample of healthy students the neural correlates of mentalizing were investigated by functional magnetic resonance imaging (MRI) during a socially interactive theory-of-mind task (ToM, Prisoner's Dilemma Game, n=164). Brain morphometry was assessed as brain gray matter volume applying structural MRI and voxel-based morphometry (VBM, n=196). Questionnaires were administered to assess childhood attachment security (CAS, Hazan-Shaver Scale), adult attachment style (Relationship Scales Questionnaire, subscales „anxiety“ (ANX) and „avoidance“ (AV)), alexithymia (Toronto Alexithymia Scale 20) and the number of affective loss experiences (AL, List of Threatening Experiences Questionnaire, VBM: n=192). The OXTR gene variant rs53576 (G/A) was assessed by genotyping of DNA from blood samples (ToM: n=163, VBM: n=195).

Results: Rs53576 and CAS significantly interacted (i.e. GxE-interaction) on ANX, alexithymia, brain morphometry and function: Structural GxE-interaction effects were

observed in a bilateral fronto-parietal and left temporal network (including hippocampus and amygdala). Functional GxE-interaction effects were found in a right frontal and bilateral parieto-temporo-occipital ToM-related neural network. GG-homozygotes compared to A-allele carriers were more susceptible to CAS with regard to brain morphometry, ANX and alexithymia. Some of the observed GxE-interaction effects were sexually dimorphic. Structural and functional GxE-interaction effects partially overlapped in several brain regions. Exploratory regression analyses showed that both structural and functional GxE-interaction effects were associated with each other and with ANX and alexithymia. Additionally GG-homozygotes compared to A-allele carriers displayed significantly higher gray matter volume in temporal poles and hippocampus. The adult attachment styles AV and ANX significantly differed in their association with ToM-related neural activations (in bilateral inferior frontal gyri (IFG), right middle cingulate cortex and amygdala among others) and brain gray matter volume in the pars opercularis of the left IFG.

Discussion: Interaction effects of CAS and rs53576 on brain gray matter volume and ToM-related neural activations were particularly observed in brain areas that are involved in social cognition like ToM and the mirror neuron system. Structural GxE-interaction effects and genetic main effects were found in brain regions that are related to memory functions. Genetic effects on memory and/or epigenetic mechanisms could play a role in the observed GxE-interaction effects on brain structure, brain function and socioemotional personality traits. AV and ANX significantly differed in their association with ToM-associated neural activations and with the volume of brain regions which play a role in emotion regulation.

The results of this research are a further step in understanding the biological underpinnings of attachment. They offer new insights how the neurobiological foundations of social cognition might develop in an interplay of attachment security and genetic variations.

1. Einleitung

Bei Säugetieren ist die Hirnreifung nicht vollständig genetisch determiniert, sondern sie ist abhängig von angemessener, spezies-typischer Stimulation durch die Umgebung und wird geprägt durch Umgebungsreize, die individuell sein können (Greenough, Black, & Wallace, 1987). Wie in Studien an Tier und Mensch gezeigt werden konnte, haben insbesondere frühe Fürsorgeerfahrungen einen wichtigen Einfluss auf die Hirnentwicklung (Bernier, Calkins, & Bell, 2016; Champagne & Curley, 2009; Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012) sowie auf die soziokognitive und -emotionale Entwicklung (Bowlby, 1969; Champagne & Curley, 2009; Sroufe, 2005). Das Ausmaß, in dem die individuelle Entwicklung für Variationen in frühen Fürsorgeerfahrungen empfänglich ist, wird auch vom individuellen Genotyp bestimmt (z. B. Bradley et al., 2011; Caspi et al., 2003; Salo et al., 2011).

1.1 Die Bindungstheorie

1.1.1 Einführung

Ein Konzept für das Verständnis der sozioemotionalen und -kognitiven Entwicklung des Menschen als Folge früher Fürsorgeerfahrungen und biologischer Veranlagungen liefert die Bindungstheorie von Bowlby (1969). Nach Bowlby (1969) gibt es eine angeborene Motivation des Menschen, eine Bindung, d.h. ein selektives emotionales Band, zu einer anderen Person aufzubauen. Bowlby (1969) postuliert, dass das Bindungsverhalten dem Schutz und der Fürsorge des Kindes dient und sich aus phylogenetischen Selektionsbedingungen heraus entwickelt hat. Darüber hinaus wird vermutet, dass eine sichere Bindung die Entwicklung von Hirnstrukturen fördert, die in soziale Kognitionen involviert sind, und das Bindung damit das Kind für die Kooperation mit anderen vorbereitet (Fonagy, Gergely, & Target, 2007).

Das Bindungssystem wird durch physiologische und psychologische Bedrohungen aktiviert und veranlasst das Kind, die Nähe der Bindungsperson zu suchen, um Schutz und emotionale Beruhigung zu erfahren. Bindungspersonen können auf das nähesuchende Verhalten des Kindes unterschiedlich verfügbar und schutzgebend reagieren und damit das kindliche Bindungsverhalten prägen. Gemäß Bowlby (1969) speichert das Kind (wiederholte) Bindungserfahrungen in inneren Arbeitsmodellen (d.h. kognitiv-

affektiven Strukturen, „internal working models") ab, die bis ins Erwachsenenalter hinein moderat stabil bleiben (Fraley, 2002). Interaktionen mit verfügbaren Bindungspersonen fördern die Entwicklung bzw. Aufrechterhaltung eines sicheren Bindungsstils, der sich durch Vertrauen in die Unterstützung durch die Bindungspersonen auszeichnet. Interaktionen mit unzureichend verfügbaren und/oder abweisenden Bindungspersonen führen zur Ausbildung „sekundärer Bindungsstrategien", die charakteristisch für unsicher gebundene Individuen sind (Mikulincer & Shaver, 2007). Der individuelle Bindungsstil entwickelt sich damit im Laufe des Lebens zu einer relativ stabilen Persönlichkeitseigenschaft und beeinflusst im Erwachsenenalter soziale Kognitionen und soziales Verhalten, insbesondere die Gestaltung von Beziehungen mit nahestehenden Personen, aber auch mit Fremden (Mikulincer & Shaver, 2007). Genetische Faktoren spielen für die Ausprägung des Bindungsstils im Erwachsenenalter ebenfalls eine Rolle, die Varianzaufklärung beträgt bis zu 45% (Brussoni, Jang, Livesley, & Macbeth, 2000; Crawford et al., 2007; Donnellan, Burt, Levendosky, & Klump, 2008).

In der Regel werden zwei Dimensionen des Bindungsstils im Erwachsenenalter unterschieden: der vermeidende Bindungsstil (AV) und der ängstliche Bindungsstil (ANX). Diese können als orthogonale Skalen reliabel über Selbstberichte erfasst werden (Kurdek, 2002; Mikulincer & Shaver, 2007; Simpson, Rholes, & Nelligan, 1992). Hohe Werte in AV und/oder ANX kennzeichnen einen unsicheren Bindungsstil, ein sicherer Bindungsstil zeichnet sich durch niedrige Werte sowohl in AV als auch in ANX aus (Mikulincer & Shaver, 2007).

AV ist charakterisiert durch Strategien der Hemmung des Bindungssystems, wie z.B. eine inhibierte Aufmerksamkeit für Emotionen, die das Bindungssystem anregen könnten, und eine Bevorzugung kognitiver Informationsverarbeitung. Vermeidend gebundene Personen streben nach Unabhängigkeit und Distanz und sie vermeiden das Aufsuchen von Hilfe, zeigen jedoch eine erhöhte physiologische Stressreaktion in belastenden Situationen (Crittenden, 1995; Mikulincer & Shaver, 2007).

ANX zeichnet sich durch ein übermäßig angeregtes Bindungssystems aus. Dies zeigt sich z.B. in einer erhöhten Aufmerksamkeit für oder auch in einer Intensivierung von angstauslösenden Gedanken, Gefühlen und Erinnerungen. Typischerweise werden ein überwiegend affektiver Kommunikationsstil und übertriebenes Nähesuchen, aber auch eine Ambivalenz bzgl. der Unterstützung durch andere beobachtet (Crittenden, 1995;

Mikulincer & Shaver, 2007).

In einigen bildgebenden Studien wurde die Assoziation der Bindungsstile AV und ANX mit neuralen Aktivierungen bei der Verarbeitung affektiver und/oder bindungsrelevanter Reize untersucht: Hierbei wurde beobachtet, dass AV die Aktivierung von Hirnregionen moduliert, die bei der Verarbeitung von Schmerz, Konflikt, Emotionsregulation und Belohnung rekrutiert werden, wie z.B. dorsales anteriores Cingulum (ACC), Insula, lateraler präfrontaler Kortex und ventrales Striatum (DeWall et al., 2012; Gillath, Bunge, Shaver, Wendelken, & Mikulincer, 2005; Suslow et al., 2009; Vrtička, Andersson, Grandjean, Sander, & Vuilleumier, 2008; Vrtička, Bondolfi, Sander, & Vuilleumier, 2012). Dagegen war ANX mit der Aktivierung des dorsalen ACC, der Amygdala und des Hippocampus assoziiert; dies wurde als erhöhte Vigilanz, Salienz und Gedächtnisbildung für emotionale Reize interpretiert (DeWall et al., 2012; Donges et al., 2012; Gillath et al., 2005; Vrtička et al., 2008; Vrtička et al., 2012). Des Weiteren wurde in zwei Studien untersucht, in wieweit AV und ANX mit dem Volumen der grauen Hirnsubstanz zusammenhängen: AV und ANX waren jeweils mit einem reduzierten Hippokampusvolumen assoziiert (Quirin, Gillath, Pruessner, & Eggert, 2010) und ANX modulierte das Volumen des linken lateralen Orbitalgyrus und des rechten anterioren Temporalpols (Benetti et al., 2010). Dies wurde als mit dem Bindungsstil assoziierte Variation des Volumens in Hirnregionen, die in die Emotions- und Stressregulation involviert sind, interpretiert (Benetti et al., 2010; Quirin et al., 2010).

1.1.2 Bindungsstile und emotionale Verlusterfahrungen

Emotionale Verlusterfahrungen (wie z.B. Verluste der Bindungsperson/des Partners durch Tod oder Trennung) stellen belastende Lebensereignisse dar (Boyle, Feng, & Raab, 2011; Tennant & Andrews, 1976). Die Bewältigung dieser Verlusterfahrungen ist u.a. durch Persönlichkeitseigenschaften wie den Bindungsstil beeinflusst.

Nach einer Trennung vom Partner berichteten ängstlich gebundene Personen ein hohes Maß an emotionalen und physischen Stressreaktionen, nähesuchendem Verhalten und sozialen Bewältigungsstrategien. Vermeidend gebundene Personen berichteten dagegen vergleichsweise schwächere Stressreaktionen sowie mehr selbstständige und weniger soziale Bewältigungsstrategien (Davis, Shaver, & Vernon, 2003). Nach dem Tod einer nahestehenden Person war ANX mit höherer Depressivität und AV

mit mehr Somatisierung assoziiert (Wayment & Vierthaler, 2002). Ein unsicherer Bindungsstil war insgesamt mit einer langsameren emotionalen Genesung nach Trennungserfahrungen (Sbarra, 2006) und höherer Depressivität nach dem Verlust nahestehender Personen durch Tod verknüpft (Wayment & Vierthaler, 2002). In der Studie von Benetti et al. (2010) hing die Anzahl emotionaler Verlusterfahrungen (operationalisiert als Verlust eines Verwandten, eines engen Freundes und/oder als Trennung vom Ehepartner in den vergangenen 5 Jahren) mit einem erhöhten Volumen grauer Substanz im linken Cerebellum zusammen, und dieser Zusammenhang wurde von AV moderiert.

Zusammenfassend kann festgehalten werden, dass individuelle Variationen im Bindungsstil mit Unterschieden in der Bewältigung von emotionalen Verlust-erfahrungen verknüpft sind.

1.1.3 Bindung und Mentalisierung

Verhaltensstudien in der Kindheit haben gezeigt, dass die Entwicklung soziokognitiver Fähigkeiten wie z.B. der Mentalisierung (ToM) mit der Bindungssicherheit zur Mutter (De Rosnay & Harris, 2002; Fonagy, Redfern, & Charman, 1997; Meins, Fernyhough, Russell, & Clark-Carter, 1998; Symons & Clark, 2000) und zum Vater (Fonagy et al., 2007) zusammenhängt. Zudem wurde beobachtet, dass die Fähigkeit zur affektiven ToM mit dem Bindungsstil im Jugendlichen- und Erwachsenenalter assoziiert ist (Huenefeldt, Laghi, & Ortu, 2013; Huenefeldt, Laghi, Ortu, & Belardinelli, 2013). Mentalisierung (ToM) ist als Fähigkeit definiert, menschliches Verhalten durch Zuschreibung mentaler Zustände zu erklären und vorherzusagen. Diese Fähigkeit entwickelt sich im Laufe des Lebens und ist eine wichtige Voraussetzung für soziale Kompetenz (Premack & Woodruff, 1978; Repacholi & Slaughter, 2003). In einer Meta-Analyse bildgebender Studien wurde von Mar (2011) ein ToM-assoziiertes neurales Netzwerk identifiziert, dass u.a. die bilateralen medialen präfrontalen Kortizes, die Temporalpole, die Sulci temporalis superior, die posterioren Cingula, die Precunei und den linken IFG umfasst (Mar, 2011). Mentale Zustände können Wissen und Glaubensinhalte, aber auch Emotionen und Intentionen beinhalten, und dementsprechend wird häufig zwischen kognitiver und affektiver ToM unterschieden (Abu-Akel & Shamay-Tsoory, 2011; Brothers & Ring, 1992; Fonagy & Luyten, 2009).

Abu-Akel und Shamay-Tsoory (2011) postulierten, dass kognitive und affektive ToM-Prozesse unterschiedliche neurale Netzwerke involvieren, u.z. dorsale neurale Schleifen (mit den dorsalen Anteilen des lateralen und medialen präfrontalen Kortex, des ACC, des Temporalpols und Striatums) für die Verarbeitung kognitiver mentaler Zustände, und überwiegend ventrale neurale Pfade (mit Amygdala, inferiorem lateralen frontalen und orbitofrontalen Kortex sowie ventralen Teilen vom medialen präfrontalen Kortex, ACC, Temporalpol und Striatum) für die Verarbeitung affektiver mentaler Zustände.
ToM-assoziierte neurale Aktivierungen können über geschichten- oder spielbasierte Aufgaben untersucht werden (Mar, 2011). Interaktiven Spielen wie dem Gefangenen-dilemma („Prisoner's Dilemma Game“) wird die Eigenschaft zugeschrieben, reale Lebenssituationen modellieren zu können und die implizite Erfassung von ToM-Prozessen zu ermöglichen (Kircher et al., 2009; Krach et al., 2008; Rilling et al., 2012; Rilling, Sanfey, Aronson, Nystrom, & Cohen, 2004).

1.2 Das Oxytocinsystem

1.2.1 Einführung

Soziales Verhalten und soziale Kognitionen, d.h. z.B. Bindung, Mentalisierung, Emotionserkennung und soziales Gedächtnis, werden durch das Oxytocinsystem beeinflusst, wie in Studien an Mensch und Tier gezeigt werden konnte (Heinrichs, von Dawans, & Domes, 2009). Des Weiteren moduliert das Oxytocinsystem die Regulation von Angst und physiologischen Stressreaktionen (Dabrowska et al., 2011; Gimpl & Fahrenholz, 2001; Heinrichs et al., 2009; Neumann & Slattery, 2015; Olff et al., 2013) sowie die Aktivität von Hirnregionen (wie z.B. der Amygdala), die in die Verarbeitung von Emotionen und motivational salienter Reize involviert sind (Bethlehem, van Honk, Auyeung, & Baron-Cohen, 2013; Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012; Gamer, Zurowski, & Büchel, 2010). Das Neuropeptid Oxytocin (OXT) und der Oxytocinrezeptor (OXTR) werden im zentralen Nervensystem und im peripheren Gewebe exprimiert. Im Gehirn wird OXT primär in den para-ventrikulären und supraoptischen Nuclei des Hypothalamus produziert und in der Neurohypophyse und vielen extrahypothalamischen Hirnregionen freigesetzt. Im Menschen konnte der OXTR im limbischen System (u.a. in der Amygdala), im dorsalen ACC, Striatum, basalen Vorderhirn und Hirnstamm nachgewiesen werden (Boccia,

Petrusz, Suzuki, Marson, & Pedersen, 2013; Gimpl & Fahrenholz, 2001; Skuse & Gallagher, 2009). Das Oxytocinsystem wird u.a. durch Geschlechtshormone reguliert und ist zum Teil sexuell dimorph. Frauen haben eine höhere OXTR-Dichte im medialen präfrontalen Kortex und eine höhere Plasma-OXT-Konzentration, und die Paarbindung wird bei Frauen mehr durch OXT beeinflusst als bei Männern (Carter, 2007; Carter, Boone, Pournajafi-Nazarloo, & Bales, 2009; Gimpl & Fahrenholz, 2001; Insel & Hulihan, 1995; Kramer, Cushing, Carter, Wu, & Ottinger, 2004; Smeltzer, Curtis, Aragona, & Wang, 2006). Die Freisetzung von OXT in das periphere Blut- und das zentrale Nervensystem wird durch reproduktive und stressvolle Reize ausgelöst (Heinrichs et al., 2009; Neumann & Slattery, 2015). Gemäß der „sozialen Salienzhypothese“ wird vermutet, dass OXT die Aufmerksamkeit und Gedächtnisbildung für soziale Reize erhöht, indem es deren Salienz mittels Einbindung des mesolimbischen dopaminergen Systems steigert (Bartz, Zaki, Bolger, & Ochsner, 2011).

1.2.2 Genetische Variationen im OXT-System

In humangenetischen Studien wurden Variationen im OXTR-Gen untersucht, um die Bedeutung des Oxytocinsystems für das menschliche Verhalten und assoziierte Hirnfunktionen weiter aufzuklären (Donaldson & Young, 2008; Ebstein, Knafo, Mankuta, Chew, & Lai, 2012). Die am besten untersuchte Genvariation des OXTR-Gens ist der Einzelnucleotid-Polymorphismus rs53576 (G/A) (Li et al., 2015). Rs53576 ist im dritten Intron des OXTR-Gens lokalisiert, seine Funktionalität ist bislang jedoch unbekannt. Es konnte mehrfach gezeigt werden, dass GG-Homozygote von rs53576 sensibler für soziale Reize sind: Sie zeigten eine höhere affektive Empathie (Rodrigues, Saslow, Garcia, John, & Keltner, 2009), ein feinfühligeres Elternverhalten (Bakermans-Kranenburg & van Ijzendoorn, 2008), ein höheres Vertrauen im Verhalten (Krueger et al., 2012) und eine stärkere Belohnungsabhängigkeit (Tost et al., 2010).

In einer bildgebenden Studie wurde beobachtet, dass GG-Homozygote von rs53576 die Amygdala während der Verarbeitung von Gesichtsausdrücken in einem höheren Ausmaß aktivierten. Zudem korrelierte das Volumen der grauen Substanz in der Amygdala negativ mit der genotyp-assoziierten Belohnungsabhängigkeit (Tost et al., 2010).

Rs53576 war auch signifikant mit der Leistung in affektiver Mentalisierung assoziiert,

allerdings waren die Befunde inkonsistent bzgl. der Risikoallele (Lucht et al., 2013; Rodrigues et al., 2009). Zudem wurde die Bindungssicherheit durch rs53576 in depressiven (Costa et al., 2009), aber nicht in gesunden Erwachsenen (Bradley et al., 2011; Gillath, Shaver, Baek, & Chun, 2008; Rodrigues et al., 2009) moduliert. Für die weitere Aufklärung dieser letztgenannten Befunde könnten Gen-Umwelt-Interaktionsanalysen hilfreich sein.

1.2.3 Gen-Umwelt-Interaktionen im OXT-System

Es gibt Hinweise aus Tier- und Menschenstudien, dass das OXT-System durch soziale Erfahrungen im frühen Leben geformt wird, insbesondere durch Eltern-Kind-Interaktionen (Bales & Perkeybile, 2012; Ross & Young, 2009). Bei Ratten wurden langfristige Effekte mütterlicher Fürsorge auf die OXTR-Dichte und auf das soziale Verhalten im Erwachsenenalter beobachtet (Champagne, Diorio, Sharma, & Meaney, 2001; Francis, Young, Meaney, & Insel, 2002; Lukas, Bredewold, Neumann, & Veenema, 2010), die vermutlich über epigenetische Modifikationen vermittelt werden (Champagne & Curley, 2009; Kumsta, Hummel, Chen, & Heinrichs, 2013; Zhang & Meaney, 2010). Beim Menschen war Kindesmissbrauch mit langfristigen Änderungen der peripheren und zentralen OXT-Konzentration (Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005; Heim et al., 2009) und mit veränderter OXT-induzierter Modulation der funktionellen Konnektivität soziokognitiver Netzwerke verknüpft (Riem et al., 2013).

In GG-Homozygoten von rs53576, aber nicht in A-Allel-Trägern, modulierten Kindesmissbrauch bzw. kindliche Bindungssicherheit den Bindungsstil, die Emotionsregulation sowie Symptome der Internalisierung im Erwachsenenalter (Bradley et al., 2011; Hostinar, Cicchetti, & Rogosch, 2014; Raby, Cicchetti, Carlson, Egeland, & Collins, 2013). Es wurde daraufhin postuliert, dass Variationen in rs53576 mit einer unterschiedlichen Suszeptibilität für die Langzeitfolgen von Kindheitserfahrungen einhergehen.

1.3 Bildgebende Verfahren

Im Rahmen der hier vorliegenden Arbeit wurden als bildgebende Verfahren des Gehirns die strukturelle und funktionelle Magnetresonanztomographie (MRT, „magnetic resonance imaging“) eingesetzt, die sich den Kernmagnetismus des Wasserstoffprotons (^{1}H) zunutze machen. Für eine ausführliche Darstellung der MRT-

Grundlagen wird auf weiterführende Literatur verwiesen (z.B. Günther, 1992; Stöcker & Shah, 2013).

1.3.1 Funktionelle MRT

In der funktionellen MRT kann die Kontrastbildung zwischen aktiven und inaktiven Hirnarealen mittels verschiedener Techniken erzeugt werden, am häufigsten wird jedoch die nichtinvasive BOLD-fMRT angewendet (Stöcker & Shah, 2013). Diese beruht auf dem BOLD-Effekt („blood oxygen level dependency“), d.h. dem Oxygenierungsgrad des Blutes. Der zeitliche Verlauf des MR-Signals, das durch die neuronale Aktivität auf einen Stimulus hin ausgelöst wird, wird durch die hämodynamische Antwortfunktion („hemodynamic response function“, HRF) beschrieben (Huettel, Song, & McCarthy, 2004; Logothetis & Wandell, 2004; Stöcker & Shah, 2013): Diese erstreckt sich über mehrere Sekunden und zeigt eine hohe inter- und intraindividuelle Variabilität. Das BOLD-Signal steht überwiegend in einem linearen Zusammenhang mit der neuralen Aktivität (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). Das BOLD-Signal scheint jedoch vor allem mit den lokalen Feldpotentialen zu korrelieren, und damit mehr die neurale Aktivität, die mit dem Input in eine Region sowie der lokalen Verarbeitung in Verbindung steht, abzubilden, und weniger die Spiking-Aktivität des Outputs einer Region (Logothetis et al., 2001; Logothetis & Wandell, 2004).

1.3.2 Strukturelle MRT und voxelbasierte Morphometrie

Strukturelle MRT-Bilder des Gehirns erlauben die quantitative Beschreibung der Größe und Form der Makrostruktur des Gehirns (mit einer Auflösung von bis zu 1 mm). Mithilfe der voxelbasierten Morphometrie (VBM) kann die lokale Gewebeverteilung quantifiziert werden, d.h. es können lokale Unterschiede im Volumenanteil der grauen Substanz untersucht werden (Ashburner & Friston, 2000; Pieperhoff, Dickscheid, & Amunts, 2013). Insgesamt liefert die VBM ein gemischtes Maß für die kortikale graue Substanz, Oberfläche, Faltung und Dicke (Hutton, Draganski, Ashburner, & Weiskopf, 2009).

1.4 Fragestellungen und Ziele

Ziel dieser Arbeit war es zu untersuchen, wie menschliche Bindungssicherheit unter Berücksichtigung genetischer Faktoren bzw. emotional belastender Bindungserfahrungen mit neuralen Aktivierungen bei einer sozialen Kognitionsaufgabe, mit der makroanatomischen Hirnstruktur und/oder mit der Persönlichkeit im Erwachsenenalter zusammenhängt.

Konkret sollte im Rahmen dieser Arbeit zum einen die Frage bearbeitet werden, wie kindliche Bindungssicherheit in Interaktion mit dem Polymorphismus des OXTR-Gens rs53576 mit neuralen Aktivierungen bei einer sozial interaktiven ToM-Aufgabe, dem Volumen der grauen Hirnsubstanz sowie dem Bindungsstil und der Alexithymie im Erwachsenenalter zusammenhängt. Die Interaktionseffekte von kindlicher Bindungssicherheit und rs53576 auf die neuralen Korrelate der Mentalisierung, auf die Hirnstruktur und die Alexithymie waren bis dahin nicht untersucht worden.

Zum anderen sollte erstmals untersucht werden, wie der Bindungsstil im Erwachsenenalter mit neuralen Aktivierungen bei einer sozial interaktiven ToM-Aufgabe zusammenhängt.

Schließlich sollte untersucht werden, wie der Bindungsstil im Erwachsenenalter, emotionale Verlusterfahrungen und deren Interaktion mit dem Volumen der grauen Substanz im Erwachsenenalter zusammenhängen. Diese Frage war bereits Gegenstand von Studien, und es sollte in dieser Arbeit erforscht werden, ob die vorhandenen Befunde repliziert werden können.

2. Zusammenfassung eigener Arbeiten

Im Folgenden werden die Hypothesen und Ergebnisse der einzelnen Manuskripte dargestellt und diskutiert. Für Tabellen und Grafiken wird auf die entsprechenden Manuskripte (inklusive Supplement) verwiesen.

2.1 Manuskript #1

2.1.1 Hypothesen

Ausgehend von den unter 1.1 beschriebenen theoretischen Überlegungen und den empirischen Befunden zum Zusammenhang zwischen Bindungssicherheit/-stil und Mentalisierungsfähigkeit (De Rosnay & Harris, 2002; Fonagy et al., 2007, 1997; Huenefeldt, Laghi, & Ortu, 2013; Huenefeldt, Laghi, Ortu, et al., 2013; Mikulincer & Shaver, 2007; Symons & Clark, 2000) lautete die Hypothese, dass individuelle Unterschiede im Bindungsstil des Erwachsenenalters mit unterschiedlichen neuralen Hirnaktivierungsmustern bei der Bearbeitung einer ToM-Aufgabe einhergehen. Als ToM-Aufgabe wurde ein interaktives Spiel, das Gefangenendilemma, ausgewählt. Die erste Hypothese war, dass Personen mit einem vermeidenden Bindungsstil (AV) verstärkt Hirnareale aktivieren, die nach Abu-Akel und Shamay-Tsoory (2011) in kognitive ToM-Prozesse involviert sind (d.h. dorsolateraler und -medialer präfrontaler Kortex sowie die dorsalen Bereiche von anteriorem Cingulum, Temporalpol und Striatum). Die zweite Hypothese lautete, dass ein ängstlicher Bindungsstil (ANX) mit einer erhöhten neuralen Aktivierung des affektiven ToM-Netzwerkes einhergeht (d.h. Amygdala, ventromedialer/inferiorer lateraler präfrontaler und orbitofrontaler Kortex, ventrale Bereiche von anteriorem Cingulum, Temporalpol, Striatum) (Abu-Akel & Shamay-Tsoory, 2011; Crittenden, 1995; Fonagy et al., 2007; Huenefeldt, Laghi, & Ortu, 2013).

2.1.2 Ergebnisse

Während der ToM-Aufgabe im Kontrast zur Kontrollbedingung (T>C) wurden u.a. folgende Hirnareale signifikant aktiviert: bilaterale superiore (SPL) und inferiore (IPL) Parietallappen, superiore Frontalgyri (SFG), Precunei, Cingula, Temporalpole (TP), IFG, Hippocampi, temporoparietale Übergänge (TPJ), superiore mediale Frontalgyri und in der rechten Hemisphäre medialer orbitaler Frontalgyrus, superiorer Temporalgyrus,

mittlerer Frontalgyrus (MFG) und Amygdala (s. Abb. 1).

Der vermeidende im Vergleich zum ängstlichen Bindungsstil (Kontrast AV > ANX) war signifikant positiver mit ToM-assoziierten Hirnaktivierungen (T>C) in mehreren Hirnarealen korreliert, u.z. im mittleren Cingulum (MCC), basolateraler Amygdala (BLA), MFG und SPL der rechten Hemisphäre und im bilateralen IFG. Korrelative Analysen mit den Skalenwerten von ANX und AV und den extrahierten beta-Gewichten der signifikanten Cluster des Kontrastes (AV > ANX) zeigten, dass AV positiv und ANX negativ mit den neuralen Aktivierungen in den obengenannten Hirnarealen korrelierten (s. Tab. 2 und Abb. 2). Diese Ergebnisse wurden durch ergänzende Analysen gestützt, dazu zählten a) die separate Betrachtung der Korrelationen von AV bzw. ANX mit den ToM-assoziierten Hirnaktivierungen (T>C) (s. Tab. 2), b) ein Vergleich von Personen mit sehr hohen vs. sehr niedrigen ANX-Werten, der aufgrund der unsymmetrischen Verteilung von ANX durchgeführt wurde, sowie c) eine Analyse der ToM-Aufgabe, die die vier möglichen Spielausgänge bzgl. Kooperation („L“) und Nicht-Kooperation („R“) der beiden Spieler („LL“, „LR“, „RL“, „RR“) differenzierte (s. Tab. 3). In den Analysen wurde für die Persönlichkeitsdimensionen Angst und Depressivität kontrolliert. Eine zusätzliche Kontrolle von Geschlecht und Alter änderte die Ergebnisse nicht.

2.1.3 Diskussion

Bei der Bearbeitung der ToM-Aufgabe im Vergleich zur Kontrollbedingung (T>C) wurde in Übereinstimmung mit Arbeiten unserer (Kircher et al., 2009; Krach et al., 2008) und anderer Arbeitsgruppen (Rilling et al., 2004) ein ausgedehntes ToM-assoziiertes neurales Netzwerk aktiviert (Abu-Akel & Shamay-Tsoory, 2011; Mar, 2011).

Es konnte erstmals gezeigt werden, dass AV und ANX im Erwachsenenalter signifikant unterschiedlich die Aktivierung des neuralen ToM-Netzwerks modulieren. Personen mit einem vermeidenden Bindungsstil aktivierten Teile des kognitiven ToM-Netzwerkes (MCC, MFG) signifikant stärker als solche mit einem ängstlichen Bindungsstil (Abu-Akel & Shamay-Tsoory, 2011). Dieses Ergebnis unterstützt die Annahme der Bindungstheorie, der zufolge AV im Vergleich zu ANX mit einer bevorzugten Anwendung kognitiver Strategien einhergeht (Crittenden, 1995; Mikulincer & Shaver, 2007). Allerdings war AV auch mit einer - im Vergleich zu ANX - verstärkten Aktivierung

von Teilen des affektiven ToM-Netzwerkes (IFG, BLA) assoziiert (Abu-Akel & Shamay-Tsoory, 2011). Dieses Ergebnis entsprach nicht der Hypothese, dass eine höhere ANX mit einer stärkeren Rekrutierung von affektiven ToM-Prozessen einhergeht. Diese Hypothese beruhte zum einen auf Ergebnissen aus einer Verhaltensstudie mit Frauen, die darauf hindeuteten, dass ANX mit einer höheren Fähigkeit in affektiver ToM zusammenhängt (Huenefeldt, Laghi, & Ortu, 2013), und zum anderen darauf, dass ANX gemäß der Bindungstheorie durch einen stärker affektiv orientierten Kommunikationsstil charakterisiert ist (Crittenden, 1995; Mikulincer & Shaver, 2007). Es kann nicht ausgeschlossen werden, dass eine ToM-Aufgabe mit stärker affektiven Stimuli (z.B. Gesichtsstimuli wie bei der „Reading the Mind in the Eyes"-Aufgabe) das affektive ToM-Netzwerk bei Personen mit einem ängstlichen Bindungsstil verstärkt ansprechen würde (Huenefeldt, Laghi, & Ortu, 2013). Andererseits ist es auch möglich, eine niedrigere neurale Aktivierung als Zeichen effizienterer Verarbeitung zu interpretieren, so dass die höhere Aktivierung von Teilen des affektiven Netzwerkes eine weniger effiziente Verarbeitung bei höherer AV indizieren würde.

Allerdings sind IFG und BLA gemeinsam mit den anderen im Kontrast (AV > ANX) identifizierten Hirnregionen in vielfältige weitere kognitive und affektive Funktionen involviert. Für eine ausführliche Diskussion wird auf das Manuskript #1 verwiesen. Zusammenfassend war in dieser Studie ein höherer AV-Wert mit stärkeren neuralen Aktivierungen in Hirnregionen assoziiert, die auch für Funktionen der Aufmerksamkeit (SPL, IFG, MFG, MCC) (Abu-Akel & Shamay-Tsoory, 2011; Fox, Corbetta, Snyder, Vincent, & Raichle, 2006), der inhibitorischen und kognitiven Kontrolle (SPL, MFG, MCC) [z.B. Fonagy et al., 2007; Miller and Cohen, 2001; Shackman et al., 2011; Sylvester et al., 2003], der Emotionsregulation (IFG, MCC, MFG, BLA) (Beauregard, Lévesque, & Bourgouin, 2001; Buchheim et al., 2006; Domes et al., 2010), des Vermeidungsverhaltens (MCC, BLA) (Balleine & Killcross, 2006; Klein et al., 2007; Pereira et al., 2010; Shackman et al., 2011) sowie des Empfindens von negativem Affekt (MCC) (Shackman et al., 2011) rekrutiert werden. Diese Interpretationsmöglichkeiten lassen sich gut in die Annahmen der Bindungstheorie und die Ergebnisse von Verhaltens- und bildgebenden Studien einfügen, denen zufolge AV u.a. mit der Herunterregulierung von Emotionen (Mikulincer & Shaver, 2007; Vrtička et al., 2012), besserer Aufmerksamkeitsleistung (Gillath, Giesbrecht, & Shaver, 2009) und höherer

inhibitorischer und kognitiver Kontrolle (Edelstein & Gillath, 2008; Mikulincer, Birnbaum, Woddis, & Nachmias, 2000; Mikulincer & Shaver, 2007; Vrtička et al., 2012) verknüpft ist.

Insgesamt lassen diese Ergebnisse vermuten, dass AV im Vergleich zu ANX während einer interaktiven ToM-Aufgabe mit einer stärkeren Aktivierung neuraler Netzwerke der Emotionsregulation und der kognitiven Kontrolle assoziiert ist.

2.2 Manuskript #2

2.2.1 Hypothesen

Ausgehend von den unter 1.2 beschriebenen Befunden, dass Variationen im OXTR-Genpolymorphismus rs53576 (G/A) mit einer unterschiedlichen Suszeptibilität für die Langzeitfolgen von Kindheitserfahrungen einhergehen (Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013), lautete die Hypothese, dass rs53576 in Abhängigkeit von der kindlichen Bindungssicherheit (CAS) die Persönlichkeitsdimensionen Alexithymie und Bindungsstil im Erwachsenenalter sowie die Hirnstruktur und neuralen Korrelate sozialer Kognition im Erwachsenenalter moduliert (GxU-Interaktion). Die Erwartung war, dass GG-Homozygote von rs53576 empfänglicher für die kindlichen Bindungserfahrungen in Bezug auf den Bindungsstil im Erwachsenenalter und die Ausprägung von Alexithymie sind. Des Weiteren lautete die Hypothese, dass rs53576 und CAS gemeinsam die Hirnstruktur, gemessen als Volumen grauer Substanz, modulieren, u.z. insbesondere in Hirnarealen, die in die Verarbeitung sozialer Salienz (Amygdala) und sozialer Kognition (z.B. ToM-assoziiertes Netzwerk) involviert sind (im Folgenden strukturelle GxU-Interaktionseffekte genannt). Weiterhin lautete die Hypothese, dass die Interaktion von rs53576 und CAS neurale Hirnaktivierungen bei einer ToM-Aufgabe in ToM-assoziierten Hirnarealen moduliert (im Folgenden funktionelle GxU-Interaktionseffekte genannt). Der Annahme folgend, dass Hirn-struktur und -funktion assoziiert sind, lautete die Vermutung, dass sich strukturelle und funktionelle GxU-Interaktionseffekte räumlich im Hirn überlappen. Schließlich wurde explorativ untersucht, ob strukturelle GxU-Interaktionseffekte mit den funktionellen Interaktionseffekten assoziiert sind. Außerdem wurde exploriert, ob die strukturellen und funktionellen GxU-Interaktionseffekte mit den individuellen Ausprägungen im Bindungsstil des Erwachsenenalters und der Alexithymie verknüpft sind.

2.2.2 Ergebnisse

Für die Persönlichkeitsdimensionen Alexithymie und Bindungsstil im Erwachsenenalter wurden signifikante GxU-Interaktionseffekte beobachtet. GG-Homozygote, aber nicht A-Allel-Träger von rs53576, berichteten signifikant höhere Alexithymiewerte bei einer unsicheren im Vergleich zu einer sicheren kindlichen Bindung (s. Abb. 1). Für den ängstlichen Bindungsstil im Erwachsenenalter zeigte sich ein signifikanter geschlechts-spezifischer GxU-Interaktionseffekt: GG-homozygote Frauen berichteten eine höhere Bindungsängstlichkeit bei einer unsicheren im Vergleich zu einer sicheren kindlichen Bindung; bei Frauen mit dem A-Allel und bei Männern beiderlei Genotyps wurde kein vergleichbarer Zusammenhang mit CAS beobachtet (s. Abb. 1). Für die untersuchten Persönlichkeitsdimensionen wurden keine signifikanten Haupteffekte des Genotyps und der kindlichen Bindungssicherheit gefunden.

Des Weiteren wurden signifikante strukturelle GxU-Interaktionseffekte beobachtet: GG-Homozygote mit einer sicheren kindlichen Bindung zeigten ein signifikant höheres Volumen der grauen Substanz in einem bilateralen fronto-parietalen und links-temporalen Netzwerk sowie ein niedrigeres Volumen im Hippokampus und in der Amygdala der linken Hemisphäre im Vergleich zu GG-Homozygoten mit einer unsicheren kindlichen Bindung (s. Tab. 1). Bei den A-Allel-Trägern fand sich ein gegensätzliches Muster, das aber (mit Ausnahme für die linkshemisphärischen Regionen Hippokampus, Amygdala und supramarginalen Gyrus) weniger ausgeprägt war (s. Tab. 1, Abb. 2 und SI-1). In der ROI-Analyse der Amygdala zeigte sich, dass sich GG-Homozygote mit einer sicheren im Vergleich zu einer unsicheren kindlichen Bindung durch ein signifikant niedrigeres Volumen der grauen Substanz in der links-hemisphärischen BLA auszeichneten, während A-Allel-Träger ein höheres Volumen in der BLA bei einer sicheren im Vergleich zu einer unsicheren kindlichen Bindung aufwiesen. Zusätzlich wurden Haupteffekte von CAS und Genotyp beobachtet: Der OXTR-Genotyp war mit signifikanten strukturellen Unterschieden im Temporalpol und Hippokampus (GG > A) sowie im Cerebellum (GG < A) assoziiert. CAS modulierte das Volumen der grauen Substanz u.a. im Precuneus (sicher > unsicher).

Für den funktionellen Kontrast ToM-Aufgabe *vs.* Kontrollbedingung (T>C; siehe auch Manuskript #1) wurden keine Haupteffekte von Genotyp oder CAS, aber GxU-

Interaktionseffekte für die neuralen Aktivierungen in ToM-assoziierten Hirnarealen gefunden (s. Tab. 2). GG-Homozygote mit einer sicheren im Vergleich zu einer unsicheren kindlichen Bindung aktivierten signifikant weniger ein (überwiegend) rechtsfrontales und bilaterales parieto-temporo-okzipitales Netzwerk, während sich bei A-Allel-Trägern das gegensätzliche Muster fand (s. Tab. 2, Abb. 3 und SI-2). Keine signifikante GxU-Interaktion wurde für die Aktivierung der Amygdala, auch nicht in der ROI-Analyse, beobachtet. Strukturelle und funktionelle GxU-Interaktionseffekte überlappten räumlich im rechten SPL, MCC sowie in sensumotorischen Arealen (s. Abb. 4).

In exploratorischen Regressionsanalysen der strukturellen und funktionellen GxU-Interaktionseffekte zeigte sich, dass ein höheres Volumen der grauen Substanz im rechten SPL [(GG > A) x (sicher > unsicher)] signifikant mit niedrigeren neuralen ToM-assoziierten Aktivierungen im bilateralen parieto-okzipitalen Cluster einschließlich der Precunei (Cuneus/Precun/SoccG/SPL) einherging. Des Weiteren war ein höheres Volumen im linken IFG, TP und PreCG [(GG > A) x (sicher > unsicher)] mit signifikant niedrigeren neuralen Aktivierungen im parieto-okzipitalen Cluster (Cuneus/Precun/ SoccG/SPL) und mit höheren Aktivierungen im frontalen Cluster (SFG/MFG/ParaCL/ PostCG/PreCG) assoziiert. Ein höheres Volumen in Hippokampus und Amygdala der linken Hemisphäre [(A > GG) x (sicher > unsicher)] ging mit höheren ToM-assoziierten neuralen Aktivierungen im bilateralen okzipitalen Cluster (LG/CG/FG/IoccG) einher.

Weitere Regressionsanalysen ergaben, dass ein höheres Volumen der grauen Substanz in Hippokampus und Amygdala der linken Hemisphäre [(A > GG) x (sicher > unsicher)] sowie höhere neurale Aktivierungen in bilateralen frontalen Regionen (SFG/MFG/ ParaCL/PostCG/PreCG) signifikant mit höherer ANX zusammenhingen. Höhere Alexithymie-Werte waren mit einem niedrigeren Volumen der grauen Substanz im rechten SPL [(GG > A) x (sicher > unsicher)] verknüpft.

2.2.3 Diskussion

In dieser Studie konnte gezeigt werden, dass der Oxytocinrezeptor-Genpolymorphismus rs53576 (G/A) und die kindliche Bindungssicherheit gemeinsam die Persönlichkeitseigenschaften Alexithymie, ängstlicher Bindungsstil im Erwachsenenalter sowie die Hirnstruktur und ToM-assoziierte neurale Aktivierungen modulieren. Die Daten

stützen die Hypothese, dass eine genetische Variation in rs53576 mit unterschiedlicher Suszeptibilität für frühe soziale Umwelterfahrungen verbunden ist, und sie zeigen die mit dieser GxU-Interaktion verbundenen strukturellen und funktionellen neuralen Korrelate auf.

GG-Homozygote von rs53576, aber nicht A-Allel-Träger, zeigten eine höhere Varianz der Alexithymie-Werte in Abhängigkeit von der kindlichen Bindungssicherheit. Diese Beobachtung lässt sich mit der Hypothese der „differentiellen Suszeptibilität“ in Einklang bringen, die von Belsky und Kollegen (2009) als Alternative zum klassischen Diathese-Stress-Modell aufgestellt wurde: Belsky et al. (2009) postulierten, dass sogenannte „Plastizitätsgene“ ein Individuum empfänglicher für sowohl positive als auch negative Umwelterfahrungen machen. Die Daten dieser Studie bestätigen vorliegende Befunde einer höheren Suszeptibilität von GG-Homozygoten für soziale Faktoren (Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013). Zudem lassen sie vermuten, dass (GG-homozygote) Frauen in Bezug auf ANX suszeptibler für frühe soziale Umweltfaktoren sind als Männer. Der Oxytocinrezeptor wird u.a. durch Sexualhormone reguliert (Gimpl & Fahrenholz, 2001) und geschlechtsspezifische Variationen im Oxytocinsystem als Folge von Fürsorgeerfahrungen sowie ein größerer Einfluss von OXT auf die weibliche im Vergleich zur männlichen Paarbildung sind bereits im Tiermodell beschrieben worden (Bales et al., 2007; Francis et al., 2002; Insel & Hulihan, 1995).

Eine differentielle genotyp-abhängige Suszeptibilität für die kindliche Bindungssicherheit wurde in dieser Studie auch bei der Hirnstruktur beobachtet: Bei GG-Homozygoten im Vergleich zu A-Allel-Trägern variierte in Abhängigkeit von der kindlichen Bindungssicherheit das Volumen der grauen Substanz in einem größeren Ausmaß, u.z. in Hirnregionen, die als Teile des parietofrontalen Spiegelneuronensystems (Cattaneo & Rizzolatti, 2009) und der Theory-of-mind (Abu-Akel & Shamay-Tsoory, 2011; Mar, 2011; Vogeley et al., 2001) angesehen werden. Dieses Ergebnis bestätigte auch die Hypothese, dass rs53576 und kindliche Bindungssicherheit gemeinsam die Struktur von Hirnregionen modulieren, die in soziale Kognitionsprozesse involviert sind.

In Bezug auf das Amygdala-Volumen und auf ToM-assoziierte neurale Aktivierungen fanden sich in dieser Studie bei GG-Homozygoten und A-Allel-Trägern eine

vergleichbare bzw. für A-Allel-Träger teilweise sogar etwas höhere Suszeptibilität für kindliche Bindungserfahrungen. Interessanterweise bildete sich damit im ToM-assoziierten neuralen Aktivierungsmuster nicht der für die (selbstberichtete) Alexithymie beobachtete Suszeptibilitätseffekt ab, obgleich Alexithymie mit kognitiver und affektiver ToM assoziiert ist (Luminet, Vermeulen, Demaret, Taylor, & Bagby, 2006; Moriguchi et al., 2006). Allerdings wirkten sich unsichere und sichere Bindungserfahrungen bei GG-Homozygoten und A-Allel-Trägern gegensätzlich auf die neurale ToM-assoziierte Aktivierungshöhe aus. Eine höhere neurale Aktivierung kann als erhöhte Rekrutierung von ToM-Prozessen oder z.B. als kompensatorische Aktivierung eines weniger funktionalen ToM-Netzwerkes interpretiert werden. Eine weitere Aufklärung dieses Ergebnisses wäre wünschenswert, es könnte jedoch die inkonsistenten Befunde zum Zusammenhang von rs53576 und ToM-Fähigkeiten erklären (Lucht et al., 2013; Rodrigues et al., 2009).

Bei der funktionellen GxU-Interaktion wurde die ToM-assoziierte neurale Aktivität (u.a.) der bilateralen Temporalpole und Precunei moduliert: Den Temporalpolen wird eine zentrale Bedeutung für ToM-Prozesse zugesprochen (Frith and Frith, 2006), da sie vermutlich Wissen über soziale Konzepte vermitteln (Olson, Plotzker, & Ezzyat, 2007). Bezüglich der Precunei konnte in einigen Studien gezeigt werden, dass deren Aktivierung mit kognitiven Prozessen der ToM (Abu-Akel & Shamay-Tsoory, 2011; Mar, 2011) sowie der Handlungsfähigkeit und der Selbstbezüglichkeit (Abu-Akel & Shamay-Tsoory, 2011; Vogeley et al., 2001) assoziiert ist. Insgesamt stützen die Daten dieser Studie die Hypothese, dass die ToM-assoziierte neurale Aktivität durch eine Interaktion von rs53576 und CAS moduliert wird.

Einen weiteren wichtigen Bestandteil des von der GxU-Interaktion modulierten neuralen Netzwerkes stellte der rechte superiore Parietallappen (SPL) dar, innerhalb dessen in dieser Studie eine regionale Überlappung funktioneller und struktureller GxU-Interaktionseffekte beobachtet wurde. Der SPL wird als multimodales Assoziationsareal angesehen, dem Funktionen bei der zielgerichteten Aufmerksamkeit (Corbetta & Shulman, 2002), dem parietofrontalen Spiegelneuronensystem (Cattaneo & Rizzolatti, 2009) und der ToM (Abu-Akel & Shamay-Tsoory, 2011) zugesprochen werden. Des Weiteren war ein höheres lokales SPL-Volumen mit niedrigeren ToM-assoziierten neuralen Aktivierungen in einem parieto-okzipitalen Cluster (der die

bilateralen Precunei umfasste) assoziiert. Dies läßt vermuten, dass strukturelle Änderungen im SPL mit funktionellen GxU-Interaktionseffekten zusammenhängen. Zudem ging ein höheres lokales SPL-Volumen mit niedrigeren Alexithymie-Werten einher; dies stützt und ergänzt bisherige Befunde bildgebender Studien, die zeigten, dass der rechte SPL in Alexithymie involviert ist (Berthoz et al., 2002; Karlsson, Naatanen, & Stenman, 2008; Moriguchi et al., 2009).

Die Hypothese, dass das lokale Volumen der Amygdala gemeinsam durch den OXTR-Genotyp und kindliche Bindungssicherheit moduliert wird, konnte im Rahmen dieser Studie bestätigt werden, da eine GxU-Interaktion in der Amygdala und dem Hippokampus der linken Hemisphäre beobachtet wurde. Ein höheres Volumen dieses Amygdala-Hippokampus-Clusters hing auch signifikant mit einem ängstlicheren Bindungsstil im Erwachsenenalter zusammen, und dieses Ergebnis lässt sich gut vereinbaren mit dem Postulat der Bindungstheorie, dass ein ängstlicher Bindungsstil mit Veränderungen in der Verarbeitung von Salienz und Angstreizen einhergeht (Mikulincer & Shaver, 2007), d.h. Funktionen, in die die Amygdala involviert ist (z.B. Gamer et al., 2010; Lindquist et al., 2012).

Ein weiteres Ergebnis war, dass sich GG-Homozygote im Vergleich zu A-Allel-Trägern durch ein erhöhtes Volumen grauer Substanz in solchen Hirnregionen auszeichneten, die in autobiographisches, episodisches und semantisches Gedächtnis sowie Speicherung und Abruf von sozialen Skripten und Konzepten involviert sind (d.h. bilaterale Temporalpole und rechter Hippokampus) (Insausti & Amaral, 2012; Olson et al., 2007). Dies legt die Vermutung nahe, dass dieser strukturelle Effekt in Beziehung zu einem umfangreicheren sozialen Gedächtnis in GG-Homozygoten stehen könnte, und dies wiederum könnte die größere Bedeutung kindlicher Bindungserfahrungen für diesen Genotyp erklären. So werden z.B. soziale Lernprozesse als relevanter Faktor in der Ätiologie der Alexithymie (Cook, Brewer, Shah, & Bird, 2013; Lemche, Klann-Delius, Koch, & Joraschky, 2004; Levant, Hall, Williams, & Hasan, 2009) und des ängstlichen Bindungsstils im Erwachsenenalter (Bowlby, 1969; Fraley, 2002; Mikulincer & Shaver, 2007) angesehen. Gemäß der „sozialen Salienzhypothese“ steigert OXT über meso-limbische dopaminerge Projektionen die Aufmerksamkeit, Salienz und Gedächtnis-speicherung für soziale Reize (Bartz et al., 2010; Insel & Young, 2001; Skuse & Gallagher, 2011). Die funktionelle Bedeutung von rs53576 im OXT-System ist zwar noch

unbekannt, aber möglicherweise beeinflusst rs53576 die soziale Salienzverarbeitung. Es gibt jedoch auch Hinweise aus Tierexperimenten, dass epigenetische Modifikationen (z.B. DNA-Methylierungen) eine wichtige Rolle bei der Vermittlung von Langzeitfolgen früher sozialer Umgebungfaktoren spielen, u.z. über die Regulation von Transkription. Genetische Polymorphismen wie rs53576 könnten mit epigenetischen Mechanismen interferieren und damit das Ausmaß epigenetischer Modifikationen infolge sozialer Erfahrungen beeinflussen. Daher könnten neben genetischen Gedächtniseffekten auch epigenetische Mechanismen den beobachteten GxU-Interaktionseffekten zugrunde liegen.

2.3 Manuskript #3

2.3.1 Hypothesen

Wie bereits in Kapitel 1.1 ausgeführt, bestehen Zusammenhänge zwischen dem Bindungsstil im Erwachsenenalter und dem Umgang mit Emotionen (Mikulincer & Shaver, 2007) sowie der Bewältigung von emotionalen Verlusterfahrungen (Davis et al., 2003; Sbarra, 2006; Wayment & Vierthaler, 2002). In neurowissenschaftlichen Studien konnten der Bindungsstil, die Anzahl emotionaler Verlusterfahrungen innerhalb der letzten 5 Jahre (AL) und deren Interaktion mit Variationen des Volumens der grauen Substanz in emotions- und/oder stressregulierenden Hirnregionen in Verbindung gebracht werden (Benetti et al., 2010; Quirin et al., 2010). Das Ziel dieser Studie war es, die Ergebnisse von Benetti et al. (2010) ($p<0.05$ „False Discovery Rate“-korrigiert und $p<0.001$ unkorrigiert) und Quirin et al. (2010) in einer unabhängigen Stichprobe zu replizieren. Basierend auf den Ergebnissen dieser bildgebenden Studien (Benetti et al., 2010; Quirin et al., 2010) lautete die Hypothese, dass AV negativ mit dem Volumen grauer Substanz im bilateralen Hippokampus (Quirin et al., 2010) und positiv mit dem lokalen Volumen im linken anterioren Temporalpol und Cerebellum zusammenhängt (Benetti et al., 2010). Des Weiteren wurde erwartet, dass ANX negativ mit dem lokalen Volumen im linken Hippokampus (Quirin et al., 2010) und rechten vorderen Temporalpol sowie positiv mit dem Volumen im linken lateralen Orbitalgyrus assoziiert ist (Benetti et al., 2010). Schließlich wurde vermutet, dass AL und der Interaktionsterm von AV und ANX positiv mit dem lokalen Volumen im linken Cerebellum zusammenhängen, und dass AV den Zusammenhang von AL mit dem lokalen Volumen

des linken Cerebellums moduliert (Benetti et al., 2010).

2.3.2 Ergebnisse

In der multiplen Regressionsanalyse, die für das gesamte Gehirn („whole-brain analysis“) durchgeführt wurde, wurden keine signifikanten FWE-korrigierten Zusammenhänge zwischen AV, ANX oder AL mit dem Hirnvolumen beobachtet, außer für den Kontrast (ANX > AV): ANX war signifikant positiver mit dem lokalen Volumen im Pars opercularis des linken IFG verknüpft als AV (s. Tab. SI-1 und Abb. 1). Die Korrelationsanalyse für die extrahierten beta-Werte des signifikanten Voxels/Clusters im IFG und ANX bzw. AV ergab, dass ANX positiv und AV negativ mit dem lokalen Volumen im IFG assoziiert ist (s. Abb. 1). Im umgekehrten Kontrast (ANX < AV) wurden keine signifikanten Assoziationen gefunden.

In den ROI-Analysen, die entsprechend den Hypothesen mit den in Quirin et al. (2010) und Benetti et al. (2010) berichteten Koordinaten durchgeführt wurden, ergaben sich keine signifikanten FWE-korrigierten Zusammenhänge. Wenn die Signifikanzschwelle herabgesetzt wurde (auf $P<0.05$ unkorrigiert mit einer minimalen Clustergröße von 20 Voxeln (siehe Paulus et al., 2013), zeigte sich in den ROI-Analysen, dass AV mit dem linken Temporalpol-Volumen positiv und dem rechten basolateralen Amygdala-Volumen negativ assoziiert war. ANX war mit dem Volumen im linken IFG (Pars orbitalis) und AL mit dem Volumen im linken Cerebellum positiv assoziiert (s. Tab. 2). Die Ergebnisse der faktoriellen Analyse mit AL als dichotomen Faktor (kein emotionaler Verlust vs. mindestens eine emotionale Verlusterfahrung), die aufgrund der Rechtsschiefe der AL-Verteilung zusätzlich durchgeführt wurde, zeigten keine signifikanten FWE-korrigierten Zusammenhänge (s. Tab. SI-2 und Abb. 2), bestätigten aber in den ROI-Analysen bei einem Signifikanzniveau von $p<0.05$ unkorrigiert den positiven Zusammenhang zwischen AL und dem linken Cerebellum-Volumen. Sie wiesen auch auf einen negativen Zusammenhang von AL mit dem Volumen des Pars orbitalis des linken IFG hin.

2.3.3 Diskussion

Gemäß der Bindungstheorie werden den Bindungsstilen AV und ANX unterschiedliche Emotionsregulationsstrategien zugeschrieben. AV ist charakterisiert durch die Hemmung und Unterdrückung von Emotionen, ANX dagegen durch die

Aufrechterhaltung und Intensivierung insbesondere von negativen Emotionen (Mikulincer & Shaver, 2007). Das Ergebnis dieser Studie war, dass ANX positiv und AV negativ mit dem lokalen Volumen des Pars opercularis des linken IFG zusammenhängen. Der linke Pars opercularis ist Teil des Broca-Areals und wird mit der Sprachproduktion und anderen motorischen Funktionen in Verbindung gebracht. Er ist in die Nachahmung von Bewegungen und emotionalen Gesichtsausdrücken involviert (Heiser, Iacoboni, Maeda, Marcus, & Mazziotta, 2003; Molnar-Szakacs, Iacoboni, Koski, & Mazziotta, 2005) und wird als Teil des parietofrontalen Spiegelneuronensystems angesehen (Cattaneo & Rizzolatti, 2009), das u.a. Empathie ermöglicht (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003). In anderen Studien wurde beobachtet, dass die bewußte Hoch- und Herunterregulation von Emotionen (Domes et al., 2010) sowie die Erregungshemmung (Beauregard et al., 2001) den linken Pars opercularis aktivieren. Daraus folgend wird der IFG (inklusive des Pars opercularis) als Bestandteil eines Emotionsregulationsnetzwerkes konzeptualisiert, das präfrontale und subkortikale limbische Hirnareale mit einschließt (Domes et al., 2010).

Aktivierungen des IFG konnten auch im Zusammenhang mit dem Bindungssystem beobachtet werden, allerdings nicht für den Pars opercularis: Die Aktivität des Pars triangularis des rechten IFG korrelierte mit der Aktivierung des Bindungssystems (Buchheim et al., 2006), und für ANX und AV konnten gegensätzliche Aktivierungen des Pars triangularis und orbitalis des bilateralen IFG in einer sozial interaktiven ToM-Aufgabe gezeigt werden (Manuskript #1) sowie eine Assoziation der Aktivierung der BA 47 des linken IFG mit ANX beim Betrachten maskierter glücklicher Gesichtsausdrücke (Donges et al., 2012). Allerdings legen die Ergebnisse dieser Studie die Vermutung nahe, dass das Volumen der grauen Substanz des Pars opercularis des linken IFG, der in die Emotionsregulation involviert ist, mit dem Bindungsstil im Erwachsenenalter zusammenhängt.

Des Weiteren fanden sich in den ROI-Analysen schwache Hinweise, dass AL mit dem lokalen Volumen einer Region im Cerebellum zusammenhängt, die in die Emotionsregulation, kognitive Kontrolle und in psychiatrische Erkrankungen (wie Depression, Angststörung und Schizophrenie) involviert ist (Benetti et al., 2010; Schmahmann, Weilburg, & Sherman, 2007; Stoodley & Schmahmann, 2010).

In dieser Studie konnten einige der von Quirin et al. (2010) und Benetti et al. (2010)

berichteten Zusammenhänge zwischen dem Bindungsstil bzw. AL und der Hirnmorphometrie replizieren werden, allerdings nur bei einer wesentlich niedrigeren Signifikanzschwelle: Es zeigte sich eine positive Assoziation a) von ANX und dem Volumen im Pars orbitalis des linken IFG, b) von AV und dem linken Temporalpol-Volumen, und c) von AL und dem Volumen des linken Cerebellums. Zudem fand sich eine schwache negative Assoziation zwischen AV und dem rechten basolateralen Amygdala-Volumen. Der Zusammenhang zwischen AV, ANX und dem Volumen der Hippokampi, zwischen ANX und dem Volumen des rechten anterioren Temporalpols und/oder zwischen AV bzw. dem Interaktionsterm von AV und ANX und dem Volumen des linken Cerebellums konnte in dieser Studie nicht beobachtet werden. Es ist möglich, dass die Verwendung unterschiedlicher Fragebögen bei der Erfassung der Bindungsstile zu diesen divergenten Ergebnissen beigetragen hat (siehe z.B. auch Mikulincer & Shaver (2007)). Aufgrund der vergleichsweise großen Stichprobe in dieser Studie ist es unwahrscheinlich, dass die inkonsistenten Ergebnisse auf eine zu geringe Teststärke dieser Studie zurückzuführen sind (Button et al., 2013). Eventuell zeigen die Ergebnisse dieser Studie auch an, dass die berichteten Zusammenhänge zwischen ANX, AV und Hirnmorphometrie (Benetti et al., 2010; Quirin et al., 2010) in der Population geringer als erwartet sind. Im Gegensatz dazu könnten die in dieser Studie gefundenen und im Vergleich zur Studie von Benetti et al. (2010) schwachen Zusammenhänge zwischen AL und dem lokalen Volumen des linken Cerebellums auf die vergleichsweise geringe Anzahl emotionaler Verlusterfahrungen in dieser Stichprobe zurückzuführen sein.

3. Zusammenfassende Diskussion

3.1 Bindungssicherheit, Oxytocinsystem und Gehirn

Im Rahmen dieser Arbeit konnte gezeigt werden, dass Hirnstruktur und ToM-assoziierte Hirnaktivierungen zum einen von der kindlichen Bindungssicherheit in Abhängigkeit vom OXTR-Genotyp rs53576 (Manuskript #2), zum anderen vom Bindungsstil im Erwachsenenalter moduliert werden (Manuskripte #1 und #3).

Die kindliche Bindungssicherheit war in Abhängigkeit von rs53576 mit individuellen Unterschieden im Bindungsstil ANX und der Alexithymie im Erwachsenenalter sowie der Hirnstruktur und -funktion von Regionen, die in soziale Kognitionen wie ToM (MFG, SFG, TP, SPL, ParaCL und präzentraler Kortex) (Abu-Akel & Shamay-Tsoory, 2011; Mar, 2011; Vogeley et al., 2001), das Spiegelneuronensystem (SPL, prämotorischer Kortex) (Cattaneo & Rizzolatti, 2009), und die Speicherung sozialer Skripte (Temporalpol) (Olson, McCoy, Klobusicky, & Ross, 2013; Olson et al., 2007) involviert sind, assoziiert. Strukturelle GxU-Interaktionseffekte fanden sich auch in Hirnregionen, die eine Rolle bei der Gedächtnisbildung und Stressregulation (Hippokampus) (Herman, Ostrander, Mueller, & Figueiredo, 2005; Insausti & Amaral, 2012) sowie der Verarbeitung salienter Reize (Amygdala) (Lindquist et al., 2012) spielen. GG-Homozygote waren empfänglicher für die kindliche Bindungssicherheit in Bezug auf das Volumen der grauen Substanz (außer in Hippokampus, Amygdala und supramarginalem Gyrus), in Bezug auf Alexithymie und ANX. Einige dieser GxU-Interaktionseffekte waren geschlechts-spezifisch, u.z. für ANX und das Volumen in fronto-temporo-parietalen Hirnregionen. Es konnte darüber hinaus gezeigt werden, dass strukturelle und funktionelle GxU-Interaktionseffekte nicht nur teilweise regional überlappen (im SPL, MCC und sensumotorischen Arealen), sondern auch statistisch assoziiert sind, und dass strukturelle und funktionelle GxU-Interaktionseffekte mit der Ausprägung von ANX und Alexithymie zusammenhängen (Manuskript #2).

Es wurde zudem beobachtet, dass sich die Bindungsstile des Erwachsenenalters AV und ANX bei einer sozial interaktiven ToM-Aufgabe signifikant in ihrem Zusammenhang mit neuralen Aktivierungen in Hirnregionen unterscheiden, die in die Emotionsregulation und kognitive Kontrolle involviert sind (MCC, BLA, MFG, SPL, IFG) (Beauregard et al., 2001; Domes et al., 2010; Fonagy et al., 2007; Miller & Cohen, 2001; Shackman et al.,

2011; Sylvester et al., 2003) (Manuskript #1). Die Bindungsstile AV und ANX waren zudem signifikant unterschiedlich mit Variationen im Volumen der grauen Substanz des Pars opercularis (IFG) verknüpft, der u.a. im Spiegelneuronensystem (Carr et al., 2003; Cattaneo & Rizzolatti, 2009; Heiser et al., 2003) und in der Emotionsregulation (Beauregard et al., 2001; Domes et al., 2010) eine wichtige Funktion übernimmt. Darüber hinaus lieferten die Daten dieser Studie schwache Hinweise, dass die Anzahl emotionaler Verlusterfahrungen mit dem Volumen einer Region im Cerebellum, die mit Emotionsregulation, kognitiver Kontrolle und psychiatrischen Erkrankungen (wie Depression, Angststörung und Schizophrenie) in Verbindung gebracht wird (Benetti et al., 2010; Schmahmann, Weilburg, & Sherman, 2007; Stoodley & Schmahmann, 2010), zusammenhängt (Manuskript #3).

Die Ergebnisse dieser Arbeit unterstützen die Annahme, dass frühe Fürsorgeerfahrungen die sozioemotionale Entwicklung sowie die Hirnstruktur und -funktion von Arealen, die in soziale Kognitionen wie z.B. die ToM und das Spiegelneuronensystem eingebunden sind, modulieren (Bowlby, 1969; F. A. Champagne & Curley, 2009; Fonagy et al., 2007; Sheridan et al., 2012; Sroufe, 2005). Die Ergebnisse dieser Arbeit unterstreichen auch die Bedeutung des Genotyps rs53576 für die Auswirkungen früher Fürsorgeerfahrungen. Für die Hirnstruktur, Alexithymie und ANX wurde für GG-Homozygote eine höhere Suszeptibilität für die kindliche Bindungssicherheit beobachtet als für A-Allel-Träger. Dieses Muster entspricht der Hypothese der differentiellen Suszeptibilität von Belsky et al. (2009), die als Alternative zum Diathese-Stress-Modell aufgestellt wurde und postuliert, dass es genetische Faktoren gibt, die Menschen empfänglicher für sowohl negative als auch positive Umweltfaktoren machen. Die in dieser Arbeit beobachtete höhere Suszeptibilität der GG-Homozygoten (von rs53576) im Vergleich zu den A-Allel-Trägern stimmt mit anderen Befunden aus Verhaltensstudien überein (Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013). Die hier berichteten Daten zeigten jedoch erstmalig, dass der Genotyp rs53576 und kindliche Bindungssicherheit in Bezug auf Alexithymie sowie in Bezug auf die ToM-assoziierten neuralen Aktivierungen und die Hirnstruktur interagieren. In Tierstudien wurde beobachtet, dass die Auswirkungen früher Fürsorgeerfahrungen auf das Verhalten im Erwachsenenalter durch epigenetische Mechanismen (wie z.B. die DNA-Methylierung) vermittelt werden können (F. A. Champagne & Curley, 2009; Kumsta et

al., 2013; Zhang & Meaney, 2010). Möglicherweise beeinflusst der untersuchte Genpolymorphismus rs53576 epigenetische Prozesse, die mit funktionellen Unterschieden im OXT-System einhergehen könnten (Jack, Connelly, & Morris, 2012; Mizumoto, Kimura, & Ivell, 1997). Interessanterweise deuten die strukturellen Hirndaten dieser Arbeit auch daraufhin, dass Gedächtnisbildungsprozesse für soziale Stimuli an der genotyp-abhängigen Suszeptibilität beteiligt sein könnten. Gemäß der „sozialen Salienzhypothese“ fördert OXT die Aufmerksamkeit, Salienz und Speicherung sozialer Reize über Einbindung mesolimbischer dopaminerger Strukturen [Bartz et al., 2010; Insel and Young, 2001; Skuse and Gallagher, 2011], und in dieser Arbeit wurde eine genotyp-abhängige Modulation des Volumens gedächtnis-assoziierter Hirnregionen (Hippokampus, Temporalpole) gefunden. Die Frage des Wirk-mechanismus von rs53576, der bislang unbekannt ist, kann im Rahmen dieser Arbeit jedoch nicht beantwortet werden.

Die vorliegenden Ergebnisse liefern weitere Hinweise, dass das OXT-System sexuell dimorph ist (Bethlehem et al., 2013; Carter, 2007; Gimpl & Fahrenholz, 2001). Neben geschlechtsspezifischen strukturellen GxU-Interaktionseffekten waren nur GG-homozygote Frauen, aber nicht GG-homozygote Männer suszeptibler für die kindliche Bindungssicherheit in Bezug auf ANX. In Tierstudien konnte gezeigt werden, dass die Paarbindung bei Frauen mehr als bei Männern von OXT beeinflusst wird (Carter, 2007; Carter et al., 2009) (Manuskript #2).

Des Weiteren unterstützen die in dieser Arbeit berichteten Ergebnisse (Manuskript #1, Manuskript #3) das Postulat der Bindungstheorie und erweitern bisherige neuro-wissenschaftliche und verhaltensbezogene Befunde, dass der Bindungsstil im Erwachsenenalter mit unterschiedlichen emotionalen Regulationsstrategien in Verbindung steht (Bowlby, 1969; Gillath et al., 2005; Mikulincer & Shaver, 2007; Vrtička et al., 2008; Vrtička et al., 2012). Gemäß der Bindungstheorie zeichnen sich vermeidend gebundene Personen dadurch aus, dass sie emotionale Zustände, die das Bindungssystem aktivieren, nicht zulassen, nicht beachten oder herunterregulieren, und dass sie eine kognitive Informationsverarbeitung bevorzugen (Crittenden, 1995; Mikulincer & Shaver, 2007). Dagegen tendieren ängstlich Gebundene dazu, insbesondere negative Emotionen aufrechtzuerhalten oder sogar zu intensivieren, in der Absicht, Aufmerksamkeit und Zuwendung von ihren Bindungspersonen zu

bekommen, und sie bevorzugen eine affektive Informationsverarbeitung (Crittenden, 1995; Mikulincer & Shaver, 2007). Sowohl bei den ToM-assoziierten neuralen Aktivierungen als auch beim Volumen der grauen Hirnsubstanz konnten mit dem Bindungsstil in Zusammenhang stehende Variationen in einer Hirnregion (IFG) beobachtet werden, die in die Emotionsregulation eingebunden ist (Manuskript #1, Manuskript #3). Allerdings betrafen die strukturellen Variationen (im Pars opercularis) und funktionellen Variationen (im Pars triangularis und orbitalis) im IFG zwar benachbarte, aber nicht überlappende Regionen. In anderen fMRT-Studien wurden Zusammenhänge des Bindungsstils mit Aktivierungen im Pars triangularis und orbitalis des IFG berichtet (Buchheim et al., 2006; Donges et al., 2012). Von der Annahme ausgehend, dass Hirnstruktur und -aktivierungen assoziiert sind (Zatorre, Fields, & Johansen-Berg, 2013), hätte man hier regionale Überlappungen erwarten können, wie sie teilweise für die strukturellen und funktionellen Interaktionseffekte von rs53576 und der kindlichen Bindungssicherheit beobachtet werden konnten (Manuskript #2). Die exploratorisch für die GxU-Interaktionseffekte durchgeführten multiplen Regressionsanalysen zeigten jedoch auch, dass strukturelle Variationen mit funktionellen Variationen nur teilweise in überlappenden Clustern, sondern häufig in benachbarten oder weiter entfernten Netzwerkregionen assoziiert waren (Manuskript #2). Dies läßt sich u.a. mit der Ausbreitung der neuralen Aktivierung in Netzwerken erklären. Zudem beruht der häufig beobachtete Zusammenhang von strukturellen Variationen mit veränderten Aktivierungsmustern (z.B. aufgrund von Trainingseffekten) auf einem komplexen und bislang wenig verstandenen Zusammenspiel verschiedener zellulärer und molekularer Mechanismen (u.a. von neuronalen und nicht-neuronalen Effekten) (Zatorre et al., 2013), so dass es auch hierdurch zu Asymmetrien zwischen strukturellen und funktionellen Effekten kommen kann (s. auch 1.3.1).

Die Ergebnisse dieser Arbeit lieferten nur schwache Hinweise, dass emotionale Verlusterfahrungen, die zu den belastenden Lebensereignissen zählen, mit Variationen der grauen Hirnsubstanz zusammenhängen (Manuskript #3). Da die vorliegende Stichprobe jedoch eine vergleichsweise geringe Anzahl an Verlusterfahrungen berichtete (Benetti et al., 2010), wäre es empfehlenswert, diese Fragestellung in weiteren Stichproben zu untersuchen.

Die Replikation der Ergebnisse zweier Studien zum Zusammenhang von AV und ANX

mit dem Volumen grauer Substanz (Manuskript #3) gelang nur teilweise, und nur auf einem deutlich schwächeren Signifikanzniveau als in den originalen Studien (Benetti et al., 2010; Quirin et al., 2010). Auch wenn dies möglicherweise mit methodischen Aspekten (bzgl. des eingesetzten Fragebogens) zusammenhängen könnte, könnte dies jedoch auch darauf hindeuten, dass die bisher berichteten Effekte in der Gesamtpopulation geringer als erwartet sind. Dieses Ergebnis hebt die Bedeutung von Replikationen in unabhängigen Stichproben für eine zuverlässigere Einschätzung der Populationseffekte weiter hervor.

3.2 Limitationen

Bei der Interpretation der vorgestellten Ergebnisse sind folgende methodische Einschränkungen zu berücksichtigen:

Die Stichprobe umfasste nur Student(inn)en, um eine hohe Homogenität der Stichprobe zu gewährleisten und dadurch den Einfluss von Störvariablen zu reduzieren. Diese Strategie kann allerdings die Generalisierbarkeit der Ergebnisse einschränken.

Die Anzahl der berichteten emotionalen Verlusterfahrungen war niedriger als in der Stichprobe von Benetti et al. (2010). Die kindliche Bindungssicherheit und die Anzahl der emotionalen Verlusterfahrungen wurden retrospektiv über Fragebögen erfasst. Daher kann nicht ausgeschlossen werden, dass die Erinnerungen bzw. mentalen Repräsentationen der Erwachsenen durch die beobachteten Zusammenhänge mit der Hirnstruktur und/oder -funktion beeinflusst oder verursacht sind (Bernier et al., 2016).

Die ToM-Leistung wurde nur über die neurale Aktivierung und nicht über Verhaltensmaße erfasst. Die Interpretation der neuralen Aktivierungshöhe in Bezug auf die Funktionalität des betreffenden Netzwerkes ist nicht eindeutig, da eine erhöhte Aktivierung sowohl eine höhere als auch eine niedrigere Funktionalität indizieren kann. Bei der ToM-Aufgabe beinhaltete die Kontrollbedingung visuelle, motorische und entscheidungsrelevante Aspekte, jedoch keine Belohnung bzw. Änderungen in der Belohnung. Daher ist es möglich, dass die aufgabenassoziierten neuralen Aktivierungen nicht nur ToM-Prozesse, sondern auch Belohnungsverarbeitung mitabbilden. Die Belohnungsverarbeitung ist primär mit der Aktivierung eines orbitostriatalen Netzwerkes verknüpft (Montague & Berns, 2002).

Die Funktionalität des OXTR-Genpolymorphismus rs53576 ist bislang unbekannt, und

dies schränkt Schlussfolgerungen über mögliche Mechanismen, die den beobachteten Zusammenhängen zugrunde liegen könnten, ein. Es ist nicht auszuschließen, dass sich rs53576 im Kopplungsungleichgewicht mit einem anderen, funktionalen Genpolymorphismus befindet, und das Variationen der Interlocus-Korrelationen in verschiedenen Populationen zu sogenannten flip-flop-Assoziationen führen können (Lin, Vance, Pericak-Vance, & Martin, 2007; Lucht et al., 2009). Aufgrund der niedrigen Häufigkeit der AA-Homozygoten wurden diese zusammen mit den AG-Genotypen zu der Gruppe der A-Allel-Träger zusammengefasst. Diese Strategie wurde bereits in anderen Studien angewandt (Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013; Rodrigues et al., 2009). Diese Genotyp-Gruppierung könnte jedoch vorhandene Assoziationen verschleiern, und eine separate Analyse aller drei Genotyp-Ausprägungen wäre wünschenswert. Es gab teilweise signifikante Unterschiede in den Allelverteilungen hinsichtlich Alter und Geschlecht, da die Genotypisierung erst nach der Rekrutierung durchgeführt wurde. Für diese beiden Variablen wurde jedoch in den Analysen kontrolliert.

In Bezug auf die in Manuskript #2 berichteten Ergebnisse ist zu berücksichtigen, dass in multimodalen Studien das Risiko für falsch positive Ergebnisse erhöht sein kann, da mehrere Phänotypen untersucht werden. Allerdings wurde das Signifikanzniveau für multiples Testen korrigiert und die Hypothesen auf der Grundlage wissenschaftlicher Evidenz entwickelt. Für die Untersuchung einer GxU-Interaktion war diese Stichprobe von moderater Größe, vergleichbar mit anderen Studien (Chen et al., 2011; McInnis, McQuaid, Matheson, & Anisman, 2015; Raby et al., 2013). In bildgebenden Studien ist jedoch die Schätzung der Effektstärken schwierig, und es gibt noch keinen Konsens bzgl. des angemessenen Vorgehens hierzu (Kriegeskorte, Lindquist, Nichols, Poldrack, & Vul, 2010). Für die Untersuchung von Gen-Haupteffekten wird in bildgebenden Studien eine Stichprobengröße von 100 als vergleichsweise groß angesehen (z.B. Krug et al., 2010; Paulus et al., 2013). Bei der Untersuchung von GxU-Interaktionen ist die erforderliche Stichprobengröße sowohl von der Stärke der Haupt- als auch der Interaktionseffekte sowie von den Allelhäufigkeiten abhängig (Dempfle et al., 2008). Bei schwachen Haupteffekten und moderaten Interaktionseffekten ist eine geringere Stichprobengröße als für die Entdeckung von Haupteffekten notwendig, aber in den anderen Fällen sind vielfach größere Stichproben erforderlich (Dempfle et al., 2008).

Insgesamt handelt es sich bei den vorliegenden Daten um korrelative Zusammenhänge, kausale Aussagen können nicht getroffen werden.

3.3 Bedeutung und Ausblick

Die Ergebnisse dieser Arbeit weisen auf die Bedeutung früher sozialer Fürsorge-erfahrungen für die Ausprägung sozioemotionaler Persönlichkeitseigenschaften (ANX, Alexithymie) sowie für die ToM-assoziierten neuralen Hirnaktivierungen und die makroanatomische Hirnstruktur im Erwachsenenalter hin. Dies kann für das Verständnis der Ätiologie psychiatrischer/psychischer Erkrankungen wichtig sein. Beeinträchtigte ToM-Fähigkeiten und veränderte ToM-assoziierte neurale Aktivierungs-muster wurden z.B. für Schizophrenie (Biedermann, Frajo-Apor, & Hofer, 2012), Autismus (Baron-Cohen, Leslie, & Frith, 1985; Mason, Williams, Kana, Minshew, & Just, 2008) und die Borderline-Persönlichkeitsstörung (Fonagy & Luyten, 2009) berichtet. Strukturelle Veränderungen im Gehirn wurden für verschiedene psychiatrische Erkrankungen wie Schizophrenie (Menon, 2011), Depression (Savitz & Drevets, 2009), bipolare (Menon, 2011; Savitz & Drevets, 2009) und Borderline-Persönlichkeitsstörung (Ruocco & Carcone, 2016) beobachtet. Vielen dieser psychiatrischen Erkrankungen wie z.B. Schizophrenie und bipolarer Störung liegen vermutlich veränderte neurale Entwicklungsprozesse während der Kindheit und Jugend zugrunde [Gogtay and Thompson, 2010].

Die Ergebnisse dieser Arbeit stützen des Weiteren die sogenannte Suszeptibilitäts-hypothese, die besagt, dass genetische Faktoren die Empfänglichkeit für Umwelt-faktoren erhöhen bzw. erniedrigen können (Belsky et al., 2009). Die vorliegenden Daten unterstreichen damit die Wichtigkeit, bei der Untersuchung der Auswirkungen genetischer Faktoren (z.B. auf das Erkrankungsrisiko oder das Gehirn) Umweltfaktoren zu berücksichtigen.

Außerdem liefern die hier vorliegenden Daten weitere Hinweise, dass das OXT-System in die Entwicklung sozialer Kognitionen und der sozioemotionalen Persönlichkeit im Erwachsenenalter involviert ist. Sie stützen Tierbefunde, die zeigten, dass frühe Fürsorgeerfahrungen für die Entwicklung des OXT-Systems im Gehirn bedeutsam sind und sich sexuell dimorph auswirken können. Diesbzgl. Studien am Menschen sind bislang selten (Riem et al., 2013). Ein besseres Verständnis des OXT-Systems und seiner

Entwicklung ist u.a. relevant für mögliche therapeutische Applikationen von OXT (z.B. bei der Behandlung von Schizophrenie, Autismus, sozialer Angst- und Borderline-Störung), wie sie seit einigen Jahren propagiert und erforscht werden (Anagnostou et al., 2014; Feifel, Shilling, & MacDonald, 2016; MacDonald & Feifel, 2012; Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). Hier zeigte sich, dass die intranasale OXT-Anwendung in Abhängigkeit von frühen sozialen Erfahrungen wirkt (Riem et al., 2013). Es wird vermutet, dass die in einer Meta-Analyse berechnete schwache Wirksamkeit der intranasalen OXT-Gabe bei psychiatrischen Erkrankungen (mit Ausnahme von Autismus) neben methodischen Aspekten (siehe z.B. Feifel et al., 2016) auch auf Auswirkungen negativer Kindheitserfahrungen zurückzuführen sein könnte (Bakermans-Kranenburg & van IJzendoorn, 2013).

Die Vulnerabilität für psychiatrische Erkrankungen (u.a. Depression und Schizophrenie) ist auch bei einem unsicheren Bindungsstil im Erwachsenenalter (wie z.B. bei ANX) erhöht. Die Ergebnisse der vorliegenden Arbeit lieferten erstmalig Hinweise, dass mögliche Zusammenhänge zwischen dem Bindungsstil und ToM-Prozessen auf veränderten Prozessen der Emotionsregulation, aber auch der kognitiven Kontrolle, beruhen könnten.

In zukünftigen Studien wäre es interessant, den Zusammenhang zwischen ToM und Bindungsstil weiter zu untersuchen: ToM-Inhalte/-Stimuli, Interaktionspartner und die methodische Erfassung des Bindungsstils könnten variiert und ein Verhaltensmaß für die ToM-Fähigkeit miterhoben werden. Es würde sich z.B. anbieten, den Zusammenhang zwischen Bindungsstil und ToM-assoziierten neuralen Aktivierungen für emotionsbezogene Stimuli wie Gesichtsausdrücke (Huenefeldt, Laghi, & Ortu, 2013), aber auch für vertraute oder ambivalente, potentiell beunruhigende oder den Selbstwert bedrohende Inhalte/Situationen zu untersuchen und den Bindungsstil mit projektiven oder Interview-Verfahren zu erfassen. Des Weiteren wäre es interessant, die beobachteten Hirnaktivierungsmuster und die strukturellen Variationen mit physiologischen Parametern (wie Stresshormonen, immunologischen Funktionen) und mit dem sozialen Funktionsniveau in Beziehung zu setzen.

Für die Untersuchung der Auswirkungen früher sozialer Erfahrungen (wie der kindlichen Bindungssicherheit oder von Verlusterfahrungen) auf die Hirnstruktur und -funktion im Zusammenspiel mit (epi)genetischen Faktoren könnten Längsschnitt-

studien eindeutigere und detailliertere Erkenntnisse über den tatsächlichen Entwicklungsprozess liefern, indem sie eine multimodale und prospektive Erfassung der untersuchten Umweltfaktoren sowie eine Beschreibung des zeitlichen Verlaufs der Hirnentwicklung erlauben. Experimentelle Ansätze, wie z.B. in Interventionen, würden nicht nur die Entdeckung korrelativer, sondern auch kausaler Zusammenhänge ermöglichen. Interessante methodische Erweiterungen wären die Untersuchung weiterer höherer kognitiver Funktionen (wie z.B. der exekutiven Funktionen) und der Emotionsregulation sowie die Nutzung weiterer Techniken zur Messung der Gehirnaktivität und -struktur (z.B. der Elektroenzephalographie und der Diffusions-Tensor-Bildgebung).

Größere Stichproben könnten eine nicht nur explorative statistische Analyse mehrerer Faktoren, wie z.B. des Geschlechts, kritischer Lebensereignisse, sozialer Unterstützung durch Bekannte/Freunde, und die Berücksichtigung mehrerer Genvariationen ermöglichen. Des Weiteren wäre es für ein besseres Verständnis der beobachteten Zusammenhänge wichtig, die Funktionalität des Genpolymorphismus rs53576 zu klären. Abschließend ist zu erwähnen, dass Replikationsversuche der hier dargestellen Ergebnisse wünschenswert wären.

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Anhang

Manuskript #1

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Authors: Schneider-Hassloff H[a1], Straube B[a], Nuscheler B[a], Wemken G[b], Kircher T[a]

Affiliations: [a]Department of Psychiatry and Psychotherapy, Philipps University Marburg, Rudolf-Bultmann St 8, 35039 Marburg, Germany, [b]Institute of Psychology, Social Psychology, Philipps University Marburg, Gutenberg St 18, 35032 Marburg, Germany

Author's present address: [1]Child and Adolescent Psychiatry and Psychotherapy, University Hospital Ulm, Steinhövel St 5, 89075 Ulm, Germany

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ADULT ATTACHMENT STYLE MODULATES NEURAL RESPONSES IN A MENTALIZING TASK

H. SCHNEIDER-HASSLOFF,[a*†] B. STRAUBE,[a] B. NUSCHELER,[a] G. WEMKEN[b] AND T. KIRCHER[a]

[a] *Department of Psychiatry and Psychotherapy, Philipps University Marburg, Rudolf-Bultmann Street 8, 35039 Marburg, Germany*

[b] *Institute of Psychology, Social Psychology, Philipps University Marburg, Gutenberg Street 18, 35032 Marburg, Germany*

Abstract—Adult attachment style (AAS) is a personality trait that affects social cognition. Behavioral data suggest that AAS influences mentalizing proficiency, i.e. the ability to predict and explain people's behavior with reference to mental states, but the neural correlates are unknown. We here tested how the AAS dimensions ''avoidance'' (AV) and ''anxiety'' (ANX) modulate neural correlates of mentalizing. We measured brain activation using functional magnetic resonance imaging (fMRI) in 164 healthy subjects during an interactive mentalizing paradigm (Prisoner's Dilemma Game). AAS was assessed with the Relationship Scales Questionnaire, including the subscales AV and ANX. Our task elicited a strong activation of the mentalizing network, including bilateral precuneus, (anterior, middle, and posterior) cingulate cortices, temporal poles, inferior frontal gyri (IFG), temporoparietal junctions, superior medial frontal gyri as well as right medial orbital frontal gyrus, superior temporal gyrus, middle frontal gyrus (MFG), and amygdala. We found that AV is positively and ANX negatively correlated with task-associated neural activity in the right amygdala, MFG, midcingulate cortex, and superior parietal lobule, and in bilateral IFG. These data suggest that avoidantly attached adults activate brain areas implicated in emotion regulation and cognitive control to a larger extent than anxiously attached individuals during mentalizing.

Key words: theory-of-mind, attachment, fMRI, social cognition.

*Corresponding author. Tel: +49-6421-58-66429; fax: +49-6421-58-68939.

E-mail address: schneid5@staff.uni-marburg.de (H. Schneider-Hassloff).

† Address: Child and Adolescent Psychiatry and Psychotherapy, University Hospital Ulm, Steinhövel Street 5, 89075 Ulm, Germany.

Abbreviations: AAS, adult attachment style; ACC, anterior cingulate cortex; ANX, anxious attachment style; AV, avoidant attachment style; BDI, Beck Depression Inventory; BLA, basolateral nucleus of amygdala; CMA, central-medial amygdala; dACC, dorsal anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; fMRI, functional magnetic resonance imaging; IFG, inferior frontal gyri/gyrus; ILFC, inferior lateral frontal cortex; MCC, middle cingulate cortex; MFG, middle frontal gyrus; OFC, orbitofrontal cortex; PDG, Prisoner's Dilemma Game; SPL, superior parietal lobule; STAI-T, State-Trait Anxiety Inventory, Trait; TPs, temporal poles; vmPFC, ventromedial prefrontal cortex.

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INTRODUCTION

''Avoidance'' (AV) and ''anxiety'' (ANX) are important dimensions of the adult attachment style (AAS; Mikulincer and Shaver, 2007), which is relevant for social behavior and cognition. That individual differences in AAS are related to neural correlates of mentalizing is likely, but has not been tested so far. With this study we investigated whether AV and ANX are associated with distinct activations in a mentalizing-related neural network.

An important framework for the understanding of human social-cognitive development is offered by attachment theory. It proposes that humans have an innate motivation to form an emotional bond with another person (attachment figure) and that its evolutionary function is to give protection and care for one's child (Bowlby, 1969). In a social constructionist view the function of attachment may go far beyond providing physical protection to the child. Attachment is assumed to foster social cognition and thereby to prepare the developing child for cooperation with others (Fonagy et al., 2007).

Caregivers differ in the way they respond to the proximity-seeking behavior of the child and thereby shape its attachment behavior. The adaptation to repeated contacts leads to cognitive-affective structures (internal working models) on the availability and security-providing features of the attachment figure. Thereby the individual attachment style evolves over time into an individual trait that remains moderately stable into adulthood (Fraley, 2002) and affects social cognition and behavior (Mikulincer and Shaver, 2007). The individual attachment style is ''activated'' not only in close relationships, but also toward unfamiliar others (to avoid confusion with ''brain activation'' in the remainder of the text we replace ''activate'' with ''trigger'' and ''deactivate'' with ''disable'' when referring to the attachment system) (Fraley et al., 2006; Vrtička et al., 2008, 2012). A secure attachment style is developed in a history of responsive and trustful interaction experiences. Attachment figure unavailability however leads to insecure attachment and forces a person to use a ''secondary attachment strategy'' (Mikulincer and Shaver, 2007). At least two different dimensions of attachment style exist that are termed ''avoidance'' (AV) and ''anxiety'' (ANX). In adulthood AV and ANX can be reliably assessed by two-dimensional self-report measures, conceptualized as two orthogonal axes (Simpson et al., 1992; Kurdek, 2002; Mikulincer and Shaver, 2007).

An avoidant attachment style (AV) is characterized by disabling strategies such as maintaining of self-reliance

and distance, and by avoiding emotional states that might trigger the attachment system. Inhibited attention to emotions of oneself and others, down-regulated emotions, and blocked emotional reactions are typical for AV, as well as enhanced attention to environmental objects and predominance of cognitive information processing (Crittenden, 1995; Mikulincer and Shaver, 2007). By contrast, an anxious attachment style (ANX) is characterized by exaggerated proximity-seeking, heightened access and attention to threat-related memories and thoughts, and a predominance of affective communication (Crittenden, 1995; Mikulincer and Shaver, 2007). Thus, the individual attachment styles represent important personality dimensions which affect social-cognitive and behavioral processes in social situations.

Few neuroimaging studies investigated neural correlates of AAS by means of affective and/or attachment-related stimuli. In avoidantly attached individuals the dorsal anterior cingulate cortex (dACC) and insula were less activated following the experience of social exclusion vs. social inclusion in a virtual ball-tossing game (DeWall et al., 2012). Positive feedback provided by pictures of happy facial expressions in a pseudogame context resulted in less activation of reward-related areas (ventral striatum, ventral tegmental area) in avoidantly attached subjects (Vrtička et al., 2008). Activation of somatosensory cortices was negatively correlated with AV during processing of sad facial expressions (Suslow et al., 2009). These data support the view that the strategy of withdrawal renders avoidant individuals less sensitive to social rejection, negative interpersonal signals, and to social reward. However in response to pictures of unpleasant social scenes and facial signals implying social conflict AV was positively associated with recruitment of brain areas related to cognitive control as well as cognitive and emotional conflict (dACC, ventral ACC), and emotion regulation (dorsolateral prefrontal cortex) (Vrtička et al., 2012). In response to pictures of pleasant social scenes AV was positively correlated with brain activity in regions implicated in motor inhibition and valuation (supplemental motor area, medial orbitofrontal cortex) (Vrtička et al., 2012). During negative thought suppression avoidantly attached individuals displayed higher activation of the ventral anterior cingulate cortex and lateral prefrontal cortex which was interpreted as less efficient suppression of negative thoughts (Gillath et al., 2005). Altogether these data suggest that AV modulates activation of brain areas implicated in pain, conflict and reward processing, and emotion regulation (e.g. dACC, insula, lateral prefrontal cortex, and ventral striatum) during the processing of emotionally significant cues.

ANX was shown to be associated with greater neural activations in dACC in response to experience of social rejection and during thinking about negative relationship scenarios (Gillath et al., 2005; DeWall et al., 2012). Negative feedback provided by angry faces and pictures of unpleasant social scenes elicited higher activation of amygdala in anxiously attached individuals (Vrtička et al., 2008, 2012). However facial cues depicting sadness did not evoke ANX-associated neural activations (Donges et al., 2012). Anxiously attached individuals further recruited several brain areas implicated in emotion processing and memory to a higher extent when they were confronted with social rejection experience, happy facial expressions, and pictures of pleasant or unpleasant social scenarios (DeWall et al., 2012; Donges et al., 2012; Vrtička et al., 2012). They activated brain areas implicated in emotion regulation (orbitofrontal cortex) to a lower extent when thinking about negative relationship scenarios (Gillath et al., 2005). In sum, the results in anxiously attached individuals show differential activation in the dACC, amygdala, and hippocampus (among others) which were interpreted as heightened vigilance, salience, and memory for emotionally significant social cues.

These previous neuroimaging studies focused on automatic brain reactivity to facial expressions, thought suppression, emotion processing, and response to social exclusion vs. inclusion. To our knowledge, the effect of AAS on the neural correlates of mentalizing has not been addressed so far. However, behavioral data have shown links between attachment quality and mentalizing ability (see below).

Mentalizing, also called theory-of-mind, is defined by ‘‘imputing mental states to oneself and others’’ (Premack and Woodruff, 1978) and provides ‘‘the ability to predict and explain people’s behavior with reference to mental states’’ (Repacholi and Slaughter, 2003). Mentalizing ability is regarded as fundamental for successful human social interactions and is typically impaired in severe mental disorders like autism (Baron-Cohen et al., 1985) and schizophrenia (Biedermann et al., 2012).

Mentalizing is regarded as a multidimensional construct and likely comprises distinct cognitive processes and cerebral networks (Frith and Frith, 2001; Hynes et al., 2006; Fonagy and Luyten, 2009). Most commonly a distinction between cognitive and affective mentalizing is made with respect to the content of mentalizing that can be either knowledge and beliefs or emotions and intentions (Brothers and Ring, 1992; Fonagy and Luyten, 2009; Abu-Akel and Shamay-Tsoory, 2011). A mentalizing-related neural network has been identified (Mar, 2011), that consists of the bilateral medial prefrontal cortices, temporoparietal junctions, superior temporal sulci, temporal poles (TPs), anterior temporal lobes, posterior cingulate cortices, precuneus, inferior frontal gyrus, and possibly the amygdala (of the right hemisphere in nonstory-based studies). Neural cognitive and affective execution loops for the processing of affective and cognitive mental states have been put forward by Abu-Akel and Shamay-Tsoory (2011). The cognitive mentalizing network is suggested to involve the dorsal parts of lateral (dlPFC) and medial prefrontal cortex (dmPFC), anterior cingulate cortex (ACC), TP, and striatum. The affective mentalizing network is assumed to engage the amygdala, inferior lateral frontal cortex (ILFC), orbitofrontal cortex (OFC), and the ventromedial prefrontal cortex (vmPFC), ACC, TP, and striatum (Abu-Akel and Shamay-Tsoory, 2011).

Different approaches have been employed to investigate the neural correlates of mentalizing, categorized in story- and nonstory-based studies (Mar, 2011). Story-based designs have been criticized to be

confounded by incidental executive and language-processing demands (Apperly et al., 2004). Additional concerns exist about the processing of fictional social agents in comparison to actual social agents (Mar, 2011). By contrast, interactive games like the iterative Prisoner's Dilemma Game (PDG) – used in nonstory-based approaches – are expected to "model a real-life social situation" (Rilling et al., 2004, 2012; Krach et al., 2008; Kircher et al., 2009). The use of the PDG allows for the implicit detection of mentalizing processes, including both affective and cognitive mentalizing.

Behavioral studies showed that mentalizing ability in childhood is influenced by attachment security to mother (Fonagy et al., 1997; Meins et al., 1998; Symons and Clark, 2000; De Rosnay and Harris, 2002) and to father (Fonagy et al., 2007) and that affective mentalizing ability in adolescence is modulated by AAS (Huenefeldt et al., 2013b). The relation of AAS and affective mentalizing in adulthood was investigated in healthy women in a behavioral study using a paradigm with stimuli from the eye region ("Reading the mind in the eyes test" = RMET) and a two-dimensional self-report measure of AAS ("Experiences in Close Relationships" questionnaire) (Huenefeldt et al., 2013a). ANX was associated with better recognition of emotionally neutral and difficult stimuli while there was no correlation with AV showing that ANX involves higher affective mentalizing proficiency. These behavioral data suggest that individual attachment styles go along with different mentalizing abilities in adulthood.

We investigated the effect of individual differences in AV and ANX on neural activity during an interactive mentalizing task, the PDG. We expected different neural activation patterns for the AAS styles "avoidance" and "anxiety" during mentalizing, considering the cognitive/affective dimensions of mentalizing and the mentalizing neural network (Abu-Akel and Shamay-Tsoory, 2011; Mar, 2011). Assuming that AV goes along with the application of cognitive strategies we predicted for AV higher activity in brain regions related to cognitive mentalizing, i.e. the dlPFC, dmPFC, dACC, dorsal TP, and dorsal striatum. For ANX we hypothesized a higher activation of regions related to affective mentalizing, i.e. the amygdala, lLFC, vmPFC, OFC, vACC, ventral TP, and ventral striatum, reflecting the ANX-associated focus on affective cues (Crittenden, 1995; Fonagy et al., 2007; Abu-Akel and Shamay-Tsoory, 2011; Huenefeldt et al., 2013a).

METHODS

Participants

In total, data from 164 healthy subjects (47.6% female; mean age = 23.97 years, s.d. = 3.09, range 19–35) were used for the analysis. All participants were students of the universities of Marburg or Gießen. Inclusion criteria were age (18–40 years), right-handedness (as assessed by the Edinburgh Inventory, Oldfield, 1971), German as native tongue, and Western- or Middle-European descent. Exclusion criteria were history of major psychiatric disorders of participants and their first-degree relatives according to ICD-10 (using the Mini-International Neuropsychiatric Interview, Ackenheil et al., 1999), relevant medical or neurological diseases, psychology students, and metal implants or other MRI contraindications. Participants gave written informed consent and the study protocol was approved by the local ethics committee according to the declaration of Helsinki.

Measures and Procedure

AAS. Individual AAS was assessed with a German version of the Relationship Scales Questionnaire (Griffin and Bartholomew, 1994; Stellmacher et al., in preparation) and analyzed according to the two-dimensional model of AAS proposed by Simpson (Simpson et al., 1992) that defines "anxiety" (ANX) and "avoidance" (AV) as two orthogonal axes (see also (Kurdek, 2002)). RSQ items were rated by the probands using a 6-point scale and were reverse coded when necessary. The composite mean scores for the attachment dimension "avoidance" (AV) and "anxiety" (ANX) were created by averaging eight resp. five item scores.

Other personality variables. To control for general anxiety, the State-Trait Anxiety Inventory, Trait version (STAI-T; (Spielberger et al., 1970; Laux et al., 1981)) was administered. The Beck Depression Inventory (BDI; (Beck and Steer, 1987; Hautzinger et al., 1994)) was used to measure the presence of depressive symptoms.

All questionnaires were administered prior to scanning (in general at least one day before).

Functional magnetic resonance imaging (fMRI) paradigm. A PDG was constructed using a modified version of a published task of our and other groups (Rilling et al., 2004; Krach et al., 2008, 2009; Kircher et al., 2009). In this game two players are simultaneously faced with the decision to press the right or left button. Depending on the decision of both players both gain a certain amount of points according to the decision matrix: If both players choose the left button, each one gains 20 points. If both players press the right button, each one receives zero points. If one player has chosen the right button and the other one the left button, the right button press wins 20 points, the left button press 10 points. Participants were instructed with two conflicting goals, "win the series of games and reach as many points as possible". As these goals could not be accomplished by choosing always the same button, the decision matrix was designed to ensure a variable pressing of both buttons and an implicit use of mentalizing (Krach et al., 2008). The control condition was designed according to previous studies (Krach et al., 2008, 2009; Kircher et al., 2009): Participants were told, that they do not face a co-player in this condition, and that they had to press one of the buttons without facing any consequences of the choice. The control condition therefore did not entail mentalizing. A computer co-player was not used as additional control condition because humans activate mentalizing-related brain areas when facing computers as co-players, albeit to a somewhat lesser extent (Rilling

et al., 2004; Krach et al., 2008; Kircher et al., 2009). It was suggested that humans have a tendency to attribute "intentions" to non-human counterparts, depending on the humanlikeness of computers/robots resp. their behavioral responses (Rilling et al., 2004; Krach et al., 2008, 2009; Kircher et al., 2009).

Prior to scanning the subjects received a comprehensive instruction and practiced the game 15 trials at minimum to become familiarized with the game rules and the decision matrix. Participants were told that they would play an online game in order to examine social decision making and that they would play consecutively against two different, but same-sex co-players who differ in their problem-solving style and whom the participants would not meet to avoid any bias by personal contact. In reality, participants played against a computer.

The fMRI paradigm was performed using Presentation software (Version 14.1, Neurobehavioral Systems, San Francisco, CA).

At the beginning of the game session in the scanner, a summary of the instructions was presented to the participants. To enhance the credibility of the co-players' existence the information was given that the game starts when every player is ready. Each player's readiness for action was indicated by a green tick on a slide. On the first slide only two of the three players were ticked off. After a short waiting period the next slide showed that all three players were ready and then the game started. The fMRI paradigm lasted 15.06 min and comprised 30 blocks: two blocks with the first co-player were followed by one block of the control condition and then two blocks with the second co-player followed. This sequence was repeated five times, resulting in 10 blocks for control and 10 blocks for the game task with each of the two co-players, i.e. 20 in total. Each block took 27.5 s plus a variable interval (jitter; mean value 623.9 ms, range 0–1000 ms) and started with one instruction screen (3.5 s + the jitter), announcing the next condition, followed by six game trials. Each trial consisted of one crosshair and one matrix screen, each appearing for 2 s. The crosshair screen indicated the time for the decision/button pressing. On the matrix screen information about the buttons both players had pressed and about the scores - of the current trial and accumulated over the trials in one block - appeared. In the control condition the matrix screen contained hash signs instead of scores. After the MR scanning participants were asked to remember experimental details to control for adequate attention to the task.

Cooperativity of playing behavior was defined as quantity of left button choices.

FMRI data acquisition. Data were acquired on a 3 Tesla whole body scanner (Siemens MAGNETOM Trio – A Tim System, Germany) at the Department of Psychiatry, University of Marburg. Functional neuroimaging data were collected using $T2^*$-weighted gradient echo planar imaging sequence sensitive to BOLD contrast (64 × 64 matrix size, 230 mm field of view, 30 ms echo time, 2.25 s repetition time, 90° flip angle, slices acquired in sequential (ascending) order with 20% distance factor, 36 axial slices orientated parallel to the AC-PC line covering the whole brain, slice thickness 3.6 mm, in plane resolution 3.6 mm × 3.6 mm). Four hundred functional images were collected and the onset of each block was synchronized to a scanner pulse.

fMRI data analysis

Functional images were analyzed using Statistical Parametric Mapping standard routines and templates (SPM8; http://www.fil.ion.ucl.ac.uk/spm/). After discarding the initial six images to remove the influence of T1 saturation effects, functional images were spatially realigned to correct for head motion, normalized into a standard stereotactic anatomical MNI-space (resulting voxel size 2 × 2 × 2 mm), smoothed with an 8-mm isotropic Gaussian FWHM kernel and high-pass filtered. The high-pass filter was adapted to the experimental design with a cut-off period of 342 s; one experimental cycle took about 171 s including the blocks with both co-players and the control condition resulting in six blocks with a maximum length of 28.5 s for each block.

Single subject (first-level) analyses. A general linear model was specified for each participant including two epoch regressors, modeling the game task and the control condition (each without instruction), as well as six regressors modeling head movement parameters. Parameter estimate (β-) images were calculated for each condition and each subject.

To provide further support for our results we additionally computed a general linear model that included five epoch regressors, modeling the control condition and the four different possible outcomes of the decisions in the game as separate epochs (see Rilling et al., 2004): mutual cooperation ("LL"), defection of subject and cooperation of co-player ("RL"), cooperation of subject and defection of co-player ("LR") and mutual defection ("RR"). The game epochs comprised the time window when the players choices were revealed and (except at the end of a block) the subsequent decision was to be made.

Group analyses: mentalizing and AAS. A first SPM8 group analysis was performed by entering the contrast images of each subject and condition into a random-effects "repeated measures" t-test, comparing game task and control. The individual composite scores of the AAS subscales AV and ANX were entered as two covariates of interest into the general linear model to investigate the relationship between the neural correlates of the mentalizing task and the individual differences in AAS. Because BDI and STAI-T scores are usually correlated with AAS scores – as they were in this study – we included BDI and STAI-T scores as covariates of no interest in order to control for possible effects of depressive symptoms and general anxiety. To

control for effects of three slightly different instructions regarding the two co-players (which we applied in a between-subject design and that are of no interest here), we included two dummy-coded covariates of no interest in the group-level analysis.

First, the mentalizing-related activity was investigated contrasting game task and control (T > C). Then the correlations of the mentalizing-related activity (T > C) with the individual AAS scores were examined. To test how ANX and AV differ in their correlation with (T > C), the two contrasts (AV > ANX) and (AV < ANX) were examined. Eigenvariates of significant clusters of the contrast (AV > ANX) were extracted for each subject using the VOI function of SPM8.

In a second SPM8 group analysis, we entered the first-level contrasts of each game epoch vs. the control condition (“LL > C”, “RL > C”, “LR > C”, “RR > C”) in a full factorial model with one four-level factor. AV and ANX as covariates of interest (and the same covariates of no interest like in the first group analysis) were entered, in order to examine the two contrasts (AV > ANX) and (AV < ANX) with respect to mentalizing-related activations for each of the possible game outcomes (“LL”, “RL”, “LR”, “RR”) separately.

We chose a voxel-wise threshold of $P < 0.001$ uncorrected for multiple comparisons. Activity was cluster extent threshold corrected for multiple comparisons employing Monte-Carlo simulation (Slotnick and Schacter, 2004). Assuming an individual voxel type I error of $P < 0.05$, a simulation with 1000 independent iterations indicated that a cluster extent of 47 contiguous resampled voxels is necessary to correct for multiple voxel comparisons at $P < 0.001$.

Localization of activation peaks is always reported as MNI-coordinates. For the anatomical localization of the functional data, probabilistic cytoarchitectonic maps were used as reference (Eickhoff et al., 2005). The automated brain mapping framework NeuroSynth (http://neurosynth.org/), enabling the search of published neuroimaging results by activation coordinates, supported the retrieval of the literature (Yarkoni et al., 2012). Statistical analyses of the behavioral data and of the eigenvariates were performed using R 2.15.2 (http://www.r-project.org/). Packages in use were “Hmisc” (Harrell, 2012), “car” (Fox and Weisberg, 2011) and “psy” (Falissard, 2012).

RESULTS

AAS – descriptive information

Avoidant attachment scores (AV) ranged from 1.25 to 5.25 with a mean value of 3.14 (SD: 0.75), anxious attachment scores (ANX) ranged from 1.00 to 6.00 with a mean value of 2.12 (SD: 0.92). AV and ANX were not correlated ($r = 0.05$, $p = 0.50$), consistent with prior results (Vrtička et al., 2012). Mean values and correlations with STAI-T and BDI scores are presented in Table 1. AV and ANX were not correlated with cooperativity (Table 1) and did not significantly differ in their correlation with cooperativity ($z = 0.81$).

Table 1. Sample description ($n = 164$). Sample characteristics ($n = 164$) showing the mean values of the RSQ-subscales AV and ANX, of BDI, STAI–T, age, of the cooperativity of the playing behavior in the game task, of the frequencies of the four distinct game outcomes (LL, RL, LR, RR), of the reaction times in the game and control condition, and showing the sex distribution. Cronbach’s α (a measure of internal consistency and a coefficient of psychometric test reliability) is listed for AV, ANX, BDI and STAI-T. The Pearson Product Moment correlations of AV and ANX are provided in the last two columns

Variable	Mean ± SD	Cronbach’s α	AV (PPC)	ANX (PPC)
AV	3.14 ± 0.75	0.71	–	0.05
ANX	2.12 ± 0.92	0.82	0.05	–
BDI	2.30 ± 2.33	0.61	0.16*	0.08
STAI-T	1.73 ± 0.40	0.90	0.26***	0.41***
Cooperativity	0.32 ± 0.13		0.08	−0.01
LL	20.15 ± 8.76		0.10	0.02
RL	39.43 ± 8.74		−0.10	−0.02
LR	17.9 ± 8.00		0.04	−0.02
RR	41.82 ± 8.03		−0.05	0.01
RT task	592.5 ± 153.0		0.05	−0.02
RT control	533.2 ± 123.8		0.07	−0.05
Age [years]	23.97 ± 3.09		0.10	−0.06
Sex [m/f]	86/78		0.51[a]	2.13[a]

AV = “Avoidance”, ANX = “Anxiety”, RT = Reaction times, LL/RL/LR/RR = Four possible game outcomes (L = left button press, R = right button press; subject’s choice is named first, co-player’s choice second), PPC = Pearson Product Moment correlation.

[a] F-value of one-way ANOVA for mean differences, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

FMRI-analysis of mentalizing-related activity

We investigated the mentalizing-related neural activity by computing the contrast game task vs control (T > C) in a SPM8 whole-brain analysis. In (T > C) a broad network was strongly activated that comprised bilateral superior and inferior parietal lobules, superior frontal gyri, precuneus, (anterior, middle, and posterior) cingulate cortices, TPs, inferior frontal gyri (IFG), hippocampi, temporoparietal junctions, superior medial frontal gyri, and on the right hemisphere medial orbital frontal gyrus, superior temporal gyrus, middle frontal gyrus (MFG), and amygdala among others. This implies that our task strongly activated a brain network implicated in both cognitive and affective mentalizing (Abu-Akel and Shamay-Tsoory, 2011; Mar, 2011) (see Fig. 1).

fMRI-analysis of AAS and mentalizing

Second, we tested the hypothesis of a differential association of the AAS subscales with neural activity in the mentalizing task (T > C). In several clusters (see Table 2) mentalizing-associated neural activity (T > C) was stronger positively correlated with AV than with ANX (contrast AV > ANX, $P < 0.001$). The opposite contrast (ANX > AV, $P < 0.001$) yielded no significant results. The inclusion of age and sex as covariates of no interest did not influence the activation clusters. A list of the significant clusters for (AV > ANX) is shown in Table 2. Correlation analyses with the beta-values of the significant clusters for (AV > ANX) and with the AAS

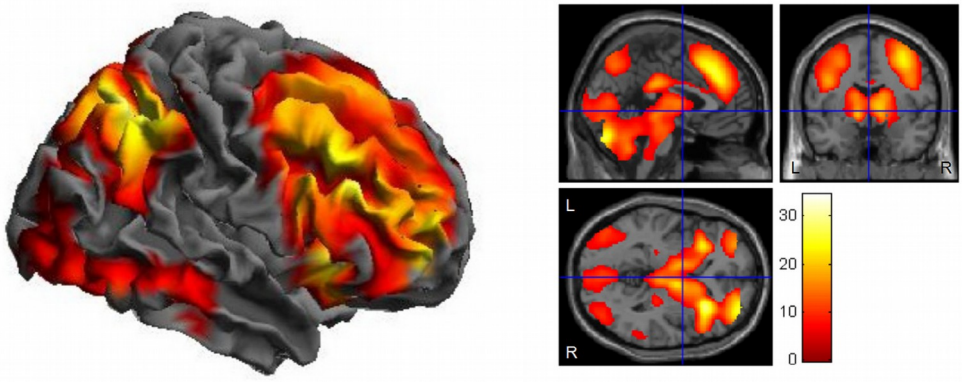

Fig. 1. Brain regions activated as a correlate of mentalizing. A large brain network was activated in the contrast game task vs. control (T > C) as a correlate of mentalizing: Bilateral inferior and superior parietal lobules, superior frontal gyri, precuneus, (anterior, middle, and posterior) cingulate cortices, temporal poles, hippocampi, inferior frontal gyri, temporoparietal junctions, superior medial frontal gyri, and on the right hemisphere medial orbital frontal gyrus, superior temporal gyrus, middle frontal gyrus, and amygdala among others. A threshold of $P < 0.001$ FWE corrected was chosen for illustrative purposes taking into account the powerful paradigm and the sample size.

Table 2. Activation peaks related to the RSQ-subscales AV and ANX. Regions of task-related neural activity (T > C) that are positively correlated with AV and negatively correlated with ANX ($P < 0.001$ uncorrected, with cluster extents of at least 47 voxels according to the cluster extent threshold correction for multiple comparisons by means of Monte-Carlo simulation). There were no significant clusters for the contrast (AV < ANX), for the negative correlation of AV, and for the positive correlation of ANX.

Anatomical region	BA		*x*	*y*	*z*	*t*	*k*	AV (PPC)	ANX (PPC)
AV > ANX									
Superior parietal lobule	7	R	22	−78	54	4.68	85	0.23**	−0.13
Inferior frontal gyrus (PT/PO)	45	R	44	24	2	4.38	482	0.25**	−0.17*
			36	32	−4	3.69			
Middle cingulate cortex	6	L/R	4	18	42	4.17	229	0.20*	−0.17*
Temporal lobe, including amygdala (BL)		R	36	0	−22	3.75	91	0.25**	−0.09
Middle frontal gyrus		R	40	48	26	3.73	63	0.17*	−0.16*
Inferior frontal gyrus (PT/PO)		L	−32	32	−2	3.51	67	0.13	−0.21**
			−40	22	−16	3.30			
								Overlap with (AV > ANX)	
								k	*P*
AV-positive correlation									
Superior parietal lobule	7	R	22	−78	54	4.14	71	62	0.003**
Amygdala (BL)/insula lobe		R	38	2	−22	3.79	129	67	0.01*
Insula lobe/inferior frontal gyrus (PT)	44/45	R	44	22	0	3.66	252	214	0.016*
			34	36	0	3.55			
			52	22	10	3.55			
Superior frontal gyrus		R	24	56	32	3.64	47		
Precentral gyrus/inferior frontal gyrus (PO)	44/45	R	48	10	32	3.42	80		
			58	18	30	3.37			
ANX-negative correlation									
Supramarginal gyrus/inferior parietal lobule	2	R	58	−30	48	3.55	60		

PT = Pars triangularis, PO = Pars orbitalis; BL = Basolateral nucleus, PPC = Pearson Product Moment correlation, *P* = *P*-value, *k* = Cluster size in voxel.
* $P < 0.05$.
** $P < 0.01$.

subscales confirmed that activity of all significant clusters was positively correlated with AV and negatively correlated with ANX. As an example the association of AV and ANX with the extracted β-values of the midcingulate cortex and amygdala is presented in Fig. 2.

For completeness we report the association between mentalizing-related neural activity and AV resp. ANX separately (see Table 2). There is a considerable overlap of several brain regions, whose activity is positively associated with AV, with the brain areas

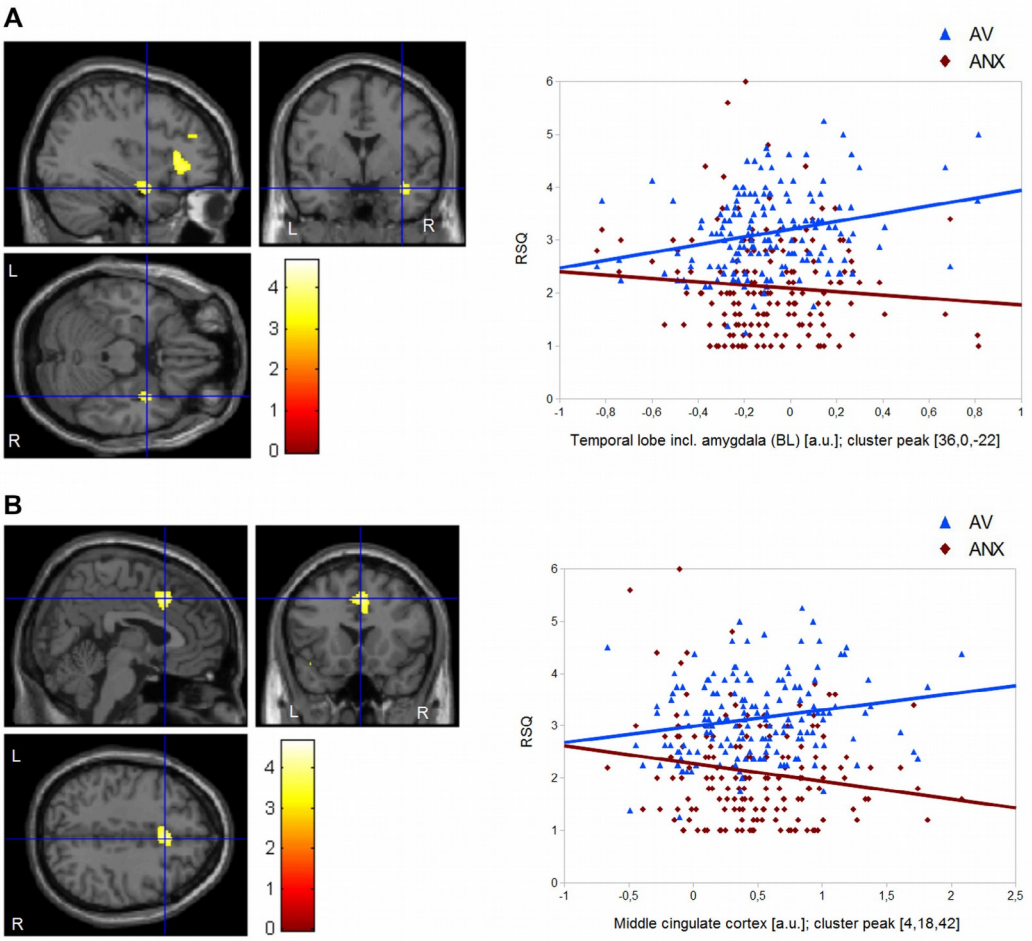

Fig. 2. Brain regional activity correlated with RSQ-subscales AV and ANX. For illustrative purpose the correlation of RSQ-subscales AV and ANX with the extracted cluster β-values and whole-brain activation maps of the contrast (AV > ANX) ($P < 0.001$ uncorrected) of (A) temporal lobe including the basolateral nucleus (BL) of the amygdala (cluster peak [36,0,−22]) and (B) the midcingulate cortex (cluster peak [4,18,42]) are shown. AV is positively and ANX negatively correlated with cluster β-values (blue = AV, red = ANX). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identified in the (AV > ANX) contrast. In the discussion we will focus on the (AV > ANX) contrast results as they allow us to directly compare the correlation differences between both attachment styles.

Because the ANX mean values in our sample were slightly skewed to the left, we contrasted subjects who scored very high and very low on ANX in an exploratory full factorial model. These results supported our findings from the correlational analysis: Subjects who scored very low in ANX compared to those with high ANX mean values activated a cluster in the right inferior parietal lobule to a higher extent (cluster peak: $x = 58$, $y = -32$, $z = 56$, $k = 184$, $t = 5.02$). The cluster significantly overlapped with the cluster identified in the negative correlation contrast of ANX ($k = 43$, $P < 0.001$, see Table 2).

Participants more often chose to defect than to cooperate, hence the game outcomes "RL" and "RR" were the most frequent outcomes, and "LL" and "LR" the least (Table 1). The results of the contrast (AV > ANX) with regard to the four different game outcomes and the associated mentalizing-related activity ("LL > C", "RL > C", "LR > C" and "RR > C") are presented in Table 3. For the opposite contrast (ANX > AV) we found one significant cluster in the left precentral gyrus in the "LR"-condition.

We were mainly interested in the overlaps between the significant clusters of the first and second group analysis for the contrast (AV > ANX). We found considerable overlaps in all four conditions. The most pronounced overlap (for all six clusters of the first group analysis) was observed in the condition "RL", in "LL"

Table 3. Activation peaks related to the RSQ-subscales AV and ANX analyzed separately for the four distinct game outcomes. Regions of task-related neural activity for the four distinct game outcomes (LL > C, RL > C, LR > C, RR > C) that are positively correlated with AV and negatively correlated with ANX ($P < 0.001$ uncorrected, with cluster extents of at least 47 voxels according to the cluster extent threshold correction for multiple comparisons by means of Monte-Carlo simulation) are listed. In the last column the overlap (in voxel) between the respective cluster and the significant clusters of the contrast (AV > ANX) of the (T > C) analysis (see Table 2) is shown

Anatomical region	BA		*x*	*y*	*z*	*t*	*k*	Overlap with (AV > ANX) of the (T > C) analysis [*k*]
(AV > ANX) for (LL > C)								
Superior parietal lobule/precuneus	7	R	22	−78	54	5.16	139	85
Rolandic operculum		R	64	−6	8	4.45	58	
Middle frontal gyrus		R	40	46	26	4.01	85	60
IFG (PO/PT)/insula lobe	45	R	36	32	−4	3.93	232	210
Supramarginal gyrus		L	−46	−34	34	3.77	66	
Superior medial gyrus/posterior medial frontal gyrus		R/L	4	20	42	3.75	221	168
Middle occipital gyrus			−24	−86	10	3.74	55	
(AV > ANX) for (RL > C)								
Superior parietal lobule/precuneus	7	R	24	−78	54	5.67	242	85
Middle frontal gyrus		R	40	48	26	5.32	280	63
IFG (PT)	45	R	44	24	0	4.71	631	457
Superior medial gyrus		R/L	4	20	42	4.47	293	195
Inferior parietal lobule/supramarginal gyrus	1	L	−50	−36	40	4.17	227	
Superior parietal lobule	7	R	38	−56	64	4.04	58	
Inferior parietal lobule	7	R	32	−48	48	4.03	61	
IFG (PO)/temporal pole		L	−38	22	−12	3.91	131	39
Supramarginal gyrus		R	64	−26	22	3.82	66	
Anterior cingulate cortex		R	8	34	22	3.82	78	
Angular gyrus/supramarginal gyrus/inferior parietal lobule		R	52	−52	28	3.77	225	
Insula/amygdala (BL)		R	40	2	−22	3.71	101	66
Superior frontal gyrus		R	26	44	44	3.68	70	
Precentral gyrus		R	50	10	34	3.52	57	
(AV > ANX) for (LR > C)								
Superior parietal lobule	7	R	22	−78	54	4.42	70	66
IFG (PT)	45	R	46	24	8	3.67	48	48
(AV > ANX) for (RR > C)								
IFG (PT)	45	R	44	24	2	4.55	310	276
Superior parietal lobule	7	R	22	−78	54	4.32	68	68
Middle frontal gyrus		R	40	46	26	3.90	155	60
Superior medial gyrus		R/L	4	20	42	3.85	100	97
Supramarginal gyrus		R	56	−48	26	3.74	93	

IFG = Inferior frontal gyrus, PT = Pars triangularis, PO = Pars orbitalis, BL = Basolateral nucleus, LL/RL/LR/RR = Four possible game outcomes (L = left button press, R = right button press; subject's choice is named first, co-player's choice second), C = Control condition, *k* = Cluster size in voxel.

and "RR" we found an overlap for four clusters and in the least frequent "LR" condition an overlap for two clusters that were associated with the highest T-values in the first group analysis.

DISCUSSION

With this study we provide first evidence, that AASs AV and ANX are distinctly associated with activations in the mentalizing-related neural network. AV was positively and ANX negatively correlated with activity in the right middle cingulate cortex (MCC), basolateral nucleus of amygdala (BLA), MFG, superior parietal lobule (SPL), and bilateral IFG. This supports our hypothesis that AV is associated with stronger engagement of cognitive mentalizing strategies than ANX. We further suggest that avoidantly attached adults activate brain areas implicated in emotion regulation and cognitive control to a larger extent than anxiously attached individuals during mentalizing.

Our mentalizing task compared to the control condition strongly activated bilateral precuneus, cingulate cortices, TPs, IFG, temporoparietal junctions, superior medial frontal gyri, superior and inferior parietal lobules, and on the right hemisphere medial orbital frontal gyrus, superior temporal gyrus, MFG, and amygdala. Therefore relevant areas of the mentalizing network have been strongly activated by our task (Abu-Akel and Shamay-Tsoory, 2011; Mar, 2011) and confirm previous results of our (Krach et al., 2008; Kircher et al., 2009) and other groups (Rilling et al., 2004).

Considering the affective and cognitive mentalizing networks put forward by Abu-Akel and Shamay-Tsoory (2011), our results showed that AV was positively and ANX negatively correlated with neural activity of parts of the cognitive mentalizing network, including regions of the dACC (MCC) and of the dlPFC (MFG), supporting our hypothesis. Interestingly our data suggest that brain areas regarded as relevant for affective mentalizing (IFG and right amygdala) were more active in avoidantly

attached and less active in anxiously attached individuals (Abu-Akel and Shamay-Tsoory, 2011). It remains to be elucidated if a different mentalizing task (with facial stimuli like in the ''Reading the mind in the eyes test'') might elicit higher activations in regions of affective mentalizing in anxiously attached individuals. As well it must be mentioned, that we did not find modulatory effects of AAS in all parts of the affective and cognitive mentalizing networks such as the medial prefrontal gyrus, ventral ACC, OFC, TP or striatum. This suggests that not all processes of the affective and cognitive mentalizing networks are affected by AAS.

Gender as covariate did not modulate the association pattern between AAS and mentalizing-related neural activity. In our sample the mean value of ANX was higher for women compared to men, and the mean value of AV was higher for men compared to women, but both mean value differences did not reach significance (ANX: $F = 2.13$, $p = 0.15$, AV: $F = 0.51$, $p = 0.47$). Gender differences in AASs are an inconsistent finding and were shown to depend on the applied measure (Collins and Read, 1990; Brennan et al., 1991). Our results suggest that attachment-related avoidance and anxiety are associated with mentalizing-related activity irrespective of gender.

The brain regions identified in the contrast (AV > ANX) in our study are involved in a broad array of cognitive and affective functions. The right inferior frontal gyrus (IFG) is regarded as part of the ventral attention system that filters signals. It interacts with the dorsal attention system (including SPL) that focuses attention. Their interaction is suggested to take place through MFG, temporoparietal junction, precuneus, and ACC (Fox et al., 2006; Abu-Akel and Shamay-Tsoory, 2011). The association of AAS with SPL, MFG, IFG, and dACC activity might be interpreted in terms of attention. Behavioral studies on attachment style have revealed that avoidantly attached individuals perform better on basic attention tasks than anxiously attached ones (Gillath et al., 2009) and our mentalizing task required basic attention.

The left inferior frontal gyrus (IFG) has been implicated in processes of arousal inhibition and the right inferior frontal gyrus (IFG) in attachment-related emotion regulation (Beauregard et al., 2001; Buchheim et al., 2006). Domes et al. (2010) proposed on the basis of their study results that the bilateral IFG may play a mediating role between dlPFC and amygdala activity as part of an emotional self-regulation circuit consisting of prefrontal regions (OFC, dlPFC, ACC) and subcortical limbic structures (amygdala, hypothalamus) (Beauregard et al., 2001). In an fMRI study that investigated social emotion perception, activity in bilateral dACC and dlPFC was positively correlated with AV (Vrtička et al., 2012). These activations were interpreted as AV associated effort in emotion regulation and cognitive conflict. In our study a considerable part of the emotional self-regulation circuit (Domes et al., 2010) was positively correlated with AV and negatively correlated with ANX. This gives support to the assumption that avoidantly attached individuals put more effort in emotion regulation than anxiously attached ones during the mentalizing task. Our task was designed to trigger attachment-related emotions because it was socially interactive. According to attachment theory avoidantly attached subjects are inclined to down-regulate attachment-related emotions while ANX is associated with sustaining or even intensifying these emotions.

SPL, dlPFC and ACC form a network that is thought to be engaged in inhibitory control processes (Miller and Cohen, 2001; Sylvester et al., 2003; Fonagy et al., 2007; Shackman et al., 2011). Increased engagement of cognitive control processes is reported for individuals with a larger MCC (Shackman et al., 2011). It has been suggested that the strategies of AV to disable the attachment behavioral system require cognitive effort, because behavioral studies showed that cognitive and emotional load tasks impede the ability of avoidantly attached individuals to inhibit attention to attachment-related emotions (Mikulincer et al., 2000; Edelstein and Gillath, 2008). Our results of AV's positive association with the activity of the SPL, dlPFC, and dACC which are implicated in inhibitory and cognitive control suit well to these behavioral results. Our data also dovetail with results of a previous neuroimaging study where avoidantly attached individuals were reported to activate ventral anterior cingulate cortex and lateral prefrontal cortex to a larger extent during negative thought suppression (Gillath et al., 2005). Altogether our data support the view that avoidantly attached participants apply more inhibitory and cognitive control processes during our interactive mentalizing task than anxiously attached ones.

Interestingly, in a previous fMRI study social exclusion experiences similarly evoked opposite activations of dACC for AV and ANX, but in a reverse pattern, i.e. a negative correlation of dACC activity with AV and a positive one with ANX (DeWall et al., 2012). dACC activity was reported to be correlated with experienced distress by the exclusion (DeWall et al., 2012). The inhibitory control circuit partially overlaps with the circuit for pain processing and instrumental avoidance behavior, mainly mediated by MCC and BLA. MCC is consistently activated during noxious stimulation and pain-related anxiety and fear (Vogt et al., 2003) and individuals with a larger MCC report higher levels of negative affect (Shackman et al., 2011). The anterior part of the MCC (aMCC) is considered as a hub where information (and cognitive schemata) about negative stimuli might be linked to motor regions responsible for facial expression of emotions and for instrumental avoidance and defensive behaviors (Pereira et al., 2010; Shackman et al., 2011). MCC is involved in avoidance learning (Klein et al., 2007), in changing behaviors as a result of changing reward properties of behavior (Bush et al., 2002; Vogt et al., 2003) and in loss aversion (Tom et al., 2007; Canessa et al., 2013). The rostral cingulate zone, a somatotopically organized premotor area, might be specifically important in this process, and its facial area seems to be part of the MCC cluster revealed in our study. aMCC is reciprocally connected with the BLA. In rodents, BLA has been proposed to be essential for calculated instrumental behavior, such as active avoidance and incentive learning with reference to sensory-specific features (Balleine and Killcross, 2006; Choi et al., 2010). Based on studies in

animals and humans, it has been put forward that BLA inhibits impulsive-affective response pathways from central-medial amygdala (CMA) to brainstem (Tye et al., 2011; van Honk et al., 2013).

Hence avoidantly attached individuals might recruit the MCC and BLA to a higher extent in consequence of an increased tendency to avoidance behavior during our game that is characterized by changing rewards, or by loss aversion. However, no absolute (monetary) losses were possible in our game. Taking into account a relative loss of game points in comparison to the co-player, the left button press was associated with a higher risk for a relative loss. Hence one might expect that loss aversion is associated with different cooperativity in the game. Interestingly, neither AV nor ANX were significantly correlated with cooperativity (nor did AV and ANX significantly differ in their association with cooperativity). Therefore it seems unlikely, that individual differences in loss aversion were involved in the attachment-related MCC activation.

Avoidantly attached individuals might also recruit the MCC and BLA to a higher extent in consequence of higher levels of negative affect, suppressed facial expression of emotions and/or an increased tendency to inhibit innate attachment motivation or other impulsive-affective responses deriving from CMA. Inhibition or masking of nonverbal expressions of emotions is regarded as typical for AV (Mikulincer and Shaver, 2007) and might have occurred during our task even though no face-to-face interaction took place. In partial support for this, AV was shown to be positively correlated with activation of brain regions associated with motor inhibition (supplemental motor area) in response to pleasant social scenes (Vrtička et al., 2012). AAS-related differences in avoidance behavior concur with attachment theory that links AV to a history of consistent reinforcement of avoidance by their caregivers. By contrast ANX has more likely developed in a history of unpredictable caregivers providing intermittent reinforcement so that anxiously attached individuals might be expected to react to negative stimuli (like omission of reward) rather with sustaining (approach) behavior than with avoidance.

Thus, our fMRI data indicate that avoidantly attached individuals activate brain areas implicated in emotion regulation and cognitive control to a higher degree than anxiously attached adults in an interactive mentalizing task. The different recruitment of neural structures during mentalizing might be a result of attachment experiences during development or (epi-)genetic factors. Evidence for genetic contributions to attachment style in adulthood exists and is consistent for attachment anxiety and conflicting for avoidance (Crawford et al., 2007; Donnellan et al., 2008).

For future research it would be interesting to investigate if mentalizing tasks with facial stimuli lead to heightened activity in the affective mentalizing network for ANX as one behavioral study showed a positive association of ANX with affective mentalizing by using the RMET (Huenefeldt et al., 2013a). Future studies could clarify if attachment-related neural modulations are stronger or different if the mentalizing task involves close friends or partners or more attachment-related scenarios. It remains to be elucidated how the different neural activation patterns during mentalizing are directly related to daily social functioning, stress responses, and immunological functions as vulnerability for mental and physical disorders is associated with attachment insecurity.

Limitations

A basic limitation is the fact that higher fMRI activation is often interpreted as higher employment of an assigned function and higher functionality (as we did), but alternative interpretations such as compensatory activation because of lowered functionality cannot be ruled out. Accordingly, AV would entail a lower functionality of processes in several brain areas that is compensated by higher brain activation in response to the task. With regard to ANX this would suggest superior functionality, more efficient processing, and therefore less neural activation.

Further our control condition involved visual, motor and decision making aspects of the task, but it did not comprise reinforcement or changes in reinforcement. Therefore task-related activity might not only depict mentalizing-associated processes, but also reward processing. Reward processing, including (monetary) rewards, reinforcement learning, prediction errors and risky behavior in decision making, is primarily associated with orbitofrontal-striatal circuit activation (Montague and Berns, 2002). The possible role of MCC and BLA in changing behaviors as consequence of changing rewards has been discussed above.

We attempted to examine a rather homogenous sample by including only students in our study in order to reduce potential confounder effects. However the generalizability of the study results may thereby be limited.

Both AV and ANX were positively correlated with STAI-T measures, a finding well supported by other research linking AV and ANX to higher general anxiety (Mikulincer and Shaver, 2007). Insecure attachment has also been associated with higher depressive symptoms in several studies, more pronounced for ANX than for AV. Our results yielded no correlation of ANX with the BDI scores, but a significant positive correlation of AV with the BDI scores. To rule out any potential confounder, in our group analyses we had controlled for BDI and STAI-T scores so that depressive symptoms and general anxiety did not account for the observed effects of AV and ANX.

CONCLUSION

Our findings provide first evidence that individual differences in AAS are associated with distinct patterns of neural activations in a mentalizing task. Our results support the assumption that avoidantly attached adults activate brain areas implicated in emotion regulation and cognitive control to a larger extent than anxiously attached individuals during mentalizing. This is a further step in understanding the biological underpinnings of attachment-related social interactions.

CONFLICTS OF INTEREST

None declared.

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Authors: Schneider-Hassloff H[a1], Straube B[a], Jansen A[a], Nuscheler B[a], Wemken G[b], Witt SH[c], Rietschel M[c], Kircher T[a]

Affiliations: [a]Department of Psychiatry and Psychotherapy, Philipps University Marburg, [b]Institute of Psychology, Social Psychology, Philipps University Marburg, Germany, [c]Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Germany

Author's present address:[1]Child and Adolescent Psychiatry and Psychotherapy, University Hospital Ulm, Steinhövel St 5, 89075 Ulm, Germany

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Oxytocin receptor polymorphism and childhood social experiences shape adult personality, brain structure and neural correlates of mentalizing

H. Schneider-Hassloff [a,*,1], B. Straube [a], A. Jansen [a], B. Nuscheler [a], G. Wemken [b], S.H. Witt [c], M. Rietschel [c], T. Kircher [a]

[a] Department of Psychiatry and Psychotherapy, Philipps University Marburg, Germany
[b] Institute of Psychology, Social Psychology, Philipps University Marburg, Germany
[c] Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Germany

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ABSTRACT

Introduction: The oxytocin system is involved in human social behavior and social cognition such as attachment, emotion recognition and mentalizing (i.e. the ability to represent mental states of oneself and others). It is shaped by social experiences in early life, especially by parent–infant interactions. The single nucleotid polymorphism rs53576 in the oxytocin receptor (OXTR) gene has been linked to social behavioral phenotypes.
Method: In 195 adult healthy subjects we investigated the interaction of OXTR rs53576 and childhood attachment security (CAS) on the personality traits "adult attachment style" and "alexithymia" (i.e. emotional self-awareness), on brain structure (voxel-based morphometry) and neural activation (fMRI) during an interactive mentalizing paradigm (prisoner's dilemma game; subgroup: n = 163).
Results: We found that in GG-homozygotes, but not in A-allele carriers, insecure childhood attachment is - in adulthood - associated with a) higher attachment-related anxiety and alexithymia, b) higher brain gray matter volume of left amygdala and lower volumes in right superior parietal lobule (SPL), left temporal pole (TP), and bilateral frontal regions, and c) higher mentalizing-related neural activity in bilateral TP and precunei, and right middle and superior frontal gyri. Interaction effects of genotype and CAS on brain volume and/or function were associated with individual differences in alexithymia and attachment-related anxiety. Interactive effects were in part sexually dimorphic.
Conclusion: The interaction of OXTR genotype and CAS modulates adult personality as well as brain structure and function of areas implicated in salience processing and mentalizing. Rs53576 GG-homozygotes are partially more susceptible to childhood attachment experiences than A-allele carriers.

1. Introduction

Investigating the neural underpinnings of human social cognition and behavior, the neuropeptide oxytocin (OXT) has received considerable attention. OXT is involved in complex social behavior and social cognition in humans such as attachment (attachment security and the tendency to share emotions with friends) [Buchheim et al., 2009; Tops et al., 2007], social memory [Bartz et al., 2011], emotion recognition [Shahrestani et al., 2013] and mentalizing [Domes et al., 2007]. Mentalizing is the ability to represent mental states such as beliefs, intentions and emotions of oneself and others. Mentalizing proficiency is considered as crucial for successful human social interactions [Premack and Woodruff, 1978].

OXT and its receptor are expressed in the central nervous system and in peripheral tissues. In the central nervous system OXT is primarily produced in the hypothalamic paraventricular and supraoptic nuclei and released into the neurohypophysis and into a variety of extrahypothalamic brain areas (for a review see e.g. Gimpl and Fahrenholz (2001)). The regional distribution of the OXT receptor (OXTR) varies between species, and in humans the OXTR is present e.g. in the limbic system including amygdala, in the dorsal ACC, striatum and hypothalamus [Boccia et al., 2013; Gimpl and Fahrenholz, 2001; Skuse and Gallagher, 2009]. The oxytocin system is regulated by gonadal steroids and is partly sexually dimorphic; e.g. animal studies showed that OXTR density in medial prefrontal cortex and plasma OXT tend to be higher in females, and that pair bonding is more dependent on OXT in females than in males [Carter, 2007; Carter et al., 2009; Gimpl and Fahrenholz, 2001; Insel and Hulihan, 1995; Kramer et al., 2004; Smeltzer et al., 2006]. Animal and

* Corresponding author at: Department of Psychiatry and Psychotherapy, Philipps University Marburg, Rudolf-Bultmann St 8, 35039, Marburg, Germany.
E-mail address: schneid5@staff.uni-marburg.de (H. Schneider-Hassloff).
[1] Present Address: ZNL TransferCenter for Neuroscience and Learning Ulm University Park St. 11 89073 Ulm Germany.

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neuroimaging studies' in humans have revealed that the amygdala, a brain structure implicated in responses to motivationally salient stimuli [Lindquist et al., 2012], is involved in the effects of OXT on social cue processing [Bethlehem et al., 2013; Gabor et al., 2012; Gamer et al., 2010]. It has been put forward that OXT alters the perceived salience of social cues as a possible mechanism of action [Bartz et al., 2011].

Neurogenetic approaches in humans have explored how variations in the oxytocin receptor gene modulate human social behavior and related brain functions [Donaldson and Young, 2008; Ebstein et al., 2012]. The single nucleotid polymorphism (SNP) rs53576 (G/A), located in the third intron of the OXT receptor gene (OXTR), has been linked to social behavior phenotypes in humans, but its functionality remains elusive. GG-homozygotes of rs53576 were shown to be more sensitive to social cues: They displayed higher affective empathy [Rodrigues et al., 2009], more sensitive parenting [Bakermans-Kranenburg and van Ijzendoorn, 2008], higher trust behavior [Krueger et al., 2012] and higher reward dependence [Tost et al., 2010]. GG-homozygotes activated the amygdala to a greater extent during face processing, and genotype-related alterations in reward dependence (i.e. reliance on social approval) negatively correlated with structural changes in local gray matter volume of the amygdala [Tost et al., 2010]. Behavioral findings regarding mentalizing have shown that rs53576 is significantly associated with performance in affective mentalizing (measured by the "Reading the mind in the eyes test" = RMET) [Lucht et al., 2013; Rodrigues et al., 2009], but risk-alleles differed. Attachment security in depressed [Costa et al., 2009], but not in healthy adults [Bradley et al., 2011; Gillath et al., 2008; Rodrigues et al., 2009] was modulated by rs53576.

To elucidate these findings further, gene-by-environment (GxE) approaches have emerged to be promising. Results from animal and human studies suggest that the OXT system is shaped by social experiences in early life, especially by parent–infant interactions [Bales and Perkeybile, 2012; Ross and Young, 2009]:

Studies in rats showed that variations in maternal care induced long-term changes in the OXT receptor expression (e.g. in the amygdala) and in social behavior in the adult offspring [Champagne et al., 2001; Francis et al., 2002; Lukas et al., 2010]. Epigenetic modulations might account for this effect of maternal care on gene expression as was shown for the glucocorticoid receptor in the rat hippocampus ([Weaver et al., 2004]; for a review see e.g. [Champagne and Curley, 2009; Kumsta et al., 2013; Zhang and Meaney, 2010]).

In humans child maltreatment was associated with alterations in peripheral OXT levels in response to physical contact in children [Fries et al., 2005], in adult women with alterations of cerebospinal fluid OXT levels [Heim et al., 2009] and with OXT-induced modulations of functional connectivity between brain regions implicated in social cognition in an fMRI resting state experiment [Riem et al., 2013].

Interestingly childhood experiences like child maltreatment and infant attachment security were shown to modulate adult attachment security/style, emotion regulation and internalizing symptoms in GG-homozygotes, but not in A-allele carriers of rs53576 [Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013]. Similarly G-allele carriers of rs53576, in contrast to AA-homozygotes, were susceptible to the quality of the childhood family environment [Bradley et al., 2013] resp. childhood maltreatment [McQuaid et al., 2013] with regard to resilient coping and positive affect resp. depressive symptomatology. Therefore variation in OXTR rs53576 seems to convey different susceptibility to long-term effects of childhood experiences on social phenotypes, with higher susceptibility in G-allele carriers.

As mentalizing - a crucial faculty during social interaction - is modulated by the OXT system, a comparable susceptibility effect of rs53576 with regard to mentalizing can be surmised. A recent quantitative meta-analysis identified a core neural network implicated in mentalizing, consisting of the bilateral medial prefrontal cortex, temporoparietal junction, superior temporal sulcus, temporal poles, anterior temporal lobes, posterior cingulate cortex, precuneus, the left inferior frontal gyrus and possibly the amygdala [Mar, 2011]. Using implicit mentalizing tasks neural activation in middle and superior frontal gyri and in superior parietal cortex was also shown to be linked to mentalizing [Kircher et al., 2009; Krach et al., 2008].

To our knowledge, the interaction effects of genetic variation in OXTR and early environmental factors on the neural correlates of mentalizing and on brain structure are still unknown. With this study we tested the hypothesis that the SNP rs53576 in OXTR and the early environmental factor childhood attachment security (CAS) interact to modulate social behavior, structure and function of the brain in adulthood. Childhood attachment describes the emotional bond between child and caregiver. It can be classified as either secure or insecure [Bowlby, 1969]. Childhood attachment security – prospectively or retrospectively assessed – is significantly related to mentalizing ability in childhood as well as to emotional regulation, social competence and psychopathology across the lifespan [Meins et al., 1998; Sroufe, 2005; Symons and Clark, 2000; Ward et al., 2006]. It therefore represents an important environmental factor during development. The interaction of OXTR genotype and childhood attachment on adult brain structure and function are yet unknown.

1.1. Hypotheses

In our study, we firstly examined the interaction effects of rs53576 and childhood attachment security (CAS) on personality traits relevant to attachment and emotion recognition, i.e. adult attachment style (AAS) and alexithymia. We assessed the AAS with the Relationship Scales Questionnaire (RSQ), using the two subscales that measure avoidance of attachment (AV) and attachment-related anxiety (ANX). Alexithymia is characterized by difficulties in identifying and describing one's own emotions and is associated with affective [Luminet et al., 2011] and cognitive mentalizing [Moriguchi et al., 2006]. It was assessed using the questionnaire Toronto Alexithymia Scale 20. We hypothesized that GG-homozygotes are more susceptible to CAS than A-allele carriers with regard to adult attachment style and alexithymia (hypothesis 1).

We secondly investigated the interaction effect of rs53576 and reported childhood attachment security (CAS) on brain gray matter volume using voxel-based morphometry (VBM). We expected interaction effects of rs53576 and CAS in brain regions related to social salience (amygdala) and social cognition (i.e. mentalizing-associated network) (hypothesis 2).

We thirdly investigated the interaction effect of rs53576 and CAS on the neural correlates of mentalizing using fMRI. Subjects performed a socially interactive game (Prisoner's Dilemma Game) that triggers implicit mentalizing processes [Kircher et al., 2009; Rilling et al., 2012]. We expected GxE-modulation in mentalizing-related brain areas [Kircher et al., 2009; Krach et al., 2008; Mar, 2011] (hypothesis 3).

Finally we assumed an association between brain morphology and function. We therefore explored brain regional overlaps of structural (VBM) and functional (fMRI) genotype-by-CAS interaction effects. We also explored whether GxE effects on brain structure are associated with GxE effects on mentalizing-related neural activity and whether GxE effects on both brain structure and mentalizing-related neural activity are associated with social adult behavior (adult attachment style and alexithymia).

2. Methods

2.1. Participants

195 subjects (97 female = 49.7%; mean age = 24.0 years, SD = 3.2, range 19–38) were included in the analysis of adult attachment style, alexithymia (RSQ/TAS) and in the structural MRI study (sMRI). A subsample of these, i.e. 163 subjects (77 female = 47.2%; mean age = 24.0 years, SD = 3.1, range 19–35), were included in the functional MRI study (fMRI). Inclusion criteria were student status, age (18–40 years), right-handedness (as assessed by the Edinburgh

Inventory, [Oldfield, 1971], inclusion criterion > + 40), German as native tongue and Western- or Middle-European descent. Exclusion criteria were history of major psychiatric disorders of participants and their first-degree relatives according to ICD-10 (using the Mini-International Neuropsychiatric Interview, [Ackenheil et al., 1999]), relevant medical or neurological diseases, psychology students and metal implants or other MRI contraindications. All participants were students of the Universities of Marburg or Gießen (Germany). Participants gave written informed consent and the study protocol was approved by the local ethics committee according to the declaration of Helsinki. Reasons for exclusion and comparison of subject demographics for the study populations are given in the supplement and are shown in Table SI-1. Subject demographics stratified by genotype are provided in the supplementary information (SI) in Table SI-2.

2.2. Measures and procedure

2.2.1. Rating scales

All questionnaires were administered prior to scanning (in general at least one day before).

2.2.1.1. Attachment security in childhood (CAS). To assess the attachment security in childhood the German version of Hazan-Shaver scale was used [Hazan & Shaver, published in [Collins and Read, 1990; Neumann, 2002]. This instrument consists of prototypical descriptions of secure, anxious-ambivalent and avoidant attachment experiences. Attachment to mother and father figure were assessed separately. The participants were asked to choose the item that best represents their remembrance of attachment to their mother and father figure in their first 16 years. The instrument was shown to yield representative distributions of attachment patterns compared to other (e.g. observational) measures [Neumann, 2002]. Participants who reported a secure attachment to both parents were coded as secure (RSQ/TAS/sMRI: n = 104, fMRI: n = 89), all others were coded as insecure. The attachment to both parents was taken into account because studies have shown that both mother and father contribute to childhood mentalizing ability [Fonagy et al., 2007] and adult attachment style [Grossmann and Grossmann, 2004].

2.2.1.2. Adult attachment style. Individual adult attachment style (AAS) was assessed with a German version of the Relationship Scales Questionnaire [Griffin and Bartholomew, 1994; Stellmacher et al., in preparation] and analyzed according to the two-dimensional model of adult attachment style proposed by Simpson et al. [1992] that defines anxiety (ANX) and avoidance (AV) as two orthogonal axes (see also [Kurdek, 2002]). RSQ items were rated by the probands using a 6-point scale. The composite mean scores for the attachment dimension "avoidance" (AV) and "anxiety" (ANX) were created by averaging eight resp. five item scores (Cronbach's α for AV: RSQ/TAS/sMRI: 0.70, fMRI: 0.71; Cronbach's α for ANX: RSQ/TAS/sMRI: 0.83, fMRI: 0.82) [Kurdek, 2002].

2.2.1.3. Toronto Alexithymia Scale 20. We applied the German version of the Toronto Alexithymia Scale 20 [Bach et al., 1996; Bagby et al., 1994] for the assessment of the personality trait alexithymia. This questionnaire with overall 20 items contains three subscales that assess difficulties in identifying and describing one's own feelings and the tendency to externally-oriented thinking. The items were rated by the probands using a 5-point scale and were combined to a total value for "alexithymia" (TAS) (Cronbach's α: RSQ/TAS/sMRI/fMRI: 0.81).

Statistical analyses were performed using R 2.15.2 (http://www.r-project.org/). In the analyses of the behavioral data rs53576, CAS and sex were included as factors and age as covariate of no interest. We chose a significance threshold of $P < 0.05$. Additionally we report P-values that are adjusted for correction of multiple testing [Hommel, 1988], taking into account that we tested three behavioral (sub)scales (ANX, AV, TAS) for GxE-interaction effects. Analyses of sex-specific effects were exploratory. More details are provided in SI.

2.2.2. MRI

Data were acquired on a 3 Tesla whole body scanner (Siemens MAGNETOM Trio - Tim System, Germany) at the Department of Psychiatry, Faculty of Medicine, University of Marburg. Localization of activation peaks is always reported as Montreal Neurological Institute (MNI)-coordinates [Brett et al., 2002]. For the anatomical localization of the functional data, probabilistic cytoarchitectonic maps according to the SPM Anatomy Toolbox (version 1.8) [Eickhoff et al., 2005] and the Wake Forest University PickAtlas software (version 2.5.2; fmri.wfubmc.edu) were used as reference.

2.2.2.1. Voxel-based morphometry (sMRI)

2.2.2.1.1. Acquisition and preprocessing of structural images. T1-weighted high-resolution anatomical images were preprocessed using VBM8. The details are provided in SI.

2.2.2.1.2. Group analyses: Rs53576 and childhood attachment security. SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) was used to calculate second-level group statistics. To investigate the joint effects of genotype (rs53576) and childhood attachment security (CAS) on brain structure, we performed a SPM8 whole brain group analysis with a random-effects full factorial model. Sex was included into the model, resulting in a model with three between-subject factors (genotype, CAS and sex) and age as covariate of no interest. For multiple regression analyses and illustration purposes eigenvariates of significant clusters were extracted using the VOI-function of SPM.

We chose a whole-brain voxel-wise threshold of $P < 0.005$ uncorrected for multiple comparisons and a cluster extent threshold corrected for multiple comparisons using Monte-Carlo simulation implemented in AFNI (http://afni.nimh.nih.gov/afni/) by means of '3dcalc', '3dFWHMx' and 'AlphaSim': Assuming an individual voxel type I error of $P < 0.05$, a simulation with 10,000 independent iterations revealed that a cluster extent of 496 contiguous resampled voxels is necessary to correct for multiple voxel comparisons at $P < 0.005$. More details about the Monte-Carlo simulation are provided in SI.

Considering that we expected GxE interaction effects in the amygdalae, we conducted a ROI-analysis for the bilateral amygdalae: The amygdala was defined according to Amunts and colleagues [Amunts et al., 2005] and the amygdala mask was created by means of the SPM Anatomy Toolbox (version 1.8, http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html). A statistical threshold of $P < 0.05$ FWE corrected for the bilateral amygdalae volumes was used.

2.2.2.2. Functional MRI (fMRI)

2.2.2.2.1. fMRI paradigm. A Prisoner's Dilemma Game was constructed using a slightly modified version of a published task of our and other groups [Krach et al., 2008; Rilling et al., 2004]. In this game two players are simultaneously faced with the decision to press the right or left button. Depending on the decision of both players they gain a certain amount of points according to the decision matrix. Participants were instructed that they would play an online game consecutively against two different, but same-sex co-players, but in reality, participants played against a computer. More details on the fMRI paradigm are provided in SI.

2.2.2.2.2. fMRI data acquisition, preprocessing and single subject analysis. Functional images were analyzed using Statistical Parametric Mapping standard routines and templates (SPM8; http://www.fil.ion.ucl.ac.uk/spm/). More details are provided in SI.

2.2.2.2.3. fMRI group analysis: Mentalizing, rs53576 and childhood attachment. To investigate the joint effects of genotype (rs53576) and childhood attachment security (CAS) on mentalizing activity, we first computed the contrast (ment > cont) at the first level for each single subject. Then we performed a SPM8 whole brain group analysis with a random-effects full factorial model using the first-level contrasts (ment > cont) (similarly to Xu et al., 2009, see also Henson and Penny, 2005). Sex was included as factor into the model, resulting in a model with three between-subject factors

(genotype, CAS and sex) and age as covariate of no interest. To control for effects of three slightly different instructions regarding the two co-players (which we applied in a between-subject design and that are of no interest here), we included two dummy-coded covariates of no interest in all group-level analyses. First, the positive effect of condition, which corresponds to the mentalizing-related activity (ment > cont), was investigated in SPM8 for the whole sample. Then the main effects of genotype and CAS and the interaction effects of genotype-by-CAS, of genotype-by-CAS-by-sex and of genotype-by-sex on mentalizing-related activity (ment > cont) were investigated. Thus, all effects refer to mentalizing-related activity, computed as first-level contrast (ment > cont). Consequently main effects of genotype, as an example, could also be interpreted as interactions of genotype (GG vs. A) by task (ment > cont).

For multiple regression analyses and illustrative purposes eigenvariates of significant clusters were extracted using the VOI-function of SPM.

We chose a whole-brain voxel-wise statistical threshold of $P < 0.005$ uncorrected for multiple comparisons and report clusters that survived a cluster-wise threshold of $P < 0.05$ family-wise error (FWE) corrected. ROI-analyses for the amygdalae were performed as described above in the sMRI method section.

2.3. Functional and structural brain regional overlaps

To explore the overlaps of clusters identified in the functional and structural contrast images ("brain regional overlaps"), the contrast images of the structural data were inclusively masked with the whole-brain activation maps of functional contrast images of the corresponding analysis. Thus we always compared the functional and structural contrast images of the same effects: the genotype main effects, the main effects of childhood attachment security (CAS), the interaction effects of rs53576-by-CAS and the interaction effects of rs53576-by-CAS-by-sex. For both structural and functional data we chose a whole-brain voxel-wise threshold of $P < 0.005$ uncorrected for multiple comparisons and the cluster extent threshold corrected for multiple comparisons (see sMRI and fMRI methods above). Given the exploratory nature of these analyses we did not correct for multiple comparisons.

2.4. Multiple regression analyses

Exploratory predictive multiple regression analyses were performed in order to test if genotype and CAS effects on individual differences in brain structure are associated with genotype and CAS effects on individual differences in brain function. Parameter estimates of all significant clusters in the structural contrast images were included as predictor variables and the eigenvariates of each significant cluster in the functional contrast images were chosen as dependent variables (in univariate models). For each multiple regression model we selected the parameter estimates for activated regions of the structural and functional contrast images of always the same effect (genotype, CAS, genotype-by-CAS or genotype-by-CAS-by-sex).

In a similar exploratory procedure we tested if genotype and CAS effects on individual differences in brain structure and function are associated with individual differences in TAS and RSQ variables. We chose the parameter estimates of all significant clusters in the structural and functional contrast images of those effects that were related to the dependent variable in the statistical analyses of the behavioral data. In a first step we performed multiple regression analyses with the eigenvariates of the structural MR clusters as predictor variables. In a second step we additionally included functional MR cluster eigenvariates as predictor variables and tested if they significantly contribute to the model.

Assumptions of the multiple regression models were tested by visual inspection of the residual vs. fitted plot (for model fit), the scale-location plot (for homoscedasticity) and the residuals vs. leverage plot (for identifying critical outliers), and by computing the variance inflation factor (VIF) and tolerance (for multicollinearity). In uncertain cases the Goldfeld-Quandt-test (for homoscedasticity) was additionally computed.

A nominal P-value < 0.05 was regarded as significant. No correction for multiple testing was carried out, given the exploratory nature of the regression analyses.

2.5. Genetics

Details on DNA extraction and genotyping are provided in SI. The allele frequencies did not significantly deviate from Hardy–Weinberg equilibrium (RSQ/TAS/sMRI: $n = 90$ GG, $n = 88$ AG, $n = 17$ AA, $P = 0.49$, fMRI: $n = 73$ GG, $n = 77$ AG, $n = 13$ AA, $P = 0.24$). The genotype groups AG and AA were combined in the analyses because of the skewed distribution, thereby adopting the same grouping strategy as e.g. in Raby et al. (2013); Bradley et al. (2011); Hostinar et al. (2014); Bakermans-Kranenburg and van Ijzendoorn (2008) and Rodrigues et al. (2009).

The genotype groups GG (GG) and AA/AG (A) significantly differed with regard to age and sex (see Table SI-2) in the fMRI-population. To control for confounding and OXT-related sex-specific effects, both age and sex were included as covariates resp. factor in statistical analyses. Analyses of sex-specific effects were exploratory. Additionally we performed analyses without the confounding variables sex and age; results are presented in SI.

3. Results

3.1. Modulation of attachment-related anxiety by a sex-specific GxE-interaction

Descriptive information and more results for adult attachment style are provided in SI (2.1). Robust models were computed because of variance inhomogeneity. In the whole sample, no significant main effects of genotype on attachment-related "anxiety" ($t(186) = -0.43$, $P > 0.05$) or "avoidance" ($t(186) = 0.20$, $P > 0.05$) were observed. Testing our first hypothesis, we found no significant genotype-by-CAS interaction on "anxiety" ($t(186) = -0.16$, $P > 0.05$) and "avoidance" ($t(186) = 0.63$, $P > 0.05$).

However, women with the GG genotype compared to A-allele carriers showed higher "anxiety" if they had experienced an insecure childhood attachment and lower "anxiety" if their childhood attachment was secure (genotype-by-CAS-by-sex: $t(186) = -2.10$, $P < 0.05$). Childhood attachment security (CAS) had less impact on "anxiety" in women who carried the A-allele and in men than in female GG-homozygotes (see Fig. 1). By contrast "avoidance" was not influenced by a significant genotype-by-CAS-by-sex interaction ($t(186) = -1.21$, $P > 0.05$).

3.2. Modulation of alexithymia by a GxE-interaction

Descriptive information and more results for "alexithymia" are provided in SI (2.2). No significant main genotype effect on "alexithymia" (TAS) was observed ($F(1,186) = 2.63$, $P = 0.107$). Testing our first hypothesis, we found that GG-homozygotes scored lower on "alexithymia" if they reported a secure compared to an insecure childhood attachment, while childhood attachment security had only small effects on A-allele carriers (genotype-by-CAS: $F(1,186) = 6.07$, $P = 0.015$, $P(\text{adjusted}) = 0.045$). No significant sex-specific genotype-by-CAS interaction was observed ($F(1,186) = 0.97$, $P = 0.326$) (see Fig. 1).

3.3. Modulation of brain gray matter volume in hippocampus, amygdala and a fronto-parieto-temporal network by a GxE-interaction

We investigated the main effects of genotype and childhood attachment security and the interaction effects of genotype-by-CAS, genotype-

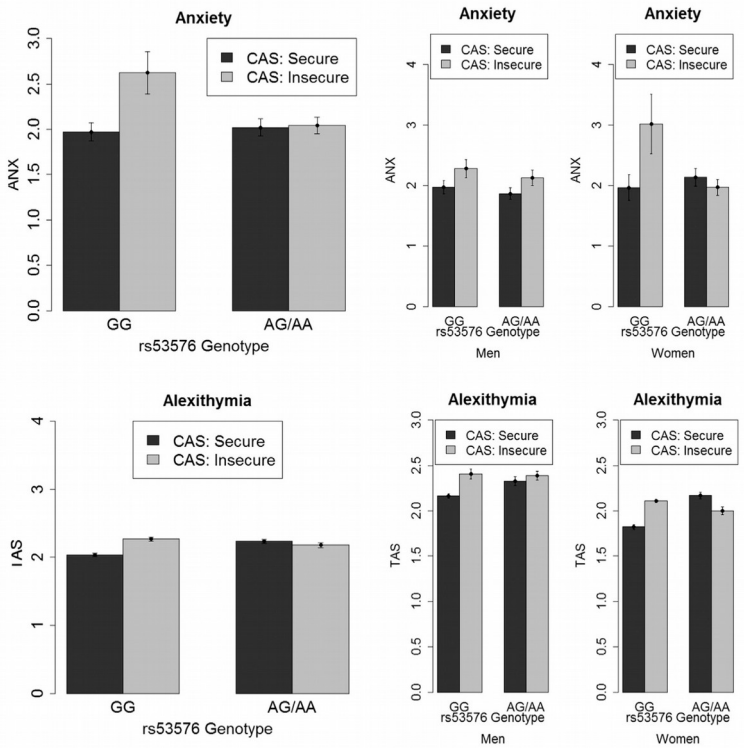

Fig. 1. Interaction effects of genotype (rs53576) and childhood attachment security (CAS) on "anxiety" (ANX) and "alexithymia" (TAS) are displayed. Error-bars illustrate SEM (SEM = standard error of the mean). The interaction effect on ANX was driven by the women subsample.

by-CAS-by-sex and genotype-by-sex on brain morphology. An overview of the results of the structural analysis is presented in Table 1.

Examining our second hypothesis, we found that GG-homozygotes with secure compared to insecure childhood attachment showed increased gray matter volume in a fronto-parieto-temporal network and decreased regional volume in left hippocampus and amygdala whereas A-allele carriers displayed the opposite, but (except for left SMG, hippocampus and amygdala) less pronounced pattern (see Fig. 2 and SI-1).

In a ROI analysis using the bilateral amygdalae as mask, we observed a genotype-by-CAS interaction effect. Insecure childhood attachment was associated with higher left basolateral amygdala gray matter volume in GG-homozygotes while the opposite pattern was observed in A-allele carriers (cluster peak x/y/z = −33/−9/−18, t = 3.57, P(Peak, FWE) = 0.034, P(Cluster, FWE) = 0.044). There was no significant sex-specific effect.

3.3.1. Modulation of brain gray matter volume in temporal, parietal and cerebellar regions by genotype

GG-homozygotes showed increased gray matter volume in bilateral temporal poles, right hippocampal area and precuneus and decreased gray matter volume in the cerebellar vermis compared to A-allele carriers.

More details and results are provided in SI (2.3).

3.4. Activation of a broad neural network by the mentalizing task

The mentalizing-related neural activity was investigated by computing the positive effect of the condition (corresponding to the task effect (ment > cont)) in a SPM8 whole brain analysis: A broad network was strongly activated that comprised bilateral superior and inferior parietal lobules, superior frontal gyri, precunei, (anterior, middle and posterior) cingulate cortices, temporal poles, inferior frontal gyri, hippocampi, temporoparietal junctions, superior medial frontal gyri and on the right hemisphere medial orbital frontal gyrus, superior temporal gyrus, middle frontal gyrus and amygdala among others.

3.5. Modulation of mentalizing-related neural activity by a GxE-interaction in a predominantly right frontal and bilateral parieto-temporo-occipital network, but not in the amygdala

We investigated the main effects of genotype and childhood attachment security and the interaction effects of genotype-by-CAS, genotype-by-CAS-by-sex and genotype-by-sex on mentalizing-related activity (ment > cont). An overview of the results of the SPM8 whole-brain analysis is presented in Table 2. Playing behavior and task reaction times did not significantly differ with regard to genotype, CAS or their interactions (for all P-values > 0.23).

Examining our third hypothesis, GG-homozygotes showed higher activations in a predominantly right frontal (including right superior and middle frontal gyri and bilateral paracentral lobules) and bilateral parieto-temporo-occipital network (encompassing bilateral TP and precunei), when they reported an insecure compared to a secure childhood attachment, whilst A-allele carriers showed the reverse pattern (see Fig. 3 and SI-2).

Table 1
Significant main and interaction effects of genotype, genotype-by-sex, childhood attachment security (CAS), genotype-by-CAS and genotype-by-CAS-by-sex on brain morphometry ($P < 0.05$ corrected at cluster level (minimal cluster size = 496 voxel)). There were no significant effects for the contrasts genotype-by-sex [(GG > A) × (m > w)], genotype (insecure > secure) and genotype-by-CAS-by-sex [(A > GG) × (secure > insecure) × (m > w)].

Anatomical region	Brain area		x	y	z	t	k
Genotype: GG > A							
TP		R	59	12	−9	4.18	766
Hipp/Precun/ParahippG/LG	CA/SUB/ BA 17/18	R	21	−39	−3	3.97	1325
TP		L	−45	17	−23	3.83	1019
Genotype: A > GG							
Cereb Vermis/Cerebellum	Lob VI/V	R/L	−5	−64	−24	3.90	2418
Genotype × sex: (A > GG) × (m > w)							
PostCG/SPL	BA 2/1 /7PC/5 L	R	38	−40	66	4.35	570
CG/LG	BA 17/18	R	14	−75	13	3.96	1136
CAS: Secure > insecure							
PostCG/Precun/ParaCL	5 M/BA 4a/5 L	R/L	−14	−43	76	4.18	1149
Genotype × CAS: (GG > A) × (secure > insecure)							
SMA/MCC	BA 6	R/L	2	11	49	4.41	921
MFG/PreCG	BA 6	L	−44	12	54	4.31	1061
SPL	7 A/7PC/5 L/BA 2	R	24	−58	69	4.16	860
PreCG/SFG	BA 6/4a	R	38	−16	61	4.11	868
SMG	PF/PFop/PFt/OP1	L	−63	−25	18	3.84	782
IFG (PO)/TP/PreCG	BA 44/45	L	−51	8	24	3.78	1055
ParaCL/SMA/MCC	BA 6/4a	R/L	−2	−34	51	3.62	1282
Genotype × CAS: (A > GG) × (secure > insecure)							
Hipp incl. Amy	CA/LB/SF/SUB	L	−18	−15	−9	4.56	542
Genotype × CAS × sex: (GG > A) × (secure > insecure) × (m > w)							
STG/SMG	PFm/PF/PGa	R	62	−46	19	4.56	1270
SFG/SMedG		R	18	59	22	3.61	697

Amy = amygdala, CG = calcarine gyrus, Cereb = cerebellar, Hipp = hippocampus, IFG = inferior frontal gyrus, LG = lingual gyrus, Lob = lobule, MFG = middle frontal gyrus, ParahippG = parahippocampal gyrus, ParaCL = paracentral lobule, PO = pars opercularis, PostCG = postcentral gyrus, PreCG = precentral gyrus, Precun = precuneus, SFG = superior frontal gyrus, SMA = supplementary motor area, SMG = supramarginal gyrus, SMedG = superior medial gyrus, SPL = superior parietal lobule, STG = superior temporal gyrus, TP = temporal pole, incl. = including. Brain areas are labeled according to the SPM Anatomy Toolbox (version 1.8) and the Wake Forest University PickAtlas software (version 2.5.2), k = number of voxels.

In a ROI analysis using the bilateral amygdalae as mask, genotype and CAS did not significantly modulate bilateral amygdalae activity (nor was a sex-specific modulation observed).

More details and results are provided in SI (2.4).

3.6. Regional overlap of GxE-interaction effects on brain structure and function in a fronto-parietal network

We expected that genotype and childhood attachment security modulate both structure and function of the same brain regions (brain regional overlaps). Our exploratory analyses revealed regional overlaps in the genotype-by-CAS interaction contrasts. GG-homozygotes showed both higher mentalizing-related activations and lower gray matter volume in the right superior parietal lobule (SPL), precentral gyrus (PreCG), superior frontal gyrus (SFG), and in bilateral paracentral lobules (ParaCL) and middle cingulate cortices (MCC), when they reported an insecure compared to a secure childhood attachment, the reverse pattern was observed for A-allele carriers (see Table 3 and Fig. 4).

3.7. Association of GxE-interaction effects on local volumes with those on mentalizing-related neural activity

Additionally to the brain regional overlaps we explored if individual differences in gray matter volumes are associated with individual differences in mentalizing-related activity by means of statistical regression models. We found that higher gray matter volume in the

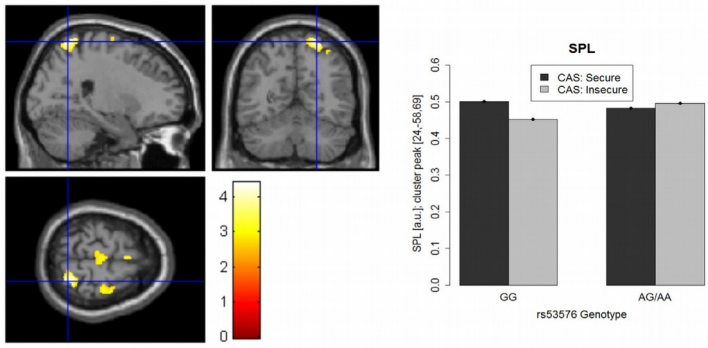

Fig. 2. Interaction effect of genotype (rs53576) and early environment (CAS) on adult brain volume [(G > A) × (secure > insecure)]: Gray matter volume of the right superior parietal lobule (SPL). β-values and whole-brain structural maps are depicted ($P < 0.05$ cluster extent threshold corrected (kmin = 496 voxel).

Table 2
Significant interaction effects of genotype and childhood attachment security (CAS) [(A > GG) × (secure > insecure)] on mentalizing-related neural correlates (ment > cont; P < 0.05 FWE cluster corrected). No significant main effects of genotype and CAS, and no significant interaction effects of genotype-by-sex, genotype-by-CAS-by-sex as well as for genotype-by-CAS [(GG > A) × (secure > insecure)] were detected.

Anatomical region	Brain area		x	y	z	t	k	P(FWE, Cluster)
Genotype × CAS: (A > GG) × (secure > insecure)								
Cuneus/Precun/SoccG/SPL	7P/7 M	R/L	16	−80	44	4.76	1276	0.017*
STG/Insula/CaudN/TP	Thal	R/L	46	0	−14	4.45	4768	<0.001***
SFG/MFG/ParaCL/PostCG/PreCG	BA 6/4a/3b /5 L	R/L	36	−8	66	4.09	2255	0.001**
LG/CG/FG/loccG	BA 17/18/ hOC3v	R/L	10	−90	−4	4.09	2139	0.001**

Abb.: CaudN = caudate nucleus, CG = calcarine gyrus, FG = fusiform gyrus, Insula = insula lobe, loccG = inferior occipital gyrus, LG = lingual gyrus, MFG = middle frontal gyrus, ParaCL = paracentral lobule, PostCG = postcentral gyrus, PreCG = precentral gyrus, Precun = precuneus, SFG = superior frontal gyrus, SoccG = superior occipital gyrus, STG = superior temporal gyrus, Thal = thalamus, TP = temporal pole. Brain areas are labeled according to the SPM Anatomy Toolbox (version 1.8) and the Wake Forest University PickAtlas software (version 2.5.2), k = number of voxels.
* P < 0.05.
** P < 0.01.
*** P < 0.001.

right SPL [(GG > A) × (secure > insecure)] was significantly associated with lower mentalizing-related neural activity in bilateral Cuneus/Precun/SoccG/SPL of the contrast [(A > GG) × (secure > insecure)] (P < 0.05). We also observed that higher local volumes in left IFG/TP/PreCG [(GG > A) × (secure > insecure)] were related to lower mentalizing-related neural activity in bilateral Cuneus/Precun/SoccG/SPL and to higher mentalizing-related neural activity in the frontal cluster encompassing right SFG/MFG/PostCG/PreCG and bilateral ParaCL. Higher local volumes in left hippocampus and amygdala [(A > GG) × (secure > insecure)] went along with higher mentalizing-related neural activity in bilateral LG/CG/FG/loccG. More details and results are provided in SI (2.5).

3.8. Association of local volume in amygdala/hippocampus and of mentalizing-related neural activity in frontal regions with individual differences in ANX

We used the contrast images of the genotype-by-CAS-by-sex and genotype-by-CAS interaction effect to explore the association between individual differences in adult attachment style and structural and functional data. We found that higher gray matter volume in left hippocampus and amygdala [(A > GG) × (secure > insecure)] was significantly associated with higher "anxiety" (t(184) = 2.04, P = 0.042). Adding the cluster eigenvariates of the mentalizing-related brain activation to the model, we found that higher brain activity in the frontal cluster (including right SFG and MFG) [(A > GG) × (secure > insecure)] was significantly associated with higher "anxiety" (t(147) = 2.25, P = 0.026). For "avoidance" we performed no regression analysis because no effects of genotype and childhood attachment security on "avoidance" were observed in the behavioral data analysis. More details and results are provided in SI (2.6).

3.9. Association of local volumes in right SPL with individual differences in alexithymia

We considered the contrast images of the genotype-by-CAS interaction effect to explore the association between individual differences in "alexithymia" and structural and functional data. We found that lower gray matter volume in the right SPL [(GG > A) × (secure > insecure)] is significantly associated with higher "alexithymia" (t(186) = −2.14, P = 0.034). Adding the cluster eigenvariates of the mentalizing-related brain activation to the model, no significant associations were observed. More details and results are provided in SI (2.7).

4. Discussion

With this study we provide evidence that the oxytocin receptor gene polymorphism rs53576 and child–parent interaction (childhood attachment security, CAS) jointly modulate - in adulthood - the personality trait "alexithymia" (TAS), attachment-related "anxiety" (ANX), brain gray matter volume and mentalizing-related neural activity. Our data support the hypothesis that rs53576 is associated with differential susceptibility to early social environmental factors and demonstrate the structural and functional neural correlates of this GxE interaction. Our data further suggest a partial sex-specificity. In more detail we found that 1) "alexithymia" is higher in rs53576 GG-homozygotes with

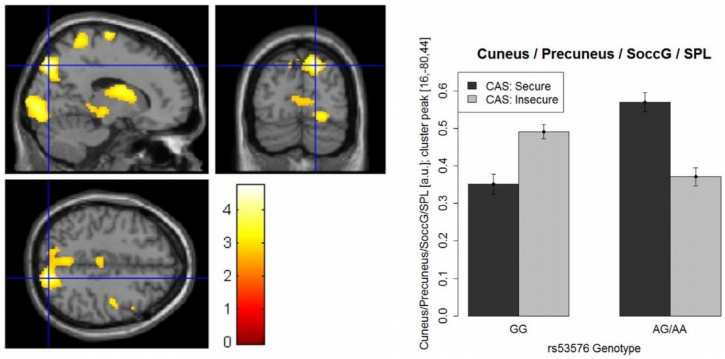

Fig. 3. Interaction effect of genotype (rs53576) and early environment (CAS) on adult brain function [(A > G) × (secure > insecure)] during mentalizing: Neural activation of the cluster encompassing the bilateral cunei, precunei, superior occipital gyri (SoccG) and superior parietal lobules (SPL). β-values and whole-brain activation maps are depicted (ment > cont; P < 0.05 FWE corrected at cluster level).

Table 3
In the right column anatomical regions and voxels per overlap (k) are listed in which both mentalizing-related activity [(A > GG) × (secure > insecure)] and gray matter volume [(GG > A) × (secure > insecure)] are modulated by the interaction of genotype and childhood attachment security (CAS) (functional and structural cluster overlap; P < 0.05 corrected at cluster level). In the left two columns the corresponding clusters of gray matter volume (confer Table 1) and mentalizing-associated activity (cf. Table 2) are shown with their respective t-values.

Anatomical region		t	Anatomical region		t	Anatomical region		k
Gray matter volume [(GG > A) × (secure > insecure)]			(Ment > cont) [(A > GG) × (secure > insecure)]			Overlap		
PreCG/SFG	R	4.11	SFG/MFG/ParaCL/PostCG/PreCG	R/L	4.09	PreCG/SFG	R	374
SPL	R	4.16	SFG/MFG/ParaCL/PostCG/PreCG	R/L	4.09	SPL	R	68
ParaCL/SMA/MCC	R/L	3.62	SFG/MFG/ParaCL/PostCG/PreCG	R/L	4.09	ParaCL/MCC	R/L	114
ParaCL/SMA/MCC	R/L	3.62	SFG/MFG/ParaCL/PostCG/PreCG	R/L	4.09	ParaCL	R/L	259

insecure compared to secure childhood attachment. GG-homozygotes with insecure compared to secure childhood attachment showed 2) decreased gray matter volume in a fronto-parieto-temporal network, 3) increased local volume in the left hippocampus and amygdala, and 4) higher mentalizing-related neural activations in a predominantly right frontal and bilateral parieto-temporo-occipital network. 5) A-allele carriers compared to GG-homozygotes displayed the opposite pattern and demonstrated less CAS-dependent differences with respect to "alexithymia" and regional volumes. 6) In GG-homozygotes insecure compared to secure childhood attachment was associated with higher attachment-related "anxiety" in women. 7) A brain regional overlap of structural and functional GxE interaction effects was observed in right superior parietal lobule (SPL), midcingulate and sensory-motor regions. Exploratory multiple regression analyses revealed that 8) GxE modulated local volumes were significantly associated with GxE modulated neural activations in the mentalizing-related network and that 9) genotype and CAS effects on brain function and/or structure were significantly associated with "alexithymia" and attachment-related "anxiety".

4.1. Gene-by-environment effects on personality traits

We did not observe main genetic effects of rs53576 on personality, neither on adult attachment style – supporting previous results [Bradley et al., 2011; Gillath et al., 2008; Rodrigues et al., 2009], nor on alexithymia. However, we did find interaction effects of genotype (rs53576) and early environment (CAS): Childhood attachment security (CAS) modulated alexithymia scores (TAS) to a significantly higher extent in GG-homozygotes than in A-allele carriers. Additionally we found a sex-specific GxE interaction effect for "anxiety", and this GxE interaction effect was significantly supported by analyzing the data without the factor sex (see also Section 4.6 and SI 2.1.2). Insecure compared to secure childhood attachment was associated with higher "alexithymia" and higher "anxiety" in GG-homozygotes. The results for "anxiety" are in line with previous research on attachment security showing that prospectively assessed CAS predicts adult attachment security only in GG-homozygotes [Raby et al., 2013]. Our data conform with previous observations that GG-homozygotes are more susceptible to the social environment [Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013]. As to ANX, they also suggest, that (GG-homozygous) women are more susceptible than men (see also 4.6).

Our results thereby dovetail with the "differential susceptibility"-hypothesis of Belsky and colleagues [Belsky et al., 2009]. Proposed as alternative to the well-known diathesis-stress framework, they assume that "plasticity genes" render individuals more susceptible to environmental factors, for worse and for better. By contrast attachment-related "avoidance" was neither modulated by genotype (rs53576) nor by a GxE interaction and this adds further evidence to the claim that "anxiety" may be more genetically determined than "avoidance" [Crawford et al., 2007].

4.2. Gene-by-environment effects on the mentalizing network: VBM

Childhood attachment security and rs53576 modulated local gray matter volumes in brain areas that are related to mentalizing (MFG, SFG, IFG, TP, SPL, ParaCL, SMG and left precentral cortex [Abu-Akel and Shamay-Tsoory, 2011; Mar, 2011; Vogeley et al., 2001]) and to the parietofrontal human mirror system (SPL, SMG, premotor cortex and caudal part of the IFG [Cattaneo and Rizzolatti, 2009]). The latter provides a "first step for mentalizing" because it helps to track the intention of others by simulation processes [Frith and Frith, 2006]. The observed interaction pattern again fits with the "differential susceptibility"-hypothesis [Belsky et al., 2009] indicating higher susceptibility of GG-homozygotes for CAS.

4.3. Gene-by-environment effects on the mentalizing network: fMRI

Our fMRI data showed that rs53576 modulates the impact of childhood attachment security (CAS) on mentalizing-associated neural activity in a predominantly right frontal and bilateral parieto-temporo-occipital network including the temporal poles and the precunei. The temporal poles are regarded as a central component of mentalizing activity [Frith and Frith, 2006]. They are suggested to mediate social conceptual knowledge that is required for mentalizing [Olson et al., 2007; Olson et al., 2013; Ross and Olson, 2010]. The activation of the precunei has been consistently associated with mentalizing [Abu-Akel and Shamay-Tsoory, 2011; Mar, 2011], agency and self-reference

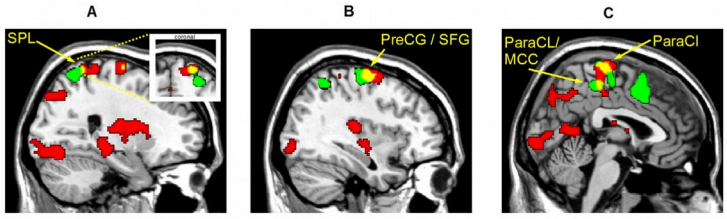

Fig. 4. Interaction effect of genotype (rs53576) and early environment (CAS) on adult brain volume (green; cf. Fig. 2, [(GG > A) × (secure > insecure)]) and function during mentalizing (red; cf. Fig. 3, [(A > GG) × (secure > insecure)]) as well as their regional overlaps in yellow (P < 0.05 corrected at cluster level) in A the SPL (x = 24; coronal: y = -50), B the precentral / superior frontal gyrus (x = 36) and C paracentral lobules / MCC (x = 2) are displayed.

[Abu-Akel and Shamay-Tsoory, 2011; Mar, 2011; Vogeley et al., 2001]. The precuneus is assumed to subserve self-representation and introspective-based processing of self mental states [Abu-Akel and Shamay-Tsoory, 2011]. It presumably also supports imagination processes by integrating signals from multiple brain areas [Mar, 2011].

Neural activity of both GG-homozygotes and A-allele carriers was susceptible to childhood attachment security, but in opposing direction and with partially even higher susceptibility to CAS in A-allele carriers. GG-homozygotes with secure childhood attachment and A-allele carriers with insecure childhood attachment recruited mentalizing-related brain areas mostly to a similarly low extent. However, A-allele carriers with insecure childhood attachment showed the lowest mentalizing-related neural activity in temporal regions including the TP, and as to precunei, A-allele carriers with secure attachment showed the highest neural activity. This finding was unexpected. Taking into account that alexithymia is associated with cognitive and affective mentalizing [Luminet et al., 2006; Moriguchi et al., 2006], it is interesting that the lower susceptibility to CAS in A-allele carriers for (self-reported) alexithymia is not paralleled by a comparable susceptibility effect in A-allele carriers for the mentalizing-related neural activations. A higher neural activation of the mentalizing network can be interpreted as higher recruitment of mentalizing processes, but also as compensatory activation due to a lower functionality of the mentalizing network. Our result thereby warrants further elucidation, but it might well account for the inconsistencies in previous studies regarding the risk allele for mentalizing performance (see also Section 4.7 for further discussion) [Lucht et al., 2013; Rodrigues et al., 2009].

In our exploratory multiple regression analyses structural GxE-effects in a cluster including the left temporal pole were associated with functional GxE-effects in bilateral precunei and frontal regions. This suggests that structural variations in the left TP are partially related to genotype-by-CAS interaction effects on (regionally distinct) mentalizing-related neural activity. We assume that this further underlines the functional role of the TP in the mentalizing network and its relevance as a target of the GxE-interaction.

4.4. Gene-by-environment effects: the right superior parietal lobule

For the interaction effects of rs53576 genotype and early environment (CAS) on adult brain structure, mentalizing-related neural activity and the personality trait alexithymia, a network including the right superior parietal lobule (SPL) emerged to be important. The SPL is regarded as a multimodal association area of high interindividual variability, whose function has been linked to the goal-directed voluntary attention system [Corbetta and Shulman, 2002], mentalizing [Abu-Akel and Shamay-Tsoory, 2011] and the parietofrontal human mirror system [Cattaneo and Rizzolatti, 2009]. Both local volume and mentalizing-related neural activity of the right SPL were modulated by a genotype-by-CAS interaction (brain regional overlap): Higher regional volume - observed in GG-homozygotes with secure childhood attachment and in A-allele carriers with insecure childhood attachment - was paralleled by lower neural activity in right SPL. Additionally in exploratory multiple regression analyses higher local volume of the right SPL was significantly associated with lower GxE modulated mentalizing-related neural activity in a cluster including bilateral precunei. This suggests that genotype-by-CAS interaction effects on mentalizing-related neural activity are partially related to structural alterations in the right SPL.

Furthermore higher local SPL volume was significantly associated with lower "alexithymia" (i.e. higher emotional self-awareness), suggesting that the SPL partially mediates the interaction effects of rs53576 and childhood attachment security on "alexithymia". In other studies higher activation of the right SPL was found in alexithymic than in nonalexithymic subjects during a mirror neuron system task [Moriguchi et al., 2009], during the observation of emotional stimuli [Karlsson et al., 2008] and the viewing of positive low-arousal pictures [Berthoz et al., 2002]. In addition, activation of the right SPL (during a mirror neuron system task) was positively correlated with the severity of alexithymia (as measured by a structured interview) [Moriguchi et al., 2009]. Moriguchi et al. (2009) suggested that insufficient self-other differentiation in alexithymia leads to the higher activation of the mirror neuron system (including right SPL), but to reduced mentalizing ability [Moriguchi et al., 2006]. Our results further support the involvement of right SPL in alexithymia. Our data also revealed that rs53576 and CAS interact to modulate both structure and neural activity of partially overlapping brain areas which are implicated in mentalizing.

4.5. Gene-by-environment effects: the amygdala

The amygdala is a key node for motivational salience processing [Lindquist et al., 2012] and considered as central for OXT's actions [Bethlehem et al., 2013]. We observed that gray matter volume of the left amygdala (basolateral and superficial groups) and hippocampus (cornu ammonis, subiculum) were jointly modulated by genotype and childhood attachment security. Insecure childhood attachment was associated with highest local volumes in GG-homozygotes and with lowest ones in A-allele carriers, but the extent of susceptibility to CAS was similar in carriers of both genotypes. In a recent study gray matter volumes in right hippocampus (cornu ammonis, subiculum) and basolateral amygdala were negatively correlated with plasma OXT levels [Andari et al., 2012]. For the left amygdala enhanced responses to fearful faces in comparison to happy faces have been reported, and OXT administration reversed the response pattern [Gamer et al., 2010]. Our exploratory multiple regression analyses revealed that higher local volume in the GxE-modulated left hippocampus/amygdala cluster is significantly associated with higher "anxiety". This result suggests that the observed GxE-effects on "anxiety" are partially mediated by alterations in the left hippocampus/amygdala volume. These data are in line with the notion of "anxiety"-related differences in salience processing and responses to fear-related cues proposed by attachment theory [Mikulincer and Shaver, 2007]. Regarding our fMRI data we did not find a significant genotype-by-CAS interaction effect on mentalizing-associated neural activity in the amygdala. It has been put forward that the amygdala is more essential for the appropriate development of mentalizing and less involved in the mentalizing process itself [Gallagher and Frith, 2003]. However, our multiple regression analyses also yielded that a higher local volume in the GxE-modulated left hippocampus/amygdala-cluster is significantly related to higher GxE-modulated mentalizing-related neural activity in occipital regions (LG/CG/FG/IoccG). Projections from the amygdala to the IoccG and FG are presumably directly involved in the modulation of attention to salient social cues like faces [Skuse and Gallagher, 2009]. Although our task did not involve facial stimuli, task behavior of the co-players was presented by visual stimuli. Our data suggest that a higher hippocampus/amygdala volume is related to higher GxE-modulated neural activity in brain areas implicated in the processing of visual social input (IoccG) [Skuse and Gallagher, 2009] and mentalizing (FG/LG) [Mar, 2011].

Yet in sum, our data confirm the link between genotype and amygdala in humans demonstrating that amygdala volume is shaped by genotype as a function of early social experiences.

4.6. Sex-specific gene-by-environment effects

By exploring sex-specific interaction effects, the susceptibility effect of rs53576 on "anxiety" was only observed in women, indicating a sexually dimorphic effect of rs53576 on attachment-related behavior. Insecure childhood attachment was associated with higher "anxiety" only in female GG-homozygotes, not in female A-allele carriers or in men. Sex-specific actions of the OXT system have been reported repeatedly [Bethlehem et al., 2013; Carter, 2007; Gimpl and Fahrenholz, 2001]. OXT receptors are regulated by gonadal steroids [Gimpl and Fahrenholz, 2001]. It has been suggested that OXT may influence pair bonding more

in women than in men, while in men the neuropeptide vasopressin may be more important [Carter, 2007; Carter et al., 2009]. Additionally, rodent studies showed that variations in maternal care resp. neonatal OXT treatment had sexually dimorphic effects on OXTR and vasopressin receptor expression in the amygdala and cingulate cortex [Bales et al., 2007; Francis et al., 2002]. In humans, both amygdala and ACC likely contain OXT and vasopressin 1a receptors [Skuse and Gallagher, 2009; Skuse and Gallagher, 2011].

In our exploratory regression analyses we found that gray matter volume of the cluster in hippocampus/amygdala and mentalizing-associated neural activity in frontal regions (SFG/MFG/ParaCL/PostCG/PreCG) were associated with "anxiety". A higher recruitment of the amygdala in anxiously attached subjects has been reported in two other fMRI studies in response to negative/unpleasant social stimuli [Vrtička et al., 2012; Vrtička et al., 2008]. On the other hand two studies (with smaller sample sizes) reported either no local volume alterations [Benetti et al., 2010] or reduced gray matter volume in the left hippocampus in association with ANX [Quirin et al., 2010]. These divergent results might indicate that the association of ANX with the left hippocampus volume depends on yet unknown factors. For example, different assessments of attachment-related anxiety may have contributed to the different results (Experiences in Close Relationships inventory (ECR-R) in the studies of Benetti et al. (2010) and Quirin et al. (2010), versus the Relationship Scales Questionnaire in our study). A positive association between ANX and the activation of frontal regions (left inferior, middle and medial frontal gyrus) has been reported in one fMRI study that investigated brain activity in response to masked happy faces [Donges et al., 2012].

While both female and male GG-homozygotes with insecure childhood attachment showed higher hippocampus/amygdala volume and a higher frontal mentalizing-related neural activation compared to those with secure childhood attachment, GG-homozygous women with an insecure childhood attachment reported more "anxiety" compared to their male counterparts: Male GG-homozygous men with insecure childhood attachment, in contrast, recruited medial frontal regions (SMedG/ACC/SMA/SFG) to a larger extent in the mentalizing task, however, this sex-specific effect on neural activity in medial frontal regions did not surpass the significance threshold (see SI 2.4.1 and 2.6.1). As our investigation of the genotype-by-CAS-by-sex effects was possibly underpowered, this insignificant result might nevertheless constitute an interesting effect. The sexually dimorphic recruitment of the ACC – an area also implicated in cognitive control [Kerns et al., 2004] – could explain why women are more vulnerable to higher "anxiety" than men if confronted with social and genetic factors associated with higher hippocampus and amygdala volume.

4.7. Oxytocin, social behavior and the early environment

The neuropeptides OXT and vasopressin are critically involved in affiliative behavior and attachment [Insel and Young, 2001]. According to Insel and Young (2001) the formation of attachment bonds involves three tasks: social approach, recognition of the partner and investment in the partner while rejecting others. As for OXT, several potential mechanisms of its action have been discussed in the literature, e.g. increasing the affiliative motivation, reducing social anxiety by decreasing physiological stress reactivity, and/or enhancing the salience of social stimuli [Bartz et al., 2011]. The social salience hypothesis postulates that OXT heightens attention to social cues, increases their salience via linking them to mesolimbic dopamine pathways and enhances the formation of social memories along with their reinforcing properties [Bartz et al., 2010; Insel and Young, 2001; Skuse and Gallagher, 2011]. Accordingly, OXT is expected to facilitate not only prosocial, but also defensive behaviors depending on the social context. In fact, several studies support this notion: OXT administration in humans increased trust and prosocial behavior in interactions with familiar or reliable others [Kosfeld et al., 2005; Zak et al., 2005], but decreased prosociality in situations of social uncertainty [Declerck et al., 2010] or social competition [De Dreu et al., 2010; Shamay-Tsoory et al., 2009]. In line with the social salience hypothesis, OXT was shown to facilitate mentalizing [Domes et al., 2007], the recognition of both positive and negative emotions from human faces [Shahrestani et al., 2013], and the formation and recall of memories of both positive and negative social information [Bartz et al., 2010; Gimpl and Fahrenholz, 2001; Guastella et al., 2008; Savaskan et al., 2008].

The functional effect of rs53576 on the OXT system is still unknown, but GG-homozygotes were shown to be more sensitive to social cues [Bakermans-Kranenburg and van Ijzendoorn, 2008; Rodrigues et al., 2009; Tost et al., 2010]. Presumably rs53576 affects the OXT-related social salience processing, involving processes of attention, reinforcement and/or memory, possibly via changes in the OXT system (e.g. density and regional distribution of OXTR). Interestingly, in our study we observed that GG-homozygotes (compared to A-allele carriers) showed increased gray matter volumes in brain areas implicated in autobiographic, episodic and semantic memory, storage and retrieval of social scripts and social concepts (bilateral temporal poles and the hippocampal area) [Insausti and Amaral, 2012; Olson et al., 2007]. We speculate that this effect on TP volume is related to increased social memory formation in GG-homozygotes. The valence of social experiences stored in TP might play a role for their recruitment in mentalizing activity, as our fMRI data suggest. Poorer recall of emotional information is also linked to alexithymia [Luminet et al., 2006]. Study results support the view that alexithymia is not caused by perceptual inability, but by deficits in the development of internal state language, consequent upon social learning and attachment experiences in childhood [Cook et al., 2013; Lemche et al., 2004; Levant et al., 2009]. Our data suggest that with regard to alexithymia A-allele carriers profited less from secure childhood attachment experiences than GG-homozygotes, and attenuated social memory formation might be implicated in this effect.

As for attachment-related "anxiety", the enhanced storage of insecure attachment experiences might increase the degree of social anxiety in GG-homozygotes. Alternatively, insecure attachment experiences in childhood could alter the anxiolytic effects of OXT. In an OXT administration study, male adults with early separation experiences (i.e. prolonged separation from one parent) showed an attenuated OXT-induced cortisol reduction compared to controls [Meinlschmidt and Heim, 2007].

We observed reduced gray matter volume in the cerebellar vermis in GG-homozygotes. The vermis is connected with the hypothalamus and OXTR-containing limbic structures (e.g. amygdala, septum), and has been conceptualized as "limbic cerebellum" [Schmahmann et al., 2007]. In more detail, the (posterior) vermis modulates neural activity in hippocampus, amygdala and septum, and damage to the vermis results in "disorders of attentional control, emotional control, autism spectrum, psychosis spectrum and social skill set" [Stoodley and Schmahmann, 2010]. It has been suggested that "the individual's ability to smoothly and automatically maintain the homeostatic, context-dependent responses that govern behavior" depends on cerebellar vermis functioning [Schmahmann et al., 2007]. Vermis and limbic brain regions might be reciprocally regulated, and OXTR might be implicated in the functioning of this circuit.

In animal studies, variations in the social rearing environment were associated with regional up- and downregulation of OXTR and changes in OXT peptide production over the lifespan [Bales and Perkeybile, 2012]. In human adults, childhood abuse was linked to changes in cerebospinal OXT concentrations [Heim et al., 2009]. Research in primate and rodent models provides evidence that epigenetic modifications play an important role in mediating long-term consequences of early social experiences via changes in gene expression [for a review see e.g. [Champagne and Curley, 2009; Kumsta et al., 2013; Zhang and Meaney, 2010]. One intensively studied epigenetic mechanism, the DNA methylation, targets CpG sites on the DNA and – depending on the location of the CpG site – can interfere with transcription and

transcriptional regulation. In the OXTR gene the methylation status of two regions was associated with transcriptional control in in vitro experiments: A region with high CpG content (CpG island) downstream of the transcription start site [Kumsta et al., 2013; Kusui et al., 2001] and a region in the third intron of OXTR [Mizumoto et al., 1997]. Methylation status in the OXTR CpG island was shown to be functional in healthy humans: Higher levels of methylation were associated with higher neural activation in STG, SMG and dorsal ACC in a social perception task [Jack et al., 2012].

It can be surmised that density and regional distribution of the OXTR is subject to regulation through methylation in the OXTR gene and that OXTR gene methylation is affected by early environmental influences. Other systems than OXT might play a role in mediating environmental influences on the OXT system, as animal studies suggest: e.g. elevated serotonin levels during early development were associated with reduced OXT expression in adulthood [Skuse and Gallagher, 2011] and high expression of estrogen receptor alpha, related to high maternal care, upregulated OXTR expression in hypothalamic regions in adults [Champagne and Curley, 2009].

Genetic polymorphisms can interfere with epigenetic and/or other regulatory mechanisms and, with regard to OXTR, thereby enhance or reduce changes in receptor density or regional distribution consequent upon early social experiences.

Taken together, altered OXTR density or distribution, mediated by genetic and/or epigenetic mechanisms, might underlie the observed GxE-interaction effects on social behavior, brain structure and activation.

Our data are in line with other studies that have demonstrated higher susceptibility of rs53576 GG-homozygotes to early life experiences [Bradley et al., 2011; Hostinar et al., 2014; Raby et al., 2013]. However, social experiences in adolescence and early adulthood were shown to be associated with higher susceptibility in A-allele carriers [Hammen et al., 2015; McInnis et al., 2015] and the developmental timing of social experiences might be critical for the genotype effect, as has been proposed by McInnis et al. (2015). Alternatively, it cannot be ruled out that rs53576 is in linkage disequilibrium with a still unknown functional genetic variation, and that variations in interlocus correlation in different populations cause flip-flop associations [Lin et al., 2007; Lucht et al., 2009].

4.8. Limitations and future directions

The functional effect of rs53576 on the OXT system is still unknown and this limits the conclusions that can be drawn on potential mechanisms of environmental modulation. Further limitations of our study have to be considered: In multimodal studies as ours several phenotypes were investigated and this raises the issue of an increased risk for false-positive results [see Tost et al., 2010]. To deal with this issue we reported FWE-corrected results when we tested our three hypotheses, and our hypotheses were developed on the basis of substantial evidence from the existing literature. However, the regression analyses have been exploratory and were not corrected for multiple testing. Probably, regression analyses have also overestimated the effects because they only included significant clusters from selected VBM and fMRI contrasts. But although the predictors (mostly) shared the same GxE-interaction, multicollinearity measures did not exceed critical values [O'Brien, 2007]). In sum, a replication of the results is warranted.

The sample size of our study was moderately large (comparable to those of Chen et al., 2011; McInnis et al., 2015 and Raby et al., 2013). In imaging genetics a sample of about 100 subjects is considered to be comparably large for the investigation of genetic main effects (e.g. [Krug et al., 2010; Paulus et al., 2013]. In neuroimaging studies the accurate estimation of effect sizes is difficult and "there is no consensus yet on the proper way to estimate power" [Kriegeskorte et al., 2010]. Power estimations for GxE-neuroimaging studies are even more challenging. The required sample size for GxE-studies depends on the strength of main (G,E) and interaction (GxE) effects and on allele frequencies (among others) [Dempfle et al., 2008]. For weak main effects and at least moderate interactions the sample requirements are less than for the detection of main effects, but otherwise a several fold higher sample size is necessary [Dempfle et al., 2008]. Therefore replications of our results in other samples are invited.

In addition, in our study the frequency of AA-homozygotes was too limited to analyze them separately. We therefore collapsed the A-allele carriers as reported in other studies [Raby et al. (2013); Bradley et al. (2011); Hostinar et al. (2014); Bakermans-Kranenburg and van Ijzendoorn (2008) and Rodrigues et al. (2009)]. However, this grouping strategy may conceal effects. The GA-genotype was shown to take either an intermediate position between the homozygotes [Tost et al., 2010], or resembled either more the GG-homozygotes [Chang et al., 2014; McQuaid et al., 2015] or the AA-homozygotes [McQuaid et al., 2015]. Therefore a separate analysis of all rs53576 genotypes would be desirable.

The genotype distribution was unbalanced with regard to sex and age, due to genotyping after the recruitment of the sample. We controlled for possible confounding effects by including sex and age in our models. We observed very similar results, when sex and age were not included in the models (see supplementary information).

Performance in mentalizing was not assessed with a behavioral measure other than our fMRI task. Further our control condition in the mentalizing task implicated motor, visual and decision making aspects, but it did not involve reinforcement or changes in reinforcement. Hence task-related activity might not only delineate mentalizing-associated processes, but might also involve reward processing. Reward processing, including reinforcement learning, prediction errors and risky behavior in decision making, is primarily associated with orbitofrontal-striatal circuit activation [Montague and Berns, 2002].

Childhood attachment security was measured retrospectively as a mental representation. Future studies would be desirable that prospectively investigate childhood attachment security and its interaction with the OXT system. Additionally a clarification of the functionality of the rs53576 polymorphism would be desirable.

Future studies could address the question if OXT administration leads to different effects on social cognition and behavior depending on the rs53576 genotype and childhood experiences. It might also be interesting to clarify if peripheral and cerebospinal fluid OXT concentrations vary in response to physical and social contact taking both genotype and childhood attachment security into account.

4.9. Conclusion

Our data show that OXTR SNP rs53576 and child–parent interaction (childhood attachment security, CAS) jointly modulate – in adulthood – the personality traits “alexithymia” and attachment-related “anxiety”, brain gray matter volume and mentalizing-related neural activity in the right superior parietal lobe (SPL), among others, possibly via changes in the OXT system. GG-homozygotes of rs53576 show higher susceptibility to early social environmental factors than A-allele carriers with regard to brain volumes and “alexithymia”, and this GxE-effect is in part sex-specific (as for attachment-related “anxiety”). Rs53576 and childhood attachment security jointly modulate structure and function of brain areas implicated in salience processing and mentalizing. Genetic effects on social memory and epigenetic mechanisms might play a role in this GxE interaction.

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Conflicts of interest

None declared.

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Supplementary Information

1. Methods

1.1 Participants

In total, neuroimaging data from 198 healthy subjects were collected. One subject had to be excluded from final analysis because no blood sample could be taken for genetic analysis and two subjects were excluded because of low structural data quality (covariance below 0.700; see sMRI analysis). From the analysis of functional data thirty-four subjects were excluded because of head movement (> 2mm/2°; n=18), indicators of insufficient participation in the fMRI mentalizing task (variability in playing behavior lower than 5%, missings more than 5%, no adequate recall of the experimental instruction in the post-assessment; n=14) and technical problems during data acquisition (n=2).

1.2 MRI

1.2.1 Voxel-based morphometry

A three-dimensional (3D) fast gradient echo sequence (GRAPPA) was used to acquire T1-weighted high-resolution anatomical images (repetition time = 1900 msec, echo time = 2.52 msec, flip angle = 9°, long term averages, inversion pre-pulse every 900 msec, field of view of 256 (feet-head [FH]) x 256 (anterior-posterior [AP]) x 176 (right-left [RL]) mm, phase encoding in AP and RL direction, voxel size = 1 mm x 1 mm x 1 mm). Structural images were preprocessed using VBM8 Toolbox standard routines and templates (version 408; http://dbm.neuro.uni-jena.de/vbm). Images were bias-corrected, tissue classified and normalized into a standard stereotactic anatomical MNI-space (resulting voxel size 1.5 x 1.5 x 1.5 mm), employing high-dimensional DARTEL normalization within a unified model. Gray and white matter segments were modulated by use of only nonlinear components to preserve actual gray and white matter values locally. The homogeneity of the resulting modulated gray matter volumes was examined by means of a covariance matrix implemented in the check data quality function. Two outliers showing covariances below 0.700 were identified and excluded. The modulated gray matter images were smoothed with a Gaussian kernel of 8mm FWHW.

We performed the Monte-Carlo simulation with the AFNI program “AlphaSim” (version

06/09/2009; Ward, 2000; http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf). As mask we entered the "mask.hdr"-file from our VBM full factorial model. The number of voxels in the mask was 469283. The cluster connectivity radius was set to rmm= 2.20 (edge-connectivity). The residual image of our data was produced by means of the AFNI program "3dcalc" from our "ResMS.hdr"-file. Smoothness of the data was computed by use of the AFNI program "3dFWHMx". Gaussian filter widths were as follows: FWHMx/y/z= 8.56/10.07/9.33.

1.2.2 Functional MRI (fMRI)

1.2.2.1 fMRI Paradigm

In this game two players are simultaneously faced with the decision to press the right or left button. Depending on the decision of both players both gain a certain amount of points according to the decision matrix. If both players choose the left button, each one gains 20 points. If both players press the right button, each one receives zero points. If one player has chosen the right button and the other one the left button, the right button press wins 20 points, the left button press 10 points. Participants were instructed with two conflicting goals, "win the series of games and reach as many points as possible". As these goals could not be accomplished by choosing always the same button, the decision matrix was designed to ensure a variable pressing of both buttons and an implicit use of mentalizing (Krach et al., 2008). The control condition was designed according to our previous studies (Kircher et al., 2009; Krach et al., 2009; Krach et al., 2008): Participants were told that they do not face a co-player in this condition, and that they had to press one of the buttons without facing any consequences of the choice. The control condition therefore did not entail mentalizing. A computer co-player was not used as additional control condition because humans activate mentalizing-related brain areas when facing computers as co-players, albeit to a somewhat lesser extent (Kircher et al., 2009; Krach et al., 2008; Rilling et al., 2004).

Prior to scanning the subjects received a comprehensive instruction and practiced the game 15 trials at minimum to become familiarized with the game rules and the decision matrix. Participants were told that they would play an online game in order to examine social decision making and that they would play consecutively against two different, but same-sex co-players who differ in their problem solving style and whom the participants would not meet to avoid any bias by personal contact. In reality,

participants played against a computer.

The fMRI paradigm was performed using Presentation software (Version 14.1, Neurobehavioral Systems, San Francisco, CA).

At the beginning of the game session in the scanner, a summary of the instructions was presented to the participants. To enhance the credibility of the co-players' existence the information was given that the game starts when every player is ready. Each player's readiness for action was indicated by a green tick on a slide. On the first slide only two of the three players were ticked off. After a short waiting period the next slide showed that all three players were ready and then the game started. The fMRI paradigm lasted 15.06 min and comprised 30 blocks: 2 blocks with the first co-player were followed by one block of the control condition and then two blocks with the second co-player followed. This sequence was repeated five times, resulting in 10 blocks for control and 10 blocks for the game task with each of the two co-players, i. e. 20 in total. Each block took 27.5 s plus a variable interval (jitter; mean value 623.9 ms, range 0-1000 ms) and started with one instruction screen (3.5 s + the jitter), announcing the next condition, followed by six game trials. Each trial consisted of one crosshair and one matrix screen, each appearing for 2 seconds. The crosshair screen indicated the time for the decision / button pressing. On the matrix screen information about the buttons both players had pressed and about the scores - of the current trial and accumulated over the trials in one block - appeared. In the control condition the matrix screen contained hash signs instead of scores. After the MR scanning participants were asked to recall experimental details to control for adequate attention to the task. Playing behavior was defined as frequency of left button presses.

1.2.2.2 fMRI data acquisition

Functional neuroimaging data were collected using T2*-weighted gradient echo planar imaging sequence sensitive to BOLD contrast (64 x 64 matrix size, 230 mm field of view, 30 ms echo time, 2.25 s repetition time, 90° flip angle, slices acquired in sequential (ascending) order with 20% distance factor, 36 axial slices orientated parallel to the AC-PC line covering the whole brain, slice thickness 3.6 mm, in plane resolution 3.6 mm x 3.6 mm). Four hundred functional images were collected and the onset of each block was synchronized to a scanner pulse.

1.2.2.3 fMRI preprocessing

Functional images were analyzed using Statistical Parametric Mapping standard routines and templates (SPM8; http://www.fil.ion.ucl.ac.uk/spm/). After discarding the initial six images to remove the influence of T1 saturation effects, functional images were spatially realigned to correct for head motion, normalized into a standard stereotactic anatomical MNI-space (resulting voxel size 2 x 2 x 2 mm), smoothed with a 8-mm isotropic Gaussian FWHM kernel and high-pass filtered. The high-pass filter was adapted to the experimental design (cut-off period 342 s).

1.2.2.4 Single subject (first-level) analysis

A general linear model was specified for each participant including two epoch regressors, modeling the game task and the control condition (each without instruction), as well as six regressors modeling head movement parameters. Parameter estimate (β-) images were calculated for each condition and each subject.

1.3 Statistical analysis

For ANOVA analysis the function “Anova (lm)” from the package “car” and in case of variance inhomogeneity robust linear models were applied (“rlm”) (Fox and Weisberg, 2011). Further packages in use were “Hmisc” (Harrell, 2012), “psy” (Falissard, 2012) and “HardyWeinberg” (Graffelman, 2012).

1.4 Genetics - DNA extraction and genotyping

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood according to standard procedures. The OXTR SNP rs53576 was genotyped on an Life Technologies 7900HT Fast Real-Time PCR System using a TaqMan 5' nuclease assay (TaqMan® SNP Genotyping Assay ID C_3290335_10; Life Technologies). Genotyping accuracy was assessed by running 15 % of the sample in duplicates. Reproducibility was 100 %.

2. Results

2. 1 Adult Attachment Style

2.1.1 Descriptive information

Avoidant attachment scores (AV; mean value: 3.17, SD: 0.75, range: 1.25-5.25) and anxious attachment scores (ANX; mean value: 2.15, SD: 0.96, range: 1-6) were not

correlated (r=0.07, P=0.34), consistent with prior study results (Vrtička, Bondolfi, Sander, & Vuilleumier, 2012).

AV and ANX were not associated with age (AV: r= 0.11, P= 0.11, ANX: r= -0.08, P= 0.27) and no significant sex differences were observed (AV: F(1,193)= 0.24, P= 0.63, ANX: F(1,193)= 1.89, P= 0.17).

2.1.2 Results

No significant main effects of CAS on ANX (CAS: t(186)= 1.40, P>0.05) or AV (CAS: t(186)= 0.40, P>0.05) and no significant genotype-by-sex interaction effects on ANX (t(186)= 0.92, P>0.05) or AV (t(186)= -0.26, P>0.05) were observed.

In a model excluding the variables sex and age, a significant genotype-by-CAS interaction effect was detected for ANX (F(1,191)= 5.6, P= 0.019, P(adjusted)= 0.041). No significant genotype-by-CAS interaction effect was observed for AV (F(1,191)= 0.0, P= 0.948).

2.2 Alexithymia

2.2.1 Descriptive information

TAS scores (mean value: 2.18, SD: 0.46, range: 1.05-3.50) were significantly correlated with AV (r= 0.33, P< 0.001) and ANX (r= 0.19, P= 0.01). TAS scores were not associated with age (r= 0.02, P= 0.79) and males showed significantly higher TAS scores (F(1,193)= 17.56, P< 0.001).

2.2.2 Results

Insecure childhood attachment was associated with a trend to higher “alexithymia” (F(1,186)= 3.25, P= 0.073). No significant genotype-by-sex interaction was found (F(1,186)= 0.09, P= 0.768).

In a model excluding the variables sex and age, the genotype-by-CAS interaction effect remained significant for TAS (F(1,191)= 5.0, P= 0.027, P(adjusted)= 0.054), but did not survive correction for multiple testing.

2.3 Voxel-based morphometry, rs53576 and CAS

2.3.1 Main and sex-specific effects on brain gray matter volume

Secure childhood attachment was associated with increased gray matter values in the left parietal lobe including the somatosensory cortex and precuneus. Female GG-homozygotes showed increased gray matter volume in parietal and occipital regions

compared to male GG-homozygotes, while local volumes in A-allele carriers were little sexually dimorphic. A sex-specific interaction effect of genotype and CAS was observed in superior frontal and temporal brain regions, indicating increased volumes for female GG-homozygotes with insecure childhood attachment compared to decreased volumes for male GG-homozygotes. More results are presented in Figure SI-1.

In a model excluding the variables sex and age, results were very similar and comprised the same significant clusters: In the genotype-by-CAS interaction contrast [(GG > A) x (secure > insecure)] we observed clusters in bilateral SMA (cluster peak x/y/z= 2/11/49, t= 4.23, k= 697), in left MFG/PreCG (cluster peak x/y/z= -44/12/54, t= 4.21, k= 1080), in right SPL (cluster peak x/y/z= 24/-58/69, t= 4.14, k= 665), in right PreCG (cluster peak x/y/z= 38/-16/61, t= 4.03, k= 785), in left SMG (cluster peak x/y/z= -63/-25/18, t= 3.64, k= 560), in left IFG (PO)/PreCG (cluster peak x/y/z= -51/8/24, t= 3.60, k= 752), and bilateral ParaCL/SMA (cluster peak x/y/z= -2/-18/58, t= 3.50, k= 966). In the genotype-by-CAS interaction contrast [(A > GG) x (secure > insecure)] we observed the cluster in hippocampus and amygdala (cluster peak x/y/z= -18/-15/-9, t= 4.51, k= 580).

2.3.2 ROI-analyses of the amygdala at an uncorrected threshold

In an exploratory test (uncorrected for multiple comparisons) we found a genotype effect: GG-homozygotes showed increased right amygdala volume (cluster peak x/y/z= 29/5/-30, t= 3.07, P(Peak,uncorr.)= 0.001, P(Peak,FWE)= 0.134, P(Cluster,FWE)= 0.279).

2.4 Mentalizing, rs53576 and CAS

2.4.1 Main, GxE-interaction and sex-specific effects on mentalizing-related neural activity

Interaction effects of rs53576 and CAS on mentalizing-related neural correlates are depicted in Figure SI-3. No significant main effects of genotype, CAS or interaction effects of genotype-by-sex or genotype-by-CAS-by-sex on mentalizing-related activity were observed.

However, a trend for significance was observed for a sex-specific genotype-by-CAS-interaction effect in the left superior medial gyrus, SFG, SMA and in right ACC (cluster peak x/y/z= -6/22/40, t= 3.70, k= 911, P(cluster, FWE) =0.064): Only male, but not female GG-homozygotes with insecure compared to secure childhood attachment activated this brain area to a higher extent [(A>GG) x (secure>insecure) x (m>w)].

In a model excluding the confounding variables sex and age, we observed a comparable activation pattern. In the genotype-by-CAS interaction contrast [(A > GG) x (secure > insecure)] the clusters in STG / Insula / CaudN / TP (cluster peak x/y/z= 44/-2/-14, t= 4.55, k= 4249, P(cluster, FWE) <0.001), in SFG / MFG / ParaCL / PostCG / PreCG (cluster peak x/y/z= 0/-28/68, t= 3.76, k= 2370, P(cluster, FWE)= 0.001) and in LG / CG / FG / IoccG (cluster peak x/y/z= 10/-90/-4, t= 3.74, k= 1561, P(cluster, FWE)= 0.008) were still significant, but the cluster in Cuneus / Precun / SoccG / SPL (cluster peak x/y/z= 16/-80/44, t= 4.67, k= 759, P(cluster, FWE)= 0.128) did no longer surpass the significance threshold at cluster-level.

2.4.2 ROI-analyses of the amygdala at an uncorrected threshold

In an exploratory test (uncorrected for multiple comparisons) GG-homozygotes with insecure childhood attachment activated the right amygdala to a higher extent than those with secure childhood attachment, while A-allele carriers showed the reversed pattern (cluster peak x/y/z= 32/-10/-10, t= 2.71, k=9, P(peak,uncorr.)= 0.004, P(peak, FWE)= 0.201, P(cluster, FWE)= 0.198).

2.5 Association of individual differences between neural activity (fMRI) and structural data (VBM)

2.5.1 Tests of assumptions for the multiple regression analyses

For all models assumptions of model fit and homoscedasticity were met (data not shown); no critical outliers were identified (Cook's distance always below 0.5). The regression models included 8 predictors for the genotype-by-CAS interaction contrasts. Expected effect sizes were unknown that would have supported the estimation of the required sample size (Green,1991). Using common rules-of-thumb (e.g. '15 subjects per predictor' or 'N ≥ 50 + 8*predictor'), sample sizes for all models were sufficient (Green, 1991; Osborne, 1999).

The variance inflation factor (VIF) for the genotype-by-CAS models varied between 1.06 and 1.98 (tolerance between 0.95 and 0.51). Multicollinearity measures therefore did not exceed critical values (i.e. variance inflation factor above 10 and tolerance below 0.1, as most commonly recommended (O'Brien, 2007)).

2.5.2 Results for the genotype-by-CAS interaction contrasts

For the genotype-by-CAS interaction contrasts we found that lower mentalizing-related

neural activity in bilateral Cuneus / Precun / SoccG / SPL was significantly associated with higher gray matter volume in the right SPL [(GG>A) x (secure>insecure)] (t(153)= -2.51, P= 0.013) and in left IFG / TP / PreCG [(GG>A) x (secure>insecure)] (t(153)= -2.21, P= 0.028). A trend for significance was observed for local volumes in the left hippocampus and amygdala [(A>GG) x (secure>insecure)] (t(153)= 1.92, P= 0.057) ($F(8,153)_{model}$=2.13, P_{model}= 0.036).

Lower mentalizing-related neural activity in bilateral STG / Insula / CaudN / TP was associated with higher local volumes in the right SPL [(GG>A) x (secure>insecure)] (t(153)= -1.97, P= 0.051), but this association failed to surpass the significance threshold ($F(8,153)_{model}$= 1.05, P_{model}= 0.398).

Lower mentalizing-related neural activity in the frontal cluster (including right SFG and MFG) was significantly related to higher gray matter volume in the left SMG [(GG>A) x (secure>insecure)] (t(153)= -2.41, P= 0.017) and lower local volumes in left IFG / TP / PreCG [(GG>A) x (secure>insecure)] (t(153)= 2.28, P= 0.024). We found a trend for significance for local volumes in left MFG / PreCG [(GG>A) x (secure>insecure)] (t(153)= 1.74, P= 0.084) ($F(8,153)_{model}$= 2.15, P_{model}= 0.034).

Lower mentalizing-related neural activity in the bilateral occipital cluster (including LG and FG) was significantly associated with lower volumes in hippocampus and amygdala [(A>GG) x (secure>insecure)] (t(153)= 2.67, P= 0.009). A trend for significance was observed for local volumes in SMA / MCC [(GG>A) x (secure>insecure)] (t(153)= -1.90, P= 0.060) ($F(8,153)_{model}$= 1.83, P_{model}= 0.075).

All other values were above P= 0.1 in the multiple regression analyses. All these analyses were uncorrected for multiple comparison; but computing a MANOVA (Pillai) with all four mentalizing-related cluster eigenvariates as dependent variables and local volumes as predictors, volumes in right SPL (Pillai(1,153,4,150)= 0.068, P= 0.031) and left IFG / TP / PreCG (Pillai(1,153,4,150)= 0.087, P= 0.008) showed significant associations.

2.6 Association between individual differences in adult attachment style and structural and functional data

2.6.1 Results

Additionally in the first model a trend towards significance (t(184)= 1.87, P= 0.063) was

observed for the association of higher volumes in right SPL with higher ANX [(GG > A) x (secure>insecure)].

Structural cluster values explained 8.2% of the variance of ANX in the first model ($F(10,184)_{model}$= 1.63, P_{model}= 0.010), and both functional and structural cluster values explained 16.2% of the variance of ANX in the second model ($F(14,147)_{model}$= 2.03, P_{model}= 0.019).

As described in SI 2.4.2, only male, but not female GG-homozygotes with insecure compared to secure childhood attachment activated left superior medial gyrus / SFG / SMA and right ACC to a higher extent [(A>GG) x (secure>insecure) x (m>w)], however this association was not significant (P= 0.064). When we additionally included the eigenvariates of this cluster in the second model - considering the exploratory nature of our genotype-by-CAS-by-sex interaction analyses and their limited cell sizes-, we observed, that not only higher mentalizing-related neural activity in bilateral frontal regions (including SFG / MFG / ParaCl / PostCG / PreCG) [(A>GG) x (secure > insecure)] (t(146)= 2.20, P= 0.030), but also lower mentalizing-related neural activity in the medial frontal regions [(A>GG) x (secure>insecure) x (m>w)] (t(146)= -2.08, P= 0.040) were significantly associated with higher “anxiety” (this model 2A explained 18.6% of the variance of ANX: $F(15,146)_{model}$= 2.23, P_{model}= 0.008).

2.6.2 Tests of assumptions for the multiple regression analyses

Model 1 included 10 predictors, model 2 14 predictors, and model 2A 15 predictors. Using common rules-of-thumb model 2 might have been, and model 2A was underpowered (see 2.5.1). For all models assumptions of model fit and homoscedasticity were met (data not shown); no critical outliers were identified (Cook's distance always below 0.5).

For model 1 the variance inflation factor (VIF) varied between 1.1 and 2.0. For model 2 VIF values varied between 1.1 and 2.7. For model 2A VIF values varied between 1.2 and 3.4. Tolerance values for model 1 varied between 0.90 and 0.50, for model 2 between 0.88 and 0.37, and for model 2A between 0.87 and 0.30. Multicollinearity measures therefore did not exceed critical values (see 2.5.1).

2.7 Association between individual differences in alexithymia and structural and functional data

2.7.1 Results

Additionally in the first model a weak trend towards significance was observed for the association of lower volumes in left hippocampus and amygdala with higher TAS [(A>GG) x (secure > insecure)] ($t(186)= -1.69$, $P= 0.094$).

Structural cluster values explained 5.3% of the variance of TAS in the first model ($F(8,186)_{model}= 1.31$, $P_{model}=0.242$), and both functional and structural cluster values explained 4.8% of the variance of TAS in the second model ($F(12,149)_{model}= 0.62$, $P_{model}= 0.820$).

2.7.2 Tests of assumptions for the multiple regression analyses

Model 1 included 8 predictors, model 2 12 predictors. Using common rules-of-thumb the second model might have been underpowered (see 2.5.1). For both models assumptions of model fit and homoscedasticity were met (data not shown); no critical outliers were identified (Cook's distance always below 0.5).

For model 1 the variance inflation factor (VIF) varied between 1.1 and 2.0. For model 2 VIF values varied between 1.1 and 2.6. Tolerance values for model 1 varied between 0.95 and 0.51 and for model 2 between 0.90 and 0.39. Multicollinearity measures therefore did not exceed critical values (see 2.5.1).

Table Legends

Tab. SI-1: Comparison of subject demographics for the study populations

	A. RSQ/TAS/sMRI	**B. fMRI**	**P**
Genotype (GG/A)	90/105	73/90	0.80[a]
CAS ratio (secure/insecure)	104/91	89/74	0.81[a]
Sex ratio (m/w)	98/97	86/77	0.64[a]
Age (mean ± SD)	24.0 ± 3.2	24.0 ± 3.1	0.83[b]
RSQ - AV (mean ± SD)	3.17 ± 0.75	3.14 ± 0.75	0.75[b]
RSQ - ANX (mean ± SD)	2.15 ± 0.96	2.12 ± 0.93	0.78[b]
TAS (mean ± SD)	2.18 ± 0.46	2.16 ± 0.45	0.64[b]

[a]= Chi-square test, [b]= T-test

Tab. SI-2: Subject demographics stratified by genotype for the structural and personality data (RSQ/TAS/sMRI, n=195) and the functional data sample (fMRI, n=163).

Sample Description RSQ/TAS/sMRI (n=195)			
rs53576 Genotype	GG n=90	AG/AA n=105	P
CAS ratio (secure/insecure)	47/43	57/48	0.77[a]
Sex ratio (m/w)	52/38	46/59	0.05[a]
Age (mean ± SD)	23.7 ± 3.2	24.3 ± 3.2	0.16[b]
RSQ - AV (mean ± SD)	3.17 ± 0.81	3.16 ± 0.69	0.91[b]
RSQ - ANX (mean ± SD)	2.28 ± 1.09	2.03 ± 0.82	0.07[b]
TAS (mean ± SD)	2.15 ± 0.43	2.21 ± 0.48	0.33[b]
Sample Description fMRI (n=163)			
	n=73	n=90	P
CAS ratio (secure/insecure)	40/33	49/41	0.96[a]
Sex ratio (m/w)	45/28	41/49	0.04*[a]
Age (mean ± SD)	23.4 ± 2.9	24.4 ± 3.2	0.04*[b]
RSQ - AV (mean ± SD)	3.15 ± 0.83	3.13 ± 0.69	0.85[b]
RSQ - ANX (mean ± SD)	2.21 ± 1.08	2.04 ± 0.78	0.24[b]
TAS (mean ± SD)	2.12 ± 0.41	2.19 ± 0.49	0.34[b]
Cooperativity (mean ± SD)	39.85 ± 15.48	37.03 ± 16.25	0.26[b]
Task reaction times (mean ± SD [ms])	606.08 ± 143.96	581.13 ± 160.15	0.3[b]

[a]= Chi-square test, [b]= T-test

Figure Legends

Fig. SI-1: Interaction effects of rs53576 and CAS on gray matter volume (P<0.05 cluster extent threshold corrected (kmin= 496 voxel)). β-values and whole-brain structural maps are depicted for the clusters in the precentral gyrus (PreCG) / superior frontal gyrus (SFG) and in the hippocampus including the amygdala.

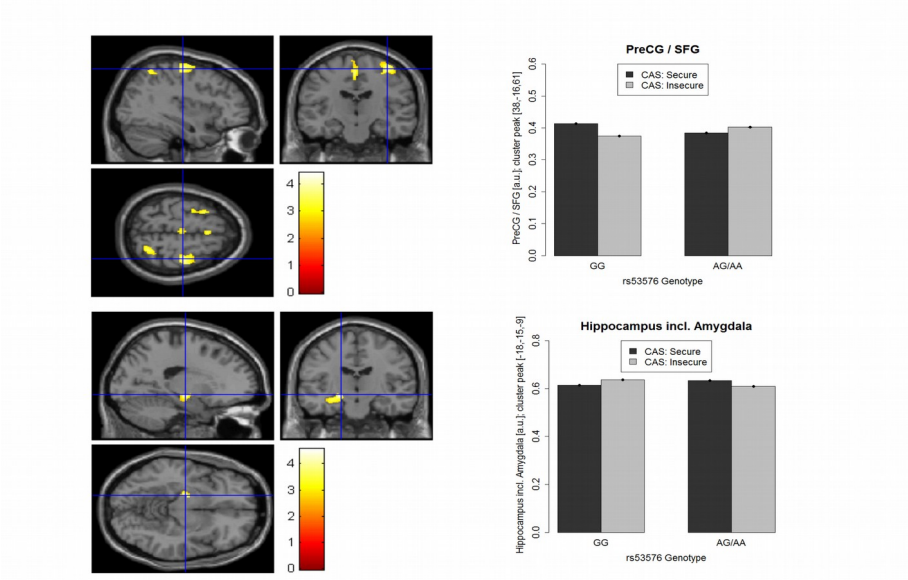
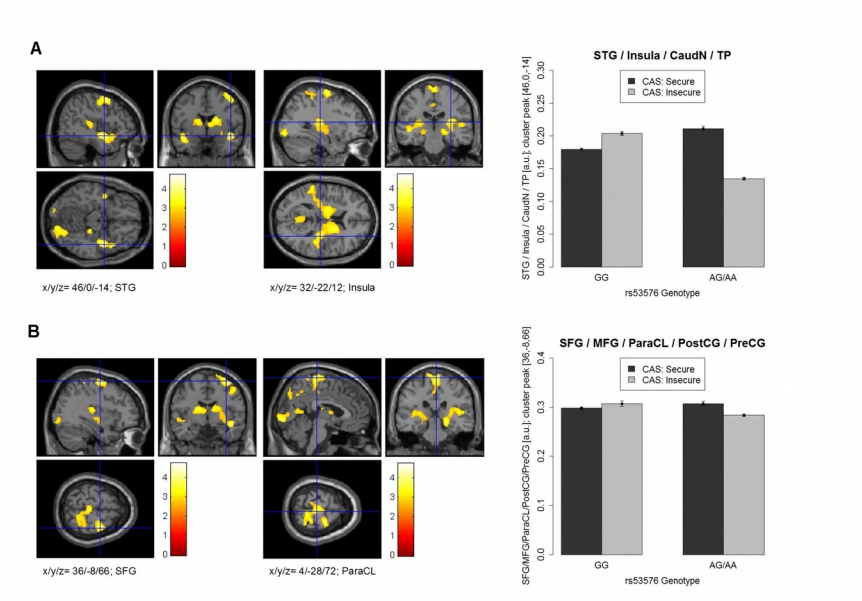


Fig. SI-2: Interaction effects of rs53576 and CAS [(A>GG) x (secure>insecure)] on mentalizing-related neural correlates (T>C; P<0.05 FWE cluster corrected). β-values and whole-brain activation maps are depicted displaying the interaction effect in the significant clusters in **A** bilateral superior temporal gyri (STG), insula lobes, caudate nuclei (CaudN) and temporal poles (TP) and **B** right superior (SFG), middle frontal (MFG), postcentral (PostCG) and precentral gyrus (PreCG) and bilateral paracentral lobules (ParaCL).



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Manuskript #3

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Title: A voxel-based morphometry study on adult attachment style and affective loss

Authors: Schneider-Hassloff H*[a,1], Jansen A[a,b], Nuscheler B[a], Kircher T[a]

Affiliations: [a]Department of Psychiatry and Psychotherapy, Philipps University Marburg, [b] Core-Facility Brainimaging, Faculty of Medicine, Philipps University Marburg

Author's present address: [1] ZNL TransferCenter for Neuroscience and Learning, Ulm University, Ulm, Germany

Abstract

The successful recovery from affective loss (i.e. bereavement, relationship breakup) has been linked to Adult Attachment Style (AAS), a personality trait. Up to now, the association between AAS, affective loss experiences and brain gray matter volume is unclear.

In 192 healthy subjects we investigated the association between MRI brain gray matter volume, applying voxel based morphometry, AAS (Relationship Scales Questionnaire, subscales “avoidance” (AV) and “anxiety” (ANX)), and number of affective losses within the last 5 years (AL; List of Threatening Experiences Questionnaire).

In a whole-brain analysis ($p<0.05$ FWE-corrected) ANX compared to AV was significantly more positively associated with brain gray matter volume in the pars opercularis of left inferior frontal gyrus. In additional region-of-interest (ROI) analyses, based on previously reported findings, positive correlations between a) ANX and local volumes in the pars orbitalis of left inferior frontal gyrus, b) AV and left temporal pole volume, and c) AL and left cerebellar volume were observed.

ANX and AV differently correlate with local volumes of the pars opercularis of the left inferior frontal gyrus which is implicated in emotion processing and regulation. The left cerebellum has been linked to affect regulation, higher-level cognitive processes and psychiatric disorders (e.g. major depression, anxiety disorders). Our results support the notion that the personality trait AAS impacts brain structure.

Keywords:

attachment; VBM; distress; inferior frontal gyrus; cerebellum; RSQ

1. Introduction

The quality and quantity of social relationships have an important impact on psychological and physiological health (House, Landis, & Umberson, 1988). Several studies have shown that a lack of social relationships (House et al., 1988) and affective loss experiences like the bereavement of a spouse (Boyle, Feng, & Raab, 2011) are associated with increased mortality. In general, affective loss experiences like bereavement and romantic relationship dissolutions are rated as stressful life events (Tennant & Andrews, 1976). On the other hand social support has beneficial effects on mental health (McEwen, 2007). Stress and emotional reactions are modulated by individual differences in the quality and quantity of social relationships (House et al., 1988; Mikulincer & Shaver, 2007).

Attachment theory provides a theoretical framework for the development of individual differences in the quality and quantity of close relationships that - in adulthood - can be assessed as the personality trait “Adult Attachment Style, AAS”. Attachment theory proposes that human attachment is an inborn drive that motivates a person to establish an emotional bond with another person (attachment figure). The attachment drive is triggered by psychological or physiological threats and motivates to seek proximity to the attachment figure in order to gain protection and to restore emotional balance (Bowlby, 1969; Mikulincer & Shaver, 2007). Attachment styles differ with regard to cognitive-affective internal working models on the availability and security-providing features of the attachment figure, likely as a consequence of interaction experiences with caregivers during childhood, and of genotype (Bowlby, 1969; Brussoni, Jang, Livesley, & Macbeth, 2000; Crawford et al., 2007; Donnellan, Burt, Levendosky, & Klump, 2008; Fraley, 2002; Mikulincer & Shaver, 2007). Interactions with available and responsive attachment figures foster the development / maintenance of a secure attachment style that is characterized by confidence in the provision of protection and support by relationship partners. Interactions with unavailable and rejecting attachment figures lead to the development of “secondary attachment strategies“ that are characteristic for insecurely attached individuals (Mikulincer & Shaver, 2007). Genotype likely also plays a role in the development of AAS, accounting for up to 45% of variance depending on adult attachment dimension and study

(Brussoni et al., 2000; Crawford et al., 2007; Donnellan et al., 2008). At least two important dimensions of consequently evolving adult attachment style have been identified, namely “avoidance” (AV) and “anxiety” (ANX). In adulthood the personality traits AV and ANX can be reliably assessed by two-dimensional self-report measures, conceptualized as two orthogonal axes (Kurdek, 2002; Mikulincer & Shaver, 2007; Simpson, Rholes, & Nelligan, 1992).

Individuals with an avoidant attachment style (AV) attempt to prevent the triggering of the attachment drive. They suppress or divert their attention away from emotions that are triggered by threats and they block emotional reactions, as for instance to the loss of an attachment figure. The striving for self-reliance and independence interferes with support seeking and problem solving. But this distancing strategy does not prevent avoidantly attached individuals from higher levels of physiological distress (Mikulincer & Shaver, 2007). An anxious attachment style (ANX) is characterized by an increased attachment drive and heightened proximity-seeking. Anxiously attached people sustain or even intensify negative emotions in the attempt to obtain attention and care of the attachment figure. They are ambivalent about support seeking and hypervigilant to threat-related feelings (emotion-focused coping style) (Mikulincer & Shaver, 2007).

Several studies investigated how AAS is related to distress reactions and coping behavior after affective loss experiences: After relationship breakups anxiously attached individuals reported high levels of physical and emotional distress, a high rate of proximity-seeking reactions and more social coping strategies. AV also showed weak, but positive associations with physical and emotional distress, however in contrast to ANX, AV was associated with more self-reliant and less social coping behavior (Davis, Shaver, & Vernon, 2003). In a study investigating the recovery after nonmarital relationship dissolution, insecure compared to secure attachment was associated with a slower emotional recovery (Sbarra, 2006). Finally, after bereavement of a close one (spouse, family member, close friend) within the past 18 months, ANX was related to higher levels of depression and AV to more somatization, while a secure attachment style was associated with less depression (each assessed by self-report) (Wayment & Vierthaler, 2002). In sum, these studies have shown that individual adult attachment styles are associated with differences in coping strategies and recovery from affective loss experiences.

The neural basis of stress regulation implicates the medial prefrontal cortex, the amygdala and the hippocampus (Ulrich-Lai & Herman, 2009), while emotion regulation recruits neural circuits encompassing the lateral prefrontal (including inferior frontal) and dorsomedial prefrontal cortex, the anterior cingulate, the striatum and inferior parietal cortex (Ochsner, Silvers, & Buhle, 2012; Phillips, Ladouceur, & Drevets, 2008). To our knowledge, only two studies have up to now investigated how brain structure (brain gray matter volume) is modulated by AAS in healthy subjects (Benetti et al., 2010; Quirin, Gillath, Pruessner, & Eggert, 2010). Quirin and colleagues (Quirin et al., 2010) investigated the association between ANX, AV and the bilateral hippocampal volume in a region-of-interest (ROI) approach (n= 22). They reported that AV is associated with reduced gray matter volume in the bilateral hippocampi and ANX with reduced local volumes in the left hippocampus. Benetti and colleagues (2010) found as a result of a whole-brain multiple regression analysis (sample size of n=32, $p<0.05$, false discovery rate (FDR) corrected) that ANX is positively correlated with gray matter volumes in the left lateral orbital gyrus and negatively correlated with volumes of the right anterior temporal pole. The results were interpreted as attachment-related variations of gray matter volumes in brain regions implicated in emotion regulation (Benetti et al., 2010; Quirin et al., 2010) and stress regulation capabilities (Quirin et al., 2010). Additionally Benetti et al. (2010) reported a positive association between AV and left anterior temporal pole and left inferior semilunar lobule of the cerebellum, as well as a positive association between an interaction term AV-by-ANX and left cerebellum, however with a lower statistical threshold ($p<0.001$ uncorrected).

Testing “the hypothesis, that the impact of affective losses on gray matter volume is moderated by attachment style”, Benetti and colleagues investigated the association between AAS, affective loss and gray matter volume (Benetti et al., 2010). Affective loss was defined as loss of a relative, of a close friend, and/or separation from a spouse within the previous five years. The number of affective losses (AL) was shown to be positively associated with local volumes in the left cerebellum, and this effect of AL in the left cerebellum was moderated by AV ($p<0.05$ FDR corrected). With a lower threshold ($p<0.001$ uncorrected) AL was shown to be negatively correlated with local volumes in left precuneus and lateral orbital gyrus.

In the present study, we aimed to replicate the findings of Benetti et al. (2010) and

Quirin et al. (2010) in an independent sample. We investigated the association between AAS, AL and brain gray matter volume in a large group of healthy subjects (n=196). We hypothesized that AV is negatively associated with bilateral hippocampal volume (Quirin et al., 2010) and positively associated with left anterior temporal pole and cerebellar volume (Benetti et al., 2010). We expected that higher ANX is associated with reduced regional volumes in the left hippocampus (Quirin et al., 2010) and right anterior temporal pole and with higher volumes in left lateral orbital gyrus (Benetti et al., 2010). We further expected that AV-by-ANX is positively correlated with left cerebellar volume (Benetti et al., 2010). Finally, we expected that higher AL is related to higher volumes in left cerebellum and lower ones in left precuneus and lateral orbital gyrus (Benetti et al., 2010). We also hypothesized that the association between AL and left cerebellar volume is modulated by AV (Benetti et al., 2010).

2. Methods

2.1 Participants

Neuroimaging data from 198 healthy subjects were collected. Inclusion criteria were student status, age (18–40 years), right-handedness (as assessed by the Edinburgh Inventory, Oldfield, 1971, inclusion criterion >+40), German as native language and Western- or Middle-European descent. Exclusion criteria were history of major psychiatric disorders of participants and their first-degree relatives according to ICD-10 (assessed by the Mini-International Neuropsychiatric Interview, Ackenheil, Stotz, Dietz-Bauer, & Vossen, 1999), relevant medical or neurological diseases, psychology students (to avoid a bias due to familiarity with a study task manipulation which is of no relevance here) and metal implants or other MRI contraindications. All participants were students of the Universities of Marburg or Gießen (Germany). Participants gave written informed consent and the study protocol was approved by the local ethics committee according to the declaration of Helsinki. Two subjects were excluded from the analysis of structural data because of low data quality (covariance below 0.700). Four subjects were excluded because they provided imprecise (n=3) or (with regard to their current age) impossible (n=1) age statements for at least one affective loss experience. The characteristics of the resulting 192 subjects were as follows: 50.0% women; mean age = 24.1 years, SD= 3.2, range 19-38.

2.2 Measures and Procedure

2.2.1 Adult attachment style

Individual adult attachment style (AAS) was assessed with a German version of the Relationship Scales Questionnaire (RSQ) (Griffin & Bartholomew, 1994; Stellmacher et al., unpublished data) and analyzed according to the two-dimensional model of adult attachment style proposed by Simpson (1992) that defines "anxiety" (ANX) and "avoidance" (AV) as two orthogonal axes (see also Kurdek, 2002). RSQ items were rated by the probands using a 6-point scale. The composite mean scores for the attachment dimension AV and ANX were created by averaging eight and five item scores respectively (Cronbach's α - a coefficient of internal consistency and reliability - for AV: 0.69; Cronbach's α for ANX: 0.83) (Kurdek, 2002). We chose the RSQ over the ECR-R questionnaire because moderate correlations between ANX and AV are frequently reported for the ECR-R subscales (Benetti et al., 2010; Mikulincer & Shaver, 2007; Quirin et al., 2010), but not for the RSQ subscales (e.g., Vrtička, Bondolfi, Sander, & Vuilleumier, 2012). All questionnaires were administered prior to scanning (in general at least one day before).

2.2.2 Affective loss

We assessed the number of affective losses (AL) using a subset of questions from the List of Threatening Experiences Questionnaire (LTE-Q) (Brugha & Cragg, 1990). We used items 3, 4, 5 and 6 of the LTE-Q to measure AL, that were loss of a relative, loss of a close friend, separation from a spouse and breakup of a steady relationship. We included breakup of a steady relationship as affective loss experience because the results of Sbarra and colleagues (2006) had revealed attachment-related differences in the emotional recovery after nonmarital relationship dissolutions. Analogous to Benetti et al. (2010), we assessed AL within the previous five years. Because the AL distribution was right-skewed, we additionally computed a dichotomous variable (no affective loss experience [AL=0; n=90] vs. at least one affective loss experience [AL >0; n= 102]).

2.2.3 Behavioral data

Statistical analyses of behavioral data were performed using R 2.15.2 (http://www.r-project.org/). For ANOVA analysis the function "Anova (lm)" from the package "car" was applied (Fox & Weisberg, 2011). Further packages in use were "Hmisc" (Harrell,

2012) and “psy” (Falissard, 2012).

2.2.4 Voxel-based morphometry (sMRI)

A three-dimensional (3D) fast gradient echo sequence (GRAPPA) was used to acquire T1-weighted high-resolution anatomical images (repetition time = 1900 msec, echo time = 2.52 msec, flip angle = 9°, long term averages, inversion pre-pulse every 900 msec, field of view of 256 (feet-head [FH]) x 256 (anterior-posterior [AP]) x 176 (right-left [RL]) mm, phase encoding in AP and RL direction, voxel size = 1 mm x 1 mm x 1 mm). Structural images were preprocessed using VBM8 Toolbox standard routines and templates (version 408; http://dbm.neuro.uni-jena.de/vbm). Images were bias-corrected, tissue classified and normalized into a standard stereotactic anatomical MNI-space (resulting voxel size 1.5 x 1.5 x 1.5 mm), employing high-dimensional DARTEL normalization within a unified model. Gray and white matter segments were modulated by use of only nonlinear components to preserve gray and white matter values locally. The homogeneity of the resulting modulated gray matter volumes was examined by means of a covariance matrix implemented in the check data quality function. Two outliers showing covariances below 0.700 were identified and excluded. The modulated gray matter images were smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM). SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) was used to calculate second-level group statistics. Localization of peaks are reported as MNI-coordinates. For the anatomical localization of the structural data, probabilistic cytoarchitectonic maps according to the SPM Anatomy Toolbox (version 2.1; http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html) (Eickhoff et al., 2005) and the Wake Forest University PickAtlas software (version 2.5.2; fmri.wfubmc.edu) were used as reference.

2.2.5 Statistical group analysis

We performed a SPM8 whole-brain multiple regression analysis as described in Benetti et al. (2010). We computed an interaction term encoding the interaction between AV and ANX by multiplying the mean-centered AV and ANX vectors. Two additional interaction terms were computed which encoded the interaction between AL and mean-centered AV and ANX respectively. The variables AV, ANX, AL and the three

interaction terms AV-by-ANX, AL-by-AV, AL-by-ANX were modeled as covariates of interest. Age and sex were included as covariates of no interest, resulting in a total of 8 covariates.

To investigate the association between AAS, AL and brain structure, we examined the extent to which gray matter volume was associated with the three main variables, the three interaction terms and with AV and ANX in comparison (AV>ANX and AV<ANX). We chose a whole-brain voxel-wise threshold of $p<0.05$ family-wise error (FWE) corrected. Comparably to Benetti et al. (2010), we additionally report clusters with a minimal cluster size of 50 voxels at a statistical threshold of $p<0.001$ uncorrected, but we will discuss only the FWE corrected results.

We further employed the small volume correction function in SPM8 with a statistical threshold of $p<0.05$ FWE corrected, using the MNI-coordinates reported by Benetti et al. (2010) and Quirin et al. (2010) as a centre of a 10mm-radius-sphere in the respective contrast images: positive association with ANX (left lateral orbital gyrus: x= -43, y= 26, z= -11), negative association with ANX (right anterior temporal pole: x= 50, y= -26, z= -26; left hippocampus: x= -33, y= -18, z= -14), positive association with AV (left superior temporal gyrus of anterior temporal pole: x= -27, y= 5, z= -23; left inferior semilunar lobule of the cerebellum: x= 24, y= -75, z= -49), negative association with AV (left hippocampus: x= -23, y= -9, z= -25; right hippocampus: x= 22, y= -8, z= -26), positive association with AV-by-ANX (left cerebellum: x= -44, y= -75, z= -34), positive association with AL (left cerebellum: x= -36, y= -75, z= -29), negative association with AL (left precuneus: x= -31, y= -80, z= -11; left lateral orbital gyrus: x= -43, y= 26, z= -11), and negative association with AL-by-AV (left cerebellum: x= -39, y= -75, z= -30).

For the small volume correction analyses we also report results with a statistical threshold of $p<0.05$ uncorrected and a minimum cluster size of 20 voxels. This rather low statistical thresholding has also been applied by other researchers in replication studies to ensure that a non-replication of previous findings is not due to strict alpha-correction levels (see Paulus et al., 2013).

As the distribution of AL was right-skewed in our sample, we additionally computed a whole-brain full-factorial model with the dichotomous AL variable (no affective loss experience vs. at least one affective loss experience, see above) as one two-level factor. We included AV, ANX and AV-by-ANX as covariates of interest (interacting with the

factor AL) and sex and age as covariates of no interest into the model.

3. Results

3.1 Adult attachment style and affective loss - descriptive information

Mean values, standard deviations (SD), ranges and correlations of AV, ANX and AL for the study sample are presented in Table 1. AV and ANX were not correlated (r= 0.06, p= 0.388), consistent with prior results of the RSQ subscales AV and ANX (Vrtička, Bondolfi, Sander, & Vuilleumier, 2012). We observed a significant positive correlation of AL with ANX (r= 0.18, p= 0.012), but not with AV (r= -0.10, p= 0.164). Furthermore women reported significantly more affective losses than men (p= 0.009).

3.2 Association of brain gray matter volume with adult attachment style and affective loss

3.2.1 Whole-brain analysis

AL, the RSQ-subscales AV and ANX and their interactions were not significantly correlated with brain gray matter volume after correction for multiple comparisons across the whole brain. But with regard to the contrast (AV < ANX), we found that ANX was significantly more positively correlated with a single voxel in the left inferior frontal gyrus (IFG) (pars opercularis) than AV (x= -47, y= 18, z= 15, t= 4.67, k=1, p(FWE)=0.048). By lowering the statistical threshold to p<0.001 uncorr., the IFG cluster size increased to 170 voxels (see Table SI-1 and Figure 1). The reverse contrast (AV > ANX) showed no significant effects. All results of the statistical whole-brain multiple regression analysis with a statistical threshold of p<0.001 uncorrected are presented in Table SI-1.

3.2.2 Small volume correction analyses

None of the small volume correction analyses yielded significant results with a significance threshold of 0.05 FWE corrected. But by lowering the statistical threshold (p<0.05 uncorrected, minimal cluster size 20 voxels), we detected in the small volume correction analyses that ANX is positively associated with local volumes in the left inferior frontal gyrus (pars orbitalis), that AV is positively associated with left temporal pole and negatively associated with right basolateral amygdala volumes, and that AL is positively associated with left cerebellar volume (see Table 2).

3.3 Association of brain gray matter volume with adult attachment style and the experience of affective loss vs. no affective loss

3.3.1 Whole-brain analysis

In the whole-brain full factorial model with the dichotomous AL variable as factor we did not observe significant results with a significance threshold of $p<0.05$ FWE corrected. The results of the whole-brain full factorial model with a significance threshold of $p<0.001$ uncorrected and a minimum cluster size of 50 voxels are presented in Fig. 2 and the supplement (Table SI-2).

3.3.2 Small volume correction analyses

Again none of the small volume correction analyses yielded significant results with a significance threshold of 0.05 FWE corrected. But by lowering the statistical threshold ($p<0.05$ uncorrected, minimal cluster size 20 voxels), in the small volume correction analyses we observed in the contrast (affective loss > no affective loss) significant effects in the left cerebellum (cluster peak: x= -39, y= -78, z= -20, t= 3.28, k= 120, p(uncorr.) = 0.001, p(FWE)= 0.024). Further we found a significant voxel in the pars orbitalis of the left IFG in the contrast (affective loss < no affective loss) (cluster peak: x= -51, y= 30, z= -6, t= 1.75, k= 1, p(uncorr.) = 0.040, p(FWE)= 0.393).

4. Discussion

We investigated the association between AAS, AL and brain gray matter volume. Our results showed that ANX significantly differs from AV in its association with gray matter volume in the pars opercularis of the left inferior frontal gyrus (IFG): ANX was positively and AV negatively correlated with IFG gray matter volume. Furthermore, in our ROI analyses, that were based on previously reported results (Benetti et al., 2010; Quirin et al., 2010), we observed positive associations a) between AL and left cerebellar volume, b) between ANX and local volumes in the pars orbitalis of left IFG and c) between AV and left temporal pole volume. Additionally, we found a weak negative association between AV and right basolateral amygdala volume. When we contrasted subjects with at least one affective loss experience vs. subjects with no affective loss experience, the results supported the positive association between AL and left cerebellar volume. They also hinted at a negative association between AL and the left IFG (pars orbitalis) volume.

4.1 Adult attachment style and brain gray matter volume

Attachment theory attributes differential emotion processing strategies to AV and ANX: Inhibition and suppression of emotions are regarded as typical for avoidantly attached individuals. In contrast sustaining and intensifying of (especially negative) emotions are frequently observed in anxiously attached people (Mikulincer & Shaver, 2007).

In this study ANX was positively associated with local volumes in the pars opercularis of the left IFG while AV was negatively associated with it, albeit only a small part of this IFG cluster surpassed the significance threshold of $p<0.05$ FWE corrected. The left pars opercularis is part of Broca's area that is implicated in language production and other motor functions. The left pars opercularis is also involved in the imitation of movements (Heiser, Iacoboni, Maeda, Marcus, & Mazziotta, 2003; Molnar-Szakacs, Iacoboni, Koski, & Mazziotta, 2005) and of facial emotional expressions (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003). It is thereby considered to be part of the parietofrontal mirror neuron system (Cattaneo & Rizzolatti, 2009) that allows the understanding of other's emotions (Carr et al., 2003). Children with high-functioning autism showed significantly reduced mirror neuron activity in the bilateral partes opercularis while imitating and observing emotional expressions and this reduced activity correlated with symptom severity in the social domain (Dapretto et al., 2006).

Additionally, in a neuroimaging study with healthy adults the left pars opercularis was shown to be activated during both emotional up- and downregulation, while the pars orbitalis and triangularis of IFG were only active during emotional downregulation (Domes et al., 2010). Activation of the pars opercularis of the left IFG was also found to be related to arousal inhibition (Beauregard, Lévesque, & Bourgouin, 2001). Subsequently the IFG (encompassing the pars opercularis) has been considered to play a mediating role in an emotional self-regulation circuit including prefrontal and subcortical limbic brain regions (Domes et al., 2010). In young adults, left pars opercularis cortex thickness was positively associated with impaired response inhibition (false alarms) in a Go/Nogo task, but only when sad faces, and not neutral or other emotional stimuli were used as 'Go' resp. 'Nogo' stimuli (Fonseka, Jaworska, Courtright, MacMaster, & MacQueen, 2016). In sum, these results suggest that the pars opercularis of left IFG is involved in empathy and emotional self-regulation.

With regard to attachment, neural activations of the IFG have been reported as well,

however, with other parts of the IFG involved: Activation of the pars triangularis of right IFG correlated with increasing arousal of the attachment system stimulated by attachment narratives (by use of the Adult Attachment Projective) (Buchheim et al., 2006). With regard to adult attachment style, left IFG (BA 47) activity was shown to be positively associated with ANX in response to masked happy facial expressions (Donges et al., 2012), and during a social interaction task bilateral IFG activation (pars triangularis and orbitalis) was significantly more negatively correlated with ANX than with AV (Schneider-Hassloff, Straube, Nuscheler, Wemken, & Kircher, 2015). However, our findings suggest that brain gray matter volume in the pars opercularis which is implicated in emotion processing and emotional self-regulation is associated with adult attachment style.

We were able to replicate some of the associations between AAS and brain gray matter volume which have been reported by Benetti et al. (2010): We observed positive associations a) between ANX and local volumes in the pars orbitalis of left IFG, b) between AV and left temporal pole volume, and c) between AL and left cerebellar volume. We also found a weak negative association between AV and right basolateral amygdala volume, i.e., a brain area that is adjacent to the reported volume modification in the hippocampus (Quirin et al., 2010). However, we were unable to find an association between ANX and brain gray matter volumes in anterior temporal pole, between AV or AV-by-ANX and left cerebellum or between AL and left precuneus or orbital gyrus (reported by Benetti (2010)), or between ANX or AV and gray matter volume in the hippocampus (reported by Quirin et al. (2010)).

First, differences in methodology may have contributed to the divergent results: AV and ANX were assessed with different questionnaires: The Experiences in Close Relationships inventory (ECR-R) in the studies of Benetti et al. (2010) and Quirin et al. (2010), versus the RSQ in our study. Although items are partially overlapping, moderate correlations between ANX and AV are frequently reported for the ECR-R subscales (Benetti et al., 2010; Mikulincer & Shaver, 2007; Quirin et al., 2010), but not for the RSQ subscales (as in our study and in e.g. Vrtička et al. (2012)) indicating differences in the conceptualization of the subscales. Second, a replication of true effects might afford a larger sample size as the original one to reach adequate statistical power (see Button et al., 2013). However, the sample size of our study was very large (six- to nine-

fold compared to those of Quirin et al. (2010) and Benetti et al. (2010)), and it is thereby unlikely that the inconsistent findings are due to a lack of power in our study. Our results could as well indicate that the reported effects of ANX and AV on gray matter volume (Benetti et al., 2010; Quirin et al., 2010) are smaller than expected or absent.

4.2 Affective loss, adult attachment style, and brain gray matter volume

We found a weak association between AL and brain gray matter volume in the left cerebellum (crus I) as reported by Benetti et al. (2010). Although our sample size allowed the detection of smaller effects (see above), the weaker correlation with AL might also be attributed to the lower prevalence of affective loss in our sample (mean(AL)= 0.85) compared to the study of Benetti et al. (2010) (mean(AL)= 1.34). When we accounted for the right-skewed distribution of AL by comparing subjects with at least one affective loss experience vs. subjects with no affective loss experience, our results again showed a weak positive association between AL and left cerebellar volume as reported by Benetti et al. (2010). However, and in contrast to Benetti et al. (2010), we did not observe a modulation of the association between AL and cerebellar volume by AV.

Our data provide evidence for a weak relation between cerebellar brain gray matter volume and affective loss experiences. As outlined by Benetti et al. (2010), the cerebellum has been linked to the regulation of affect, and psychiatric disorders like major depression and anxiety disorders were shown to be associated with functional and structural abnormalities of the cerebellum. The cerebellar crus I region, which is anatomically connected to prefrontal (e.g. anterior cingulum) and posterior parietal cortices (Stoodley & Schmahmann, 2010), has been shown to be involved in higher-level processes (i.e. attentional and executive control) and presumably plays a role in schizophrenia (Schmahmann, Weilburg, & Sherman, 2007).

4.3 Limitations

AL was assessed retrospectively, and our sample reported a rather low amount of affective losses. However, we investigated a representative sample of students. We included only students in our study in order to achieve a homogenous sample: While this strategy reduces potential confounder effects, it might also limit the

generalisability of the results. Interestingly, we observed a positive correlation between ANX and AL, comparably to Benetti et al. (2010). However, as both studies were cross-sectional it is not possible to disentangle if affective loss experiences were followed by increased ANX or reverse. A change in AAS during adulthood was found in up to 25% of subjects, however, the main direction of change was observed from an insecure attachment style toward a secure one (see Kirkpatrick & Davis, 1994). A more detailed analysis of our data (data not shown) yielded that only the number of relationship breakups was significantly related to AAS: a positive correlation was observed for ANX (driven by the female subsample) and a negative one for AV. Higher social withdrawal and increased odds of being single have been reported for AV compared to securely and anxiously attached individuals (Mikulincer & Shaver, 2007). The increased avoidance of relationships might thereby decrease the likelihood of a relationship breakup for AV. On the other hand, ANX was shown to be associated with the shortest relationship duration compared to avoidantly or securely attached subjects when the duration was retrospectively assessed (Hazan & Shaver, 1987), and this could explain the positive association between ANX and relationship breakup. To further elucidate the association between AAS, AL and brain gray matter volume a longitudinal study would be desirable.

4.4 Conclusions

Our results showed that ANX and AV differently modulate the volume of the pars opoercularis of the IFG, a brain area that is implicated in emotion processing and regulation. Furthermore, we replicated some of the previously reported associations between AAS, AL and brain gray matter volume, e.g. an association between AL and the cerebellum, a brain area related to affect regulation and psychiatric disorders. However, our data also suggest that some of recently reported correlations might be lower than expected in the population.

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Conflicts of interest

None declared

Legends to Tables

Table 1: Subject demographics (n=192)

Descriptive information for the variables “avoidance” (AV) and “anxiety” (ANX) assessed by the Relationship Scales Questionnaire (RSQ), for the variable “number of affective losses” (AL) assessed by the List of Threatening Experiences Questionnaire (LTE-Q), as well as for sex and age. PPC indicates the Pearson Product Moment correlation coefficient.

Variable	Mean ± SD (range)	RSQ - AV (PPC)	RSQ - ANX (PPC)	LTEQ - AL (PPC)
RSQ - AV	3.18 ± 0.74 (1.25 - 5.25)	-	0.06	-0.10
RSQ - ANX	2.15 ± 0.96 (1 - 6)	0.06	-	0.18*
LTEQ - AL	0.86 ± 1.05 (0 - 5)	-0.10	0.18*	-
Age	24.1 ± 3.2 (19 - 38)	0.11	-0.08	-0.01
Sex [m/f]	96 / 96	0.59[a]	-1.25[a]	-2.65*[a]

Abbr.: AL= „number of affective losses“, ANX= „anxiety“, AV= „avoidance“, PPC= Pearson Product Moment correlation, SD= standard deviation.

[a] t-value of two-sample t-test for mean differences, * <0.05 p-value

Table 2: Small volume correction analyses of the association between AAS, AL and brain gray matter volume.

The results of the small volume correction analyses with a 10mm-radius-sphere, a significance threshold of p<0.05 uncorrected and a minimum cluster size of 20 voxels are displayed. No suprathreshold clusters were observed in following small volume analyses: positive correlation with AV (x/y/z= 24/-75/-49), negative correlation with AV (x/y/z= -23/-9/-25), negative correlation with ANX (x/y/z= 50/-26/-26, x/y/z= -33/-18/-14), positive correlation with AV-by-ANX (x/y/z= -44/-75/-34), negative correlation with AL (x/y/z= -31/-80/-11, x/y/z= -43/26/-11) and negative correlation with AL-by-AV (x/y/z= -39/-75/-30).

Center of sphere (x/y/z)	**Anatomical region**	**x**	**y**	**z**	**t**	**k**	**p (uncorr)**	**p (FWE)**
AV - positive correlation								
-27/5/-23	left temporal pole	-29	9	-24	2.32	104	0.011	0.191
AV - negative correlation								
22/-8/-26	right basolateral amygdala	29	0	-24	1.73	5	0.042	0.401
ANX - positive correlation								
-43/26/-11	left IFG (pars orbitalis)	-47	24	-2	1.97	6	0.027	0.323
AL - positive correlation								
-36/-75/-29	left cerebellum (lobule VIIa crus I) (extending into fusiform and inferior occipital gyrus)	-39	-78	-20	2.30	35	0.011	0.198

Abbr.: AAS= adult attachment style, AL= “number of affective losses“, ANX= “anxiety“, AV= “avoidance“, k= number of voxels, uncorr= uncorrected.

Legends to Figures

Fig. 1: Adult attachment style modulates gray matter volume (whole-brain analysis). Whole-brain activation maps with a significance threshold of p<0.001 uncorrected are depicted on the left. For illustrative purpose the correlation of RSQ-subscales AV and ANX with the extracted cluster β-values of the left inferior frontal gyrus (pars opercularis and triangularis) (cluster peak [-47,18,15], p<0.001 uncorrected) are shown. ANX is positively (r= 0.18) and AV negatively (r= -0.14) correlated with cluster β-values (blue= AV, red= ANX). r= Pearson Product Moment Correlation coefficient.

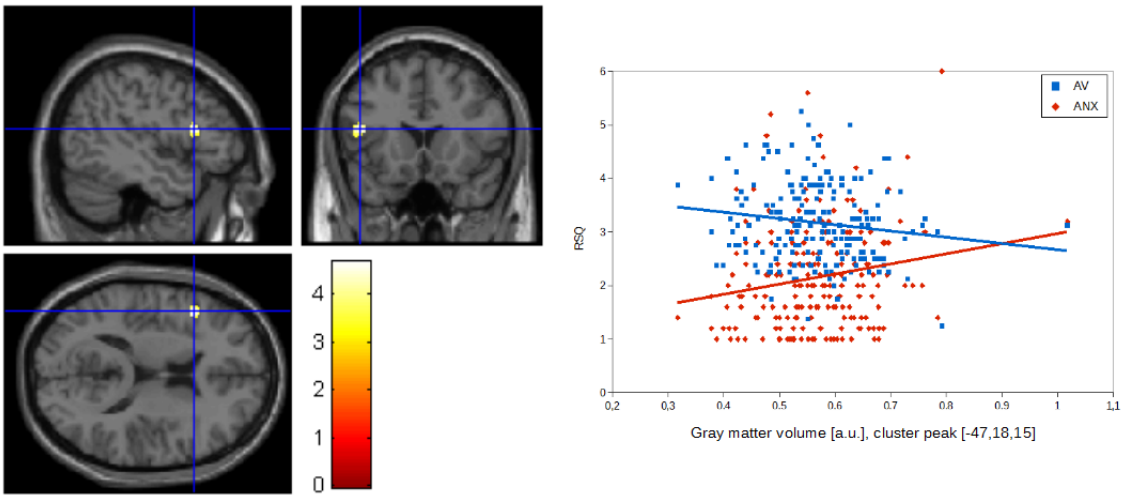
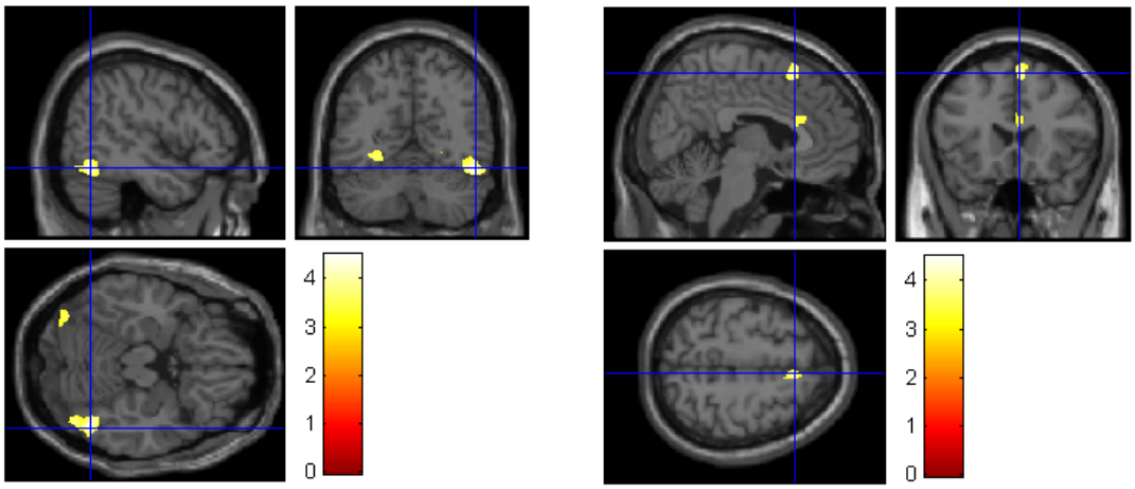


Fig. 2: Association of brain gray matter volume with the experience of affective loss vs. no affective loss

The whole-brain activation maps with a significance threshold of p<0.001 uncorrected and a cluster size threshold of 50 voxels show clusters of the contrast (affective loss > no affective loss) (crosshairs at [50,-60,-18] on the left and at [6,21,57] on the right).



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Supplementary Information

Table SI-1: Adult attachment style, AL and brain gray matter volume.

Results of the whole-brain multiple regression analysis in SPM8 with a statistical threshold of p<0.001 uncorrected and a minimum cluster size of 50 voxels, investigating the association between brain gray matter volume and the three main variables AV, ANX and AL, the three interaction terms (AV-by-ANX, AL-by-AV, AL-by-ANX) and AV and ANX in comparison (AV<ANX, ANX<AV). No results were observed for the correlation between local volumes and AL-by-AV, AL-by-ANX, the negative correlation with ANX and AL, and for (AV > ANX). FWE corrected p-values of peaks are listed for completeness. Brain areas are labeled according to the SPM Anatomy Toolbox (version 2.1) and the Wake Forest University PickAtlas software (version 2.5.2). Bold = significant at p<0.05 FWE corrected, k= cluster size in voxels.

Anatomical region	**Brain Area**		**x**	**y**	**z**	**t**	**k**	**p (FWE)**
AV - positive correlation								
Caudate nucleus		R / L	0	2	7	3.74	60	0.685
AV - negative correlation								
Insula lobe	BA 44	L	-44	9	0	4.24	142	0.203
IFG (POp)	BA 44	L	-47	17	15	4.11	95	0.303
Posterior-medial frontal gyrus	BA 6	L	-6	9	66	3.53	69	0.881
Cerebellar hemisphere	Lobule VIIa crusII	L	-17	-91	-38	3.35	115	0.967
Middle frontal gyrus		R	47	47	19	3.33	73	0.974
ANX - positive correlation								
Cerebellar vermis	Lobule IX,VIIa/b	R / L	2	-58	-36	3.53	275	0.877
AV-by-ANX - positive correlation								
Calcarine gyrus / precuneus	hOc1/2	R / L	0	-60	10	4.02	123	0.379
Inferior / middle occipital gyrus	hOc5,hOc4la	L	-42	-73	-3	3.62	50	0.809
AV-by-ANX - negative correlation								
Cerebellar hemisphere / vermis	Lobule VI, VIIIa	L	-11	-66	-29	4.30	373	0.171
Cerebellar hemisphere / vermis	Lobule VI, VIIa/b	R	11	-67	-29	4.06	228	0.346

AV-by-ANX - negative correlation								
Middle orbital gyrus	Fp1	R	24	65	-14	3.63	82	0.793
Rectus		R / L	0	36	-26	3.60	71	0.821
Cerebellar hemisphere	Lobule VIIa	L	-21	-72	-35	3.35	70	0.968
Cerebellar hemisphere	Lobule VIIa	L	-30	-82	-24	3.31	85	0.977
AV < ANX								
IFG (POp/PT**)**	**BA 44**	**L**	**-47**	**18**	**15**	**4.67**	**170**	**0.048**
Insula lobe, IFG (POp)	BA 44	L	-42	8	1	4.54	160	0.076
Middle frontal gyrus, IFG (PT)		R	30	27	30	4.31	85	0.164
Middle frontal gyrus, PreCG		R	41	3	54	3.68	308	0.749
Posterior-medial frontal gyrus		L	-6	6	67	3.44	77	0.932
Cerebellar hemisphere	Lobule VIIa	L	-15	-84	-35	3.32	49	0.963
AL - positive correlation								
Rectus, frontal superior orbital gyrus		R	11	30	-27	3.54	57	0.873

Abbr.: AL= “number of affective losses“, ANX= “anxiety“, AV= “avoidance“, IFG= inferior frontal gyrus, k= number of voxels, POp= pars opercularis, PreCG= precentral gyrus, PT= pars triangularis

Table SI-2: Dichotomous AL, AAS and brain gray matter volume

Results of the full factorial model with the dichotomous AL variable as factor, a significance threshold of p<0.001 and a minimum cluster size of 50 voxels. No significant effects were observed for the contrasts (affective loss < no affective loss), [ANX x (affective loss > no affective loss)] and [AV-by-ANX (affective loss </> no affective loss)].

Anatomical region	**Brain Area**		**x**	**y**	**z**	**t**	**k**	**p (FWE)**
Affective loss > no affective loss								
FG, inferior temporal gyrus, IocG	FG2, hOc4la/4v	R	50	-60	-18	4.50	715	0.088
Posterior-medial frontal gyrus		R/L	6	21	57	3.97	193	0.432
ACC	BA 33	R/L	3	27	24	3.80	186	0.620
FG, LG	FG1	L	-29	-60	-9	3.71	160	0.718
FG, LG, IocG	FG1/2, hOc4la/4v	L	-30	-82	-15	3.61	182	0.815
LG, FG	FG1,hOc4v/1	R	23	-63	-5	3.57	55	0.851
Cuneus	hOc2/3d	R/L	0	-94	16	3.47	52	0.918
AV x (affective loss > no affective loss)								
Caudate nucleus, pallidum	BA 33	L	-3	11	1	3.79	183	0.627
SupraMarginal gyrus / PCG /RO	OP1/3/4	L	-53	-22	16	3.63	155	0.793
Superior / middle frontal gyrus		R	23	53	16	3.52	56	0.888
Middle frontal gyrus		R	33	38	36	3.42	63	0.941
AV x (affective loss < no affective loss)								
Superior parietal lobule	5L, 7PC	R	21	-51	59	3.65	58	0.777
ANX x (affective loss < no affective loss)								
Middle frontal gyrus		R	30	29	34	4.25	84	0.203
SocG/ middle occipital gyrus		R	26	-67	28	4.11	305	0.301

Abbr.: AAS= adult attachment style, ACC= anterior cingulate cortex, ANX= “anxiety“, AV= “avoidance“, AL= “number of affective losses”, FG= fusiform gyrus, IocG= inferior occipital gyrus, k= number of voxels, LG= lingual gyrus, PCG= postcentral gyrus, RO= rolandic operculum, SocG= superior occipital gyrus

Erklärung zum eigenen Anteil an dieser Arbeit

Diese Arbeit ist im Rahmen des LOEWE-Projekts „Cultural Neuroscience - neurale Prozesse, soziale Interaktion und gesellschaftliche Konflikte“ entstanden. Die Durchführung des Projekts erfolgte im Team.

Im Rahmen dieser Arbeit habe ich bei der Auswahl der Fragebögen und des fMRT-Paradigmas mitgewirkt. Ich habe das fMRT-Paradigma programmiert und am MR-Tomographen implementiert. Die Durchführungsorganisation und das Management der Studiendaten (unter Nutzung des MRT-Datenmanagementsystems der „Kerneinheit MRT“) wurden zu schätzungsweise 90% von mir übernommen. Bei der Rekrutierung der Probanden (n=218) und der Datenerhebung (Screening, Bildgebung, neuropsychologische Tests, Fragebögen, Speichel- und Blutproben) habe ich geschätzt einen Anteil von 40 % übernommen. Die Hypothesen der Manuskripte wurden von mir generiert. Die Datenanalyse wurde von mir durchgeführt und fachlich durch Prof. Dr. Tilo Kircher, Prof. Dr. Benjamin Straube und Prof. Dr. Andreas Jansen unterstützt. Die Manuskripte wurden von mir geschrieben und von Prof. Dr. Tilo Kircher, Prof. Dr. Benjamin Straube und Prof. Dr. Andreas Jansen korrigiert.

Verzeichnis akademischer Lehrer

Meine akademischen Lehrer waren die Damen/Herren:

in **Fribourg** (Schweiz)

Agazzi, Clin-Lalande, Combe, Giraud, Imbach, Kern, Keßler, Kohler, Levinas, O'Meara, Zeller

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