Justus-Liebig-Universität Gießen

Fachbereich Psychologie und Sportwissenschaften

Einfluss von chronischem und akutem Stress auf appetitive Konditionierung

Kumulative Dissertation

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Glossar

TSST	Trier Social Stress Test
HCC	Haarcortisolkonzentration
MRT	Magnetresonanztomograph
fMRT	Funktionelle Magnetresonanztomographie
NS	Neutraler Stimulus
CS+	Konditionierter Stimulus gepaart mit Verstärker
CS-	Konditionierter Stimulus signalisiert Ausbleiben von Verstärkung
UCS	Unkonditionierter Stimulus
CR	Konditionierte Reaktion
UCR	Unkonditionierte Reaktion
MID	Monetary Incentive Delay
NAcc	Nucleus Accumbens
vACC	Ventraler anteriorer cingulärer Cortex
dACC	Dorsaler anteriorer cingulärer Cortex
OFC	Orbitofrontaler Cortex
d.h.	das heißt
z.B.	zum Beispiel

1. Überblick

In der vorliegenden Arbeit werden die Ergebnisse dreier Fragestellungen, die in einer Studie neuronaler Korrelate der appetitiven Konditionierung und ihrer Modulation durch chronischen und akuten Stress sowie des Extinktionslernens untersucht wurden, vorgestellt. Nach einer einleitenden Darstellung des Hintergrundes wird ausführlicher auf die theoretischen Grundlagen appetitiver Konditionierung und Extinktion sowie akuten und chronischen Stresses eingegangen. Daraus hervorgehend werden die drei im Rahmen der Studie bearbeiteten Fragestellungen vorgestellt. Dabei wurde der Einfluss von akutem Stress auf appetitive Konditionierung (Kruse, Tapia León, Stalder, Stark & Klucken, 2018), die Interaktion von akutem Stress mit kumulativer Cortisolausschüttung bei appetitiver Konditionierung (Kruse, Tapia León, Stalder, Stark & Klucken, in Vorb.) sowie die neuronalen Korrelate appetitiven Extinktionslernens (Kruse, Tapia León, Stark & Klucken, 2017) untersucht. Weitere meiner Arbeiten an diesem Themengebiet, die nicht Thema der Dissertation sind, befassten sich mit appetitiven Konditionierungsprozessen (Tapia León, Kruse et al., 2018) im Zusammenhang mit genetischen Polymorphismen (Klucken, Kruse, et al., 2015) sowie Verhaltenssüchten (Klucken et al., 2016). Ebenfalls nicht Teil der Dissertation sind die als Ko-Autor veröffentlichten Publikationen zu aversiver Konditionierung, darunter ein methodenfokussierter Überblicksartikel (Lonsdorf et al., 2017), eine Studie zu aversiver Konditionierung bei repressivem Coping-Stil (Klucken, Kruse et al., 2015) und die Untersuchung von Rekonsolidierungseffekten (Klucken, Kruse, et al., 2016).

Nach einem Überblick über die Methodik der durchgeführten Studie werden für jede der drei Fragestellungen zentrale Ergebnisse vorgestellt und in den Forschungskontext eingeordnet. In der abschließenden Diskussion werden die Ergebnisse integriert und Implikationen für die klinische Forschung diskutiert sowie mögliche Richtungen für sich anschließende Forschung aufgezeigt.

2. Einleitung

Belohnungslernen ist eine überlebenswichtige Fähigkeit von Menschen und anderen Lebewesen. Es stellt eine Assoziation zwischen neutralen Umweltreizen und Belohnungen wie Nahrung, Lob oder der Chance auf Fortpflanzung her. Neutrale Umweltreize, die wiederholt im Zusammenhang mit einer Belohnung auftraten, werden schließlich selbst als Belohnung erlebt. Belohnungslernen erleichtert das Erlangen einer Vielzahl positiver Verstärker und ermöglicht eine funktionale Interaktion mit der Umwelt. Ein weiterer wichtiger Aspekt des adaptiven Belohnungslernens ist das Verlernen nicht länger gültiger Zusammenhänge, das sogenannte Extinktionslernen. Die neuronalen Korrelate appetitiven Extinktionslernens sind jedoch beim Menschen noch kaum untersucht. Sowohl der Einfluss von Stress und Stresshormonen auf das Belohnungslernen als auch ein besseres Verständnis des Extinktionslernens sind insbesondere klinisch relevant. Maladaptive appetitive Akquisitions- und Extinktionslernprozesse gelten als wichtiges Modell für die Entstehung und Behandlung psychischer Störungen wie Suchterkrankungen (Martin-Soelch, Linthicum & Ernst, 2007; Millan, Marchant & McNally, 2011; Quirk & Mueller, 2008).

Ein weiterer, in der vorliegenden Arbeit wichtiger Mechanismus zur funktionalen Interaktion mit der Umwelt, ist die Stressreaktion des Körpers. Viele Studien konnten zeigen, dass emotionale Lernprozesse in vielfältiger Weise unter Stress verändert und durch Stresshormone moduliert werden (Joëls, Pu, Wiegert, Oitzl & Krugers, 2006; Mather & Lighthall, 2012; Stark et al., 2006). Im Bereich des Belohnungslernens ist dieser Zusammenhang jedoch bislang kaum untersucht worden. Während die Rolle von akutem und chronischem Stress bei der Entstehung psychischer Störungen weithin anerkannt ist (Piazza & Le Moal, 1998; Sinha, 2008), gestaltet sich die experimentelle Erforschung der dahinterstehenden Mechanismen schwierig. Herausforderungen sind beispielsweise die Variabilität des Stresseffektes in Abhängigkeit von der Tageszeit (Lupien, Maheu, Tu, Fiocco & Schramek, 2007), dem zeitlichen Abstand zwischen Stressor und Experiment (Henckens, Hermans, Pu, Joels & Fernandez, 2009), der Art des Stressors (Miller, Chen & Zhou, 2007) oder auch von der Einnahme hormoneller Verhütungsmittel bei weiblichen Probanden (Merz & Wolf, 2017).

Studien zum Effekt von akutem Stress auf appetitive Konditionierungsprozesse kommen zu widersprüchlichen Ergebnissen, von begünstigtem (Berghorst, Bogdan, Frank & Pizzagalli, 2013; Bogdan & Pizzagalli, 2006) bis reduziertem Belohnungslernen (Dagher, Tannenbaum, Hayashi, Pruessner & McBride, 2009; Kumar et al., 2014). Diese widersprüchlichen Ergebnisse zeigen, wie wichtig ein tieferes Verständnis der Einflussfaktoren und Randbedingungen beim Zusammenhang von Stress und Belohnungslernen sind. Einen solchen Einflussfaktor stellt möglicherweise chronischer Stress dar, der als zentrales Element in Entstehungsmodellen psychischer Störungen gilt. Da chronischer Stress aus ethischen Gründen beim Menschen nicht experimentell manipuliert werden kann, bedarf es anderer methodischer Vorgehensweisen. Eine innovative Möglichkeit bietet die Untersuchung kumulativer Cortisolausschüttung mittels Haarproben dar (Stalder & Kirschbaum, 2012). Ein Abbild des ausgeschütteten Cortisols im

Laufe eines Monats setzt sich in approximal einem Zentimeter Haupthaar ab und ermöglicht so eine retrospektive Analyse. Eine erhöhte kumulative Cortisolausschüttung ist hierbei nicht mit chronischem Stress gleichzusetzen, stellt jedoch als zentraler Teil der Stressreaktionen einen möglichen biologischen Marker dar.

Ziel des Dissertationsprojektes war es, die neuronalen und psycho-physiologischen Korrelate appetitiver Konditionierungs- und Extinktionsprozesse zu untersuchen. Zusätzlich sollte der Einfluss von akutem und chronischem Stress auf Konditionierungs- und Extinktionsvorgänge genauer untersucht werden, um die Auswirkungen von zwei relevanten, bis dato nicht untersuchten, Faktoren zu erforschen. Die Ergebnisse der kumulativen Dissertation sollen so zu einem besseren Verständnis emotionaler Lernprozesse auf neuronaler Ebene beitragen.

3. Theoretischer Hintergrund

3.1. Appetitive Konditionierung

Belohnungslernen kann experimentell im Paradigma der differentiellen appetitiven Konditionierung untersucht werden. Ein neutraler Reiz (engl. conditioned stimulus; CS+) wird dabei wiederholt mit einem appetitiven Stimulus (engl. unconditioned stimulus; UCS, z.B. Nahrung, Geld oder erotische Stimuli) dargeboten. Der UCS löst eine unkonditionierte Reaktion (UCR) aus, wie z.B. erhöhte Hautleitfähigkeit, erhöhte positive Valenz- oder Erregung, vermehrtes Annäherungsverhalten sowie eine erhöhte Aktivierung von belohnungsassoziierten Arealen im Gehirn. Ein weiterer neutraler Reiz (CS-) wird hingegen nie mit einem UCS dargeboten und signalisiert nach wenigen Durchgängen das Ausbleiben einer Belohnung. Nach einigen Wiederholungen führt bereits die Darbietung des CS+ im Vergleich zum CS- zu einer ähnlichen, nun konditionierten, Reaktion (engl. conditioned response; CR). Das Erlernen der Assoziation wird hierbei als Akquisition bezeichnet.

Bisherige Studien konnten unter Nutzung der funktionellen Magnetresonanztomographie die neuronalen Korrelate der Akquisition appetitiver Konditionierung identifizieren (Chase, Kumar, Eickhoff & Dombrovski, 2015; Martin-Soelch et al., 2007). Hier sind insbesondere die Amygdala, das ventrale Striatum (Nucleus Accumbens), das dorsale Striatum (Nucleus Caudatus und Putamen), der dorsale und ventrale anteriore cinguläre Cortex (ACC), die Insula und der orbitofrontale Cortex (OFC) zu nennen. In diesem Belohnungsnetzwerk vermittelt die Amygdala den Zusammenhang zwischen CS+ und UCR. Diese Funktion wurde auch in klassischen Furchtkonditionierungsparadigmen gefunden (Chase et al., 2015). Das ventrale Striatum vermittelt die motivationale Salienz des CS und ist deshalb bei zahlreichen

Lernprozessen mit motivationaler Komponente involviert (Delgado, 2007). Ein einflussreiches Modell ist dabei die Modellierung des Vorhersagefehlers („prediction error“; Schultz, Dayan & Montague, 1997). Dabei handelt es sich um ein Lernsignal, das Abweichung von bestehenden Erwartungen kodiert und insbesondere mit dem ventralen Striatum assoziiert wird. Im Verlauf der Konditionierung wird die Erwartung entwickelt, dass auf den CS+ der UCS folgt. Dadurch reduziert sich bei erfolgreichem Lernen der Vorhersagefehler. Das dorsale Striatum ist insbesondere in handlungsrelevante Prozesse involviert, wobei der Nucleus Caudatus (dorsomediales Striatum) zielgerichtete Handlungen und das Putamen (dorsolaterales Striatum) insbesondere Handlungen vermittelt, die bereits habituell ausgeführt werden (Schwabe & Wolf, 2011). Eine deutliche funktionale Unterscheidung kann im Bereich des Belohnungslernens auch zwischen dem ventralen ACC (vACC) und dem dorsalen ACC (dACC) getroffen werden. Der vACC ist in die Detektion und Entwicklung spezifischer Zusammenhänge zwischen CS+ und UCS involviert und spielt deshalb in der frühen Phase von Lernprozessen eine Rolle (Gabriel, Burhans & Kashef, 2003). Der dACC hingegen enkodiert insbesondere den Outcome (Agren, 2014; Alexander & Brown, 2011; Etkin, Egner & Kalisch, 2011), also im Fall des Belohnungslernens die erwartete Belohnung und spielt deshalb eher in der späten Phase der Akquisition und im langfristigen Abruf von Belohnungszusammenhängen eine Rolle. Das interozeptive Erleben der Belohnungsreaktion wiederum wird mit der Aktivierung der Insula in Verbindung gebracht (Hermans, Henckens, Joëls & Fernández, 2014). Eine komplexe Rolle spielt der OFC, der als kortikale Kontrollinstanz betrachtet werden kann und unter anderem bei der Anpassung des gelernten Wertes einer Belohnung an aktuelle Gegebenheiten von Bedeutung ist (Berridge, 2003).

Im Bereich der Suchterkrankungen kommt es zu mehreren dysfunktionalen Prozessen im Bereich des Belohnungslernens. Eine Vielzahl zuvor neutraler Stimuli löst bei Suchtpatienten das Bedürfnis nach Suchtmittelkonsum aus („Craving“). Der Drang zum Suchtmittelkonsum wird sehr resistent gegenüber neuen Lernerfahrungen. Man geht heute davon aus, dass appetitive Konditionierung dazu führt, dass vormals neutrale Reize über die Akquisition zu konditionierten Reizen werden und Craving auslösen. Somit eignet sich das Studium der Mechanismen von appetitiver Konditionierung zur Untersuchung der Entstehung von Suchterkrankungen.

Extinktion, als das Verlernen von konditionierten Hinweisreizen, gilt aktuell als eines der wichtigsten Grundlagenmodelle für die Behandlung von Angst, aber auch Suchterkrankungen. Experimentell lassen sich Extinktionsprozessen untersuchen, indem der CS+ nach einer erfolgreichen Akquisition ohne Paarung mit dem UCS dargeboten wird (genau wie der CS-). Nach wiederholter Darbietung des CS+ ohne Verstärkung des UCS, löst der CS+ während der Extinktion keine konditionierten Reaktionen mehr aus. Es wird hierbei jedoch davon ausgegangen, dass die erste CS+/UCS Gedächtnisspur, die während der Akquisition auf neuronaler Ebene entstanden ist, nicht gelöscht wird (Bouton, 2002). Vielmehr entsteht während der Extinktion eine neue, zweite Gedächtnisspur, die die zuvor entwickelte Assoziation inhibiert. Im Humanbereich wurden die neuronalen Korrelate des Extinktionslernen bisher ausschließlich im Bereich der Furchtkonditionierung untersucht. Neuronale Korrelate appetitiven Extinktionslernens sind bislang lediglich aus Tierstudien bekannt (Millan et al., 2011; Tye, Cone, Schairer & Janak, 2010).

3.2. Akuter und chronischer Stress

Akuter Stress wird als entscheidender Faktor bei der Entstehung psychischer Erkrankungen betrachtet. Insbesondere physische Belastung, soziale Bewertung und wahrgenommene Unkontrollierbarkeit führen zu starken Stressreaktionen (Dickerson & Kemeny, 2004). Dabei kommt es zunächst zu einer Aktivierung des sympathischen Nervensystems, das unter anderem zu einer Ausschüttung des Stresshormons Adrenalin führt, das einen direkten Effekt im Gehirn hat. Zudem findet im Gehirn die vermehrte Ausschüttung von Dopamin statt (Henckens et al., 2009). In einer zweiten, langsameren Reaktionskaskade kommt es über die Hypothalamus-Hypophysen-Nebennierenachse (HPA-Achse) zur Ausschüttung von Cortisol. Nach der Passage der Blut-Hirn-Schranke entfaltet auch Cortisol eine Wirkung im Gehirn. Cortisol folgt einer circadianen Rhythmik mit einer maximalen Cortisolausschüttung um die Zeit des Erwachens (Cortisol-Aufwachreaktion). Der tonische Cortisolspiegel fällt danach ab, wobei der Abfall besonders am Vormittag sehr ausgeprägt ist. Danach ist der tonische Cortisolspiegel vergleichsweise stabil. Akute Cortisolpeaks durch aktuellen Stress wirken sich daher auf der abfallenden Flanke des Morgenpeaks anders aus als am Rest des Tages. Cortisol wirkt insbesondere im Hippocampus, im präfrontalen Cortex, der Amygdala und dem ventralen Striatum, in denen jeweils Cortisolrezeptoren nachgewiesen werden konnten (McEwen, 2006).

Während die Auswirkungen von akutem Stress auf Konditionierungs- und Extinktionsprozesse in den letzten Jahren vermehrt untersucht wurden, sind Untersuchungen über die Auswirkungen von chronischem Stress auf emotionale Lernprozesse und/oder hirnphysiologische Veränderungen kaum untersucht. Es ist dabei anzunehmen, dass chronische Stressbelastung und dadurch angestoßene Modulationsprozesse auch mit dem Effekt von akuten Stressoren interagieren. Mittels Analyse der kumulativen Haarcortisolkonzentration (HCC) in chronisch gestressten Menschen konnte gezeigt werden, dass chronischer Stress sich in Form von erhöhter HCC niederschlägt. Dies zeigte sich z.B. bei von Arbeitslosigkeit bedrohten Menschen (Dettenborn, Tietze, Bruckner & Kirschbaum, 2010). Weitere Studien konnten zeigen, dass Stressoren wie körperliche und psychische Erkrankungen sich ebenfalls in den gemessenen HCC widerspiegeln (Kirschbaum, Tietze, Skoluda & Dettenborn, 2009; Pereg et al., 2011; Staufenbiel, Penninx, B. W. J. H., Spijker, Elzinga & van Rossum, Elisabeth F.C., 2013). Für die Messung der HCC konnte eine hohe Reliabilität beobachtet werden (Stalder et al., 2012). Interessanterweise hing die subjektive retrospektive Einschätzung der chronischen Stressbelastung ersten Untersuchungen zufolge nicht mit HCC zusammen (Gidlow, Randall, Gillman, Silk & Jones, 2016). Dadurch muss der Wert von HCC als Biomarker für chronischen Stress jedoch nicht grundsätzlich in Frage gestellt werden, da bekannt ist, dass subjektive retrospektive Einschätzung von Stress durch Fragebogenverfahren retrospektiven Verzerrungen unterliegt.

Die chronisch erhöhte Ausschüttung von Cortisol bewirkt zudem strukturelle Veränderungen im Gehirn. Dieser Effekt konnte insbesondere im Hippocampus, der Amygdala und im präfrontalen Cortex gezeigt werden (McEwen, 2006). Zudem wurde in Tierstudien gezeigt, dass besonders ein Netzwerk aus Amygdala, Pallidum und ACC durch chronischen Stress verändert ist und zu veränderten Lernvorgängen führt (Ito, Nagano, Suzuki & Murakoshi, 2010; Lin et al., 2015; Papp, Lappas, Muscat & Willner, 1992). Da diese Hirnstrukturen auch insbesondere im Kontext von Akquisitions- und Extinktionslernen eine wichtige Rolle spielen, ist die Frage nach den Auswirkungen von chronischem Stress auf Konditionierungsprozesse von großer Bedeutung, um ein besseres Verständnis im Grundlagenmodell für die Entstehung, aber auch Veränderung von emotionalen Lernvorgängen zu gewinnen.

3.3. Darstellung der Fragestellungen

Drei Forschungsfragen leiten die vorliegende Dissertation.

Fragestellung I: Wie verändert akuter Stress die Akquisition appetitiver Konditionierung?

Neben Vulnerabilitätsfaktoren, die bestimmte Menschen anfällig für die Entwicklung psychischer Erkrankungen machen, gilt akute Belastung als ein zweiter wichtiger Faktor im Vulnerabilität-Stress-Modell, das häufig zur Erklärung der Entstehung psychischer Störungen herangezogen wird. Akute Stressreaktionen beinhalten Reaktionen verschiedener Systeme: So z.B. die Ausschüttung von Adrenalin und Dopamin, die Ausschüttung von Cortisol und die psychische Verarbeitung der Stresssituation (Joëls, Sarabdjitsingh & Karst, 2012). Der Einfluss von Stressreaktionen auf die appetitive Konditionierung ist bislang unklar, mit auf den ersten Blick widersprüchlichen Befunden von erleichtertem Belohnungslernen (Lighthall et al., 2013) bis hin zu reduziertem Belohnungslernen (Berker et al., 2016).

Eine Erklärung für diese unbefriedigende Befundlage könnte in den unterschiedlichen zeitlichen Verläufen der unterschiedlichen endokrinologischen Stressantworten liegen. Die Ausschüttung von Adrenalin wirkt kurzfristig und überlappt wenig mit Cortisol. Dopamin hingegen wird gemeinsam mit Adrenalin ausgeschüttet und überlappt mit der Wirkung des verzögert ausgeschütteten Cortisols. Die Erfassung der akuten Cortisolsekretion über den Speichel ermöglicht den Effekt dieser Stressreaktion korrelativ zu erfassen. So konnte gezeigt werden, dass die verschiedenen Stressantworten Belohnungslernen unterschiedlich beeinflussen (Maier, Makwana & Hare, 2015). Dies muss berücksichtigt werden, wenn die Rolle von Stress bei der Entstehung von psychischen Störungen wie Sucht untersucht wird.

In der vorliegenden Arbeit wird davon ausgegangen, dass akuter Stress durch seinen Effekt auf den Hippocampus die Generalisierung von Belohnungslernen abhängig von verschiedenen Gegebenheiten verändert. Es wird angenommen, dass die akute Stressreaktion die Akquisition appetitiver Konditionierung nicht beeinträchtigt, sondern die Übergeneralisierung auf den CS- befördert. Weiter wird angenommen, dass dies neuronal insbesondere über reduzierte differenzielle Reaktionen (CS+ - CS-) im Hippocampus, vACC, OFC, sowie im ventralen Striatum und der Amygdala vermittelt wird.

Fragestellung II: Wie interagieren kumulative Cortisolausschüttung und akuter Stress bei der Modulation appetitiver Konditionierung?

Während akuter Stress als adaptive Reaktion verstanden wird, die Verhaltensanpassungen zum Umgang mit außergewöhnlichen Belastungen, die zeitlich begrenzt sind, erlaubt (Mc Ewen, 2006), wird chronischer Stress als maladaptiv betrachtet. Aufgrund der anhaltenden Belastung bei chronischem Stress können Anpassungsreaktionen nicht aufrechterhalten werden. Hieraus resultiert ein deutliches Risiko für Folgeerkrankungen (Sinha, 2008). Chronischer Stress kann dabei als länger anhaltende Phase wiederholter, akut belastender Ereignisse verstanden werden, die sich unter anderem als eine kumulativ erhöhte Ausschüttung von Cortisol zeigt. Aufgrund der durch langanhaltend erhöhte Ausschüttung von Cortisol induzierten neuronalen Veränderungen ist anzunehmen, dass kumulative Cortisolausschüttung den Effekt einer akuten Stressbelastung moduliert.

Bislang ist jedoch unklar, wie diese Modulation sich im Detail gestaltet. Allgemeine Theorien zum Effekt von Belastungen sagen zwei Möglichkeiten vorher: Die eine Möglichkeit postuliert eine zusätzliche Belastung durch die Addition einer akuten und einer kumulierten Stressreaktion, was zu einem reduzierten Funktionsniveau führt. Eine andere Möglichkeit ist die Annahme, dass eine Adaption an die kumulierte Stressreaktion erfolgt. Durch die Adaption an kumulierten Stress wirkt sich akuter Stress anders aus. Langfristig könnte dadurch beispielsweise sowohl bei hohem akutem und hohem kumuliertem Stress als auch bei niedrigem akutem und niedrigem kumuliertem Stress ein optimales Funktionsniveau erreicht werden (Nederhof & Schmidt, 2012). Die neuronalen Korrelate dieser Interaktion wurden bislang noch nicht untersucht, sodass eine Überprüfung dieser Theorien hier noch aussteht. In Tierstudien konnte gezeigt werden, dass chronischer Stress Belohnungsverarbeitung insbesondere im ACC und in den Basalganglien moduliert (Ito, Nagano, Suzuki & Murakoshi, 2010). Es wird angenommen, dass eine Interaktion mit akutem Stress daher neuronale Korrelate in diesen Hirnarealen moduliert.

Die vorliegende Dissertation untersuchte daher die kumulative Cortisolausschüttung bei gesunden Probanden und die Modulation appetitiver Konditionierungsprozesse unter akutem Stress. Es wird also nicht der Endpunkt einer chronischen Belastung betrachtet, zu dem keine Anpassungsreaktion mehr möglich ist, sondern eine querschnittliche Untersuchung des Effekts unterschiedlicher kumulativer Cortisolausschüttungen. Es soll dabei überprüft werden, inwieweit die neuronale Modulation der Belohnungsverarbeitung im ACC und den Basalganglien, die bei chronischem Stress gezeigt wurde, schon im Verlauf beobachtet werden kann, sowie eine Interaktion mit akutem Stress untersucht werden.

Fragestellung III: Welche neuronalen Korrelate stehen mit der Extinktion appetitiver Konditionierung in Zusammenhang?

In Bezug auf die Behandlung psychischer Störungen gilt Extinktionslernen als wichtiges Modell. Neuronale Modelle der Extinktion appetitiver Konditionierung stellen die Grundlage für neurowissenschaftliche Untersuchungen von Therapieverfahren dar, die auf appetitiver Extinktion basieren. Bislang wurden die im Tiermodell identifizierten Zielregionen jedoch nicht in Humanstudien validiert. Tierstudien konnten während des Extinktionslernens erhöhte neuronale Aktivität bei der Darbietung des CS+ in der Amygdala und im Nucleus Accumbens identifizieren, (Janak, Chen & Caulder, 2004; Tye et al., 2010).

Im Verlauf eines Extinktionstrainings wird zunächst die Akquisitionsgedächtnisspur erneut abgerufen (Quirk & Mueller, 2008). Mit ausbleibender Paarung des CS+ mit dem UCS bildet sich eine zweite Gedächtnisspur, die sogenannte Extinktionsgedächtnisspur. Neuronale Korrelate der Extinktionsgedächtnisspur sind deshalb von neuronalen Korrelaten des langfristigen Abrufs der Akquisitionsgedächtnisspur zu unterscheiden. Anders als die neuronalen Korrelate der Extinktion konditionierter Furcht, wurden die neuronalen Korrelate der Extinktion konditionierter Belohnung bislang im Menschen nicht untersucht. Es wird - ausgehend von den oben zitierten tierexperimentellen Arbeiten - angenommen, dass in der späten Phase der Extinktion erhöhte differentielle Aktivierung des NAcc und der Amygdala im Zusammenhang mit erfolgreichem Extinktionslernen stehen.

4. Studie

Zur Beantwortung der dargestellten Forschungsfragen nahmen 56 männliche Probanden an einem modifizierten *Monetary Incentive Delay* (MID) Paradigma als appetitives Konditionierungsparadigma teil. 40 Minuten vor Beginn des MID-Paradigmas nahm die eine Hälfte der Probanden zur Stressinduktion am *Trier Social Stress Test* (TSST) teil, die andere Hälfte der Probanden absolvierte einen Placebo-TSST als Kontrollbedingung. Alle Messungen fanden am Nachmittag ab 13 Uhr statt. Der Einfluss der circadianen Rhythmik des Cortisols sowie ein Einfluss von Geschlechtshormonen konnte durch diese Vorgaben gering gehalten werden. Vor der Stressinduktion und nach dem MID-Paradigma bewerteten die Probanden die zunächst neutralen Stimuli (CS+/CS-) in Bezug auf Valenz, Arousal und UCS-Erwartung. Während des MID-Paradigmas wurden EDA und fMRT aufgezeichnet. Zusätzlich wurde jeweils eine Haarprobe entnommen. Aus dieser wurde mithilfe eines Flüssigchromatographie-Massenspektrometrie(LC-MS)-Verfahrens die Konzentration von Cortisol in den zuletzt gewachsenen 2 cm Haar bestimmt (Gao et al., 2013). 24 Stunden später nahmen die Probanden an einer Extinktionsphase teil, bei der ebenfalls EDA und fMRT aufgezeichnet wurden. Im Anschluss wurden erneut die subjektiven Bewertungen erhoben.

Bisherige Studien zu den neuronalen Korrelaten appetitiver Konditionierung beim Menschen wählten häufig ein rein passives Lernparadigma. Dabei werden neutrale Stimuli als CS betrachtet, auf die dann die Darbietung einer Belohnung (UCS) folgt (Martin-Soelch et al., 2007). Dies ist konzeptuell ähnlich zur klassischen Furchtkonditionierung, in der auf die Betrachtung neutraler Stimuli die Darbietung eines Furchtreizes folgt. Das bisherige Vorgehen ist jedoch nicht vergleichbar mit Tierstudien zu appetitiver Konditionierung, in denen die Tiere nach Darbietung des CS eine Handlung ausführen müssen, um die Belohnung zu erhalten.

Daher wurde das Monetary Incentive Delay (MID) Paradigma für die vorliegende Studie adaptiert, das eine zu tierexperimentellen Studien vergleichbare Handlungskomponente beinhaltet (Knutson, Westdorp, Kaiser & Hommer, 2000).

Die einzelnen Durchgänge des MID-Paradigmas sind wie folgt aufgebaut: Nach einem neutralen Ankündigungsreiz folgt ein variables zeitliches Intervall. Darauf folgt ein Zielreiz, auf den Probanden instruiert wurden mit einem Tastendruck zu reagieren. Erfolgt der Tastendruck, während der Zielreiz noch sichtbar ist, besteht je nach zuvor präsentiertem Ankündigungsreiz die Möglichkeit auf einen Gewinn. Feedback über den erhaltenen Gewinn wird in der Regel direkt nach dem Zielreiz gezeigt. Das MID-Paradigma wird jedoch weitgehend auf die Untersuchung des Abrufs von verbal instruierten und vorab trainierten Belohnungsassoziationen beschränkt. Die Probanden wissen vor der fMRT-Untersuchung genau, welche Bedeutung die Ankündigungsreize haben. Eine Untersuchung der Akquisition dieser Kontingenzen findet dadurch nicht statt. Deshalb wurde das MID Paradigma für die aktuelle Studie angepasst. Den Probanden sind die Kontingenzen zwischen den Hinweisreizen und der Belohnung anfangs nicht bekannt, sondern sie erlernen diese im Laufe des Experiments. Ein weiteres wenig beachtetes Problem bisheriger MID Paradigmen ist zudem, dass die Zeitpunkte der verstärkten Durchgänge von den Reaktionszeiten der Probanden abhängen. Dies beeinflusst jedoch erheblich die Designeffizienz für die spätere Analyse der fMRT-Daten. Deswegen wurde in der vorliegenden Studie ein Mittelweg zwischen der Abhängigkeit der Verstärkung vom Verhalten der Probanden und einem Vorgehen von Dillon et al. (2016) gewählt, indem eine Abhängigkeit der Belohnung vom Verhalten vorgetäuscht wird.

Für diesen Mittelweg werden durchschnittliche Reaktionszeiten und deren Varianz in einer Vorübung bestimmt. Die Darbietungsdauer des Zielreizes wird anschließend in verstärkten Durchgängen verlängert und in unverstärkten Durchgängen verkürzt. Dennoch erhält der Proband die Belohnung in CS+-Durchgängen nur bei einer ausreichend schnellen Reaktion, die erfolgen muss während der Zielreiz dargeboten wird. Dadurch kann der Zeitpunkt verstärkter Durchgänge weitgehend geplant und vorab pseudorandomisiert werden, was ein effizientes Design, vergleichbare Lernkurven und möglichst ähnliche Verstärkerraten zwischen den Probanden sicherstellt. Durch die beibehaltene Abhängigkeit vom Verhalten des Probanden ist sichergestellt, dass die Probanden die korrekte Ausführung der Aufgabe einhalten. Allerdings ergibt sich hieraus die Möglichkeit, dass Probanden Gewinne erzielen, obwohl im Design ein Ausbleiben des Gewinns vorgesehen war, beziehungsweise Gewinne verfehlen, die im Design vorgesehen waren. Diese Durchgänge führen zu einer adaptiven Anpassung des weiteren

Ablaufs. Der eigentlich geplante Durchgang (verstärkt oder unverstärkt) wird nachgeholt, um die geplante Verstärkerrate einzuhalten, und die Darbietungszeit des Zielreizes für weitere Durchgänge verlängert beziehungsweise verkürzt, um das Risiko weiterer Abweichungen zu reduzieren. Diese Anpassung ist wichtig, da die Verstärkerrate während der Akquisition das Extinktionslernen beeinflusst. 24 Stunden später nahmen die Probanden dann an einem Extinktionstraining im MRT teil. Hier blieb das Paradigma weitgehend unverändert. Jedoch konnten die Probanden nun weder nach dem CS+ noch nach dem CS- bei schneller Reaktion auf den Zielreiz Geld gewinnen. Zur Analyse von Lernvorgängen wurde schließlich sowohl die Akquisition als auch die Extinktion in eine frühe (erste Hälfte) und eine späte Phase (zweite Hälfte) geteilt und diese getrennt analysiert. Es wird davon ausgegangen, dass die frühe Phase jeweils Lernvorgänge abbildet, die in der zweiten Hälfte insbesondere abgerufen werden.

4.1. Fragestellung I: Wie verändert akuter Stress die Akquisition appetitiver Konditionierung? (Kruse et al., 2018)

Zunächst wurde der Einfluss von akutem Stress auf appetitive Konditionierungsprozesse untersucht. Dazu wurde bei einer Gruppe der TSST durchgeführt (N=27 Männer; Stressgruppe), bei einer zweiten Gruppe eine nicht stressinduzierende Placebo-Variante (N=29 Männer; Kontrollgruppe). Anschließend wurde bei allen Probanden der MID als appetitives Konditionierungsparadigma im MRT durchgeführt und zudem EDA und Reaktionszeiten erhoben. Die Ergebnisse konnten 2018 in der Fachzeitschrift NeuroImaging veröffentlicht werden. Die Analyse der Speichelcortisolreaktivität zeigte eine signifikant erhöhte akute Cortisolsekretion in der Stressgruppe. Die anschließende Akquisition der appetitiven Konditionierung im fMRT war ebenfalls erfolgreich. Die Akquisition wurde in einem 2 (Stress- vs Kontrollgruppe) × 2 (CS+ vs CS-) × 2 (Frühe vs Späte Phase) Design analysiert, wobei hier nicht auf alle Analysen eingegangen werden kann. Stress- und Kontrollgruppe zeigten deutlich erhöhte Hautleitfähigkeitsreaktionen auf den CS+ im Vergleich zum CS-. Zudem wurde der CS+ im Vergleich zum CS- als affektiv positiver und aufregender bewertet und war mit einer erhöhten Erwartung eines UCS verbunden. Gruppenunterschiede zeigten sich hier nicht.

Die Analyse der Reaktionszeiten der Probanden zeigte sowohl einen Effekt der appetitiven Konditionierung als auch einen Unterschied zwischen der Stress-Gruppe und der Kontrollgruppe. Alle Probanden reagierten schneller auf den Zielreiz, dem der CS+ vorangegangen war sodass ein Gewinn bei entsprechend schneller Reaktion erzielt werden konnte, im Vergleich zu einem Zielreiz, der auf einen CS- folgte. Zusätzlich gab es Unterschiede zwischen Stress- und Kontrollgruppe abhängig von der Phase der Akquisition.

Probanden der Kontrollgruppe reagierten in der späten Phase der Akquisition (zweite Hälfte der Trials) langsamer auf den CS- als in der ersten Hälfte und auch langsamer als Probanden der Stressgruppe. In der Stressgruppe hingegen unterschieden sich die Reaktionszeiten nach dem CS- in der späten Phase nicht von der frühen Phase. Das Verhalten der Kontrollgruppe war somit adaptiv, da schnelle Reaktionen auf den Zielreiz nach dem CS- zu zeigen nicht belohnt wurde. Die Stressgruppe hingegen reagiert gleichbleibend schnell, obwohl schnelle Reaktionen nach dem CS- nicht belohnt werden.

Ähnlich dazu zeigte die Stressgruppe im Vergleich zur Kontrollgruppe eine geringere differenzielle BOLD-Antwort beim CS+ im Vergleich zum CS-. In der frühen Phase der Konditionierung zeigte sich dies in der Amygdala, im OFC und vACC, sowie in der späten Phase zusätzlich im Ncl. Caudatus, Hippocampus und der Insula. Dies konnte auf stärkere BOLD-Antworten auf den CS- in der Stressgruppe, insbesondere im linken OFC in der frühen Phase der Akquisition und im linken Ncl. Caudatus und rechten vACC in der späten Phase zurückgeführt werden. Trends in diese Richtung zeigten sich in der rechten Amygdala und bilateral im vACC in der frühen Phase. Dies deutet auf eine Übergeneralisierung der appetitiven Konditionierung vom CS+ auf den CS- auf neuronaler und behavioraler Ebene hin. Veränderte Generalisierungseffekte konnten in einer Studie mit dopaminergen Effekten auf den Hippocampus in Zusammenhang gebracht werden (Kahnt & Tobler, 2016). Die vorliegenden Ergebnisse blieben auch bei statistischer Kontrolle für die Unterschiede in der Cortisolsekretion bestehen. Dies lässt vermuten, dass nicht Cortisol sondern die dopaminerge Stressreaktion für die hier gezeigte Übergeneralisierung verantwortlich ist. Die reduzierte differentielle Aktivierung im Ncl. Caudatus könnte durch ein reduziertes zielgerichtetes Annäherungsverhalten zugunsten von habituellem Verhalten unter Stress erklärt werden, wie es von Schwabe und Wolf (2011) beschrieben wurde.

Zusätzlich wurde eine Konnektivitätsanalyse mit dem Ziel durchgeführt, das Netzwerk, das für Spezifität oder Übergeneralisierung der Konditionierung verantwortlich gemacht wird, näher zu untersuchen. Die Konnektivitätsanalyse zeigte eine signifikant stärkere Konnektivität in der Stressgruppe zwischen dem Hippocampus und der Amygdala, dem OFC, dem vACC und dem dACC mit Trends im NAcc. Diesem Netzwerk, im Kern bestehend aus Hippocampus, vACC und OFC, wurde in Tierstudien die Aufrechterhaltung der Spezifität der Akquisition auf den CS+ zugeschrieben (Xu & Sudhof, 2013). In Tierstudien führte beispielsweise chemische Deaktivierung des vACC bei appetitiver Konditionierung zu Annäherungsverhalten nicht nur auf den CS+ sondern auch vermehrt auf den CS- (Bussey, Everitt & Robbins, 1997; Cardinal et al., 2003).

Zusammenfassend zeigen die Ergebnisse eine Übergeneralisierung appetitiver Konditionierung vom CS+ auf den CS- sowohl auf behavioraler Ebene als auch neuronaler Ebene. Hier wurde reduzierte Aktivierung in Arealen des Netzwerks gefunden, das in Tierstudien mit der Spezifität von Konditionierungsprozessen in Zusammenhang gebracht wurde, bei gleichzeitig erhöhter Konnektivität. Die Ergebnisse liefern eine mögliche Erklärung für bisherige widersprüchliche Befunde, da sich Übergeneralisierung abhängig vom Paradigma als verstärktes oder reduziertes Belohnungslernen zeigen kann.

4.2. Fragestellung II: Wie interagieren kumulative Cortisolausschüttung und akuter Stress bei der Modulation appetitiver Konditionierung? (Kruse et al., in Vorb.)

Eine weitere Fragestellung war, ob appetitive Konditionierung auch durch chronischen Stress moduliert wird. Diese Fragestellung wurde an der gleichen Stichprobe wie Fragestellung I untersucht, eine Publikation ist in Vorbereitung. Dazu wurden die neuronalen Korrelate (CS+ - CS-) und die Hautleitfähigkeitsveränderungen der Stress-Gruppe und der Kontrollgruppe während der Akquisition zunächst unabhängig mit der kumulativen Haarcortisolkonzentration (HCC) korreliert und die gefundenen Zusammenhänge miteinander verglichen. Während die konditionierte EDA in der Stressgruppe positiv mit der HCC korrelierte, bestand in der Kontrollgruppe ein negativer Zusammenhang zwischen konditionierter EDA und HCC. Der Zusammenhang war in der Stressgruppe signifikant positiver als in der Kontrollgruppe sowohl in der frühen (Z=2.33; p=.020) als auch der späten Phase (Z=2.40; p=.016) der Akquisition. Während HCC unter akutem Stress also mit stärkeren konditionierten Reaktionen assoziiert war, war HCC ohne akuten Stressor mit schwächeren konditionierten Reaktionen assoziiert.

Die Analyse der fMRT-Daten zeigte ähnliche Ergebnisse. Für die Analyse wurden die Kontrastschätzer der signifikanten Peak-Voxel des differentiellen Kontrasts der Konditionierung über beide Gruppen hinweg extrahiert und mit der HCC korreliert. Auch hier korrelierte der differentielle BOLD-Kontrast für CS+ - CS- in der Stressgruppe positiv mit der HCC. Eine signifikante Modulation konnte hier in der frühen Phase der Akquisition im linken vACC, bilateralen dACC und linken Ncl. Caudatus gezeigt werden, die im linken vACC und linken Ncl. Caudatus signifikant vom Zusammenhang in der Kontrollgruppe verschieden war. In der späten Phase der Akquisition konnte eine signifikante Modulation im linken dACC und linken OFC gezeigt werden, die sich jeweils signifikant vom Zusammenhang in der Kontrollgruppe unterschied.

Dies deutet darauf hin, dass kumulative Cortisolausschüttung appetitive Konditionierung moduliert. Während Probanden der Kontrollgruppe umso geringere konditionierte EDA aufwiesen je höher die gemessene HCC lag, zeigte sich bei akut gestressten Probanden der entgegengesetzte Zusammenhang. Nach akutem Stress zeigten Probanden in der Akquisition umso höhere konditionierte EDA, je höher die gemessene HCC lag. Die neuronalen Korrelate deuten darauf hin, dass dieser Zusammenhang bereits in der frühen Phase der Akquisition zum Tragen kommt. Die Modulation des vACC, der mit frühem diskriminativem Lernen assoziiert wird, deutet auf frühe veränderte Lernprozesse hin (Gabriel et al., 2003). Die differentielle Modulation in der frühen Phase könnte sich dabei auf die Verarbeitung der Belohnungserwartung auswirken. Deren Enkodierung wird mit dACC und OFC in Verbindung gebracht, die insbesondere in der späten Phase der Akquisition differentiell moduliert werden. Dies könnte auf langfristige neuronale Adaptationsprozesse hindeuten, bei denen Hirnareale, die für die Akquisition relevant sind, an ein gewisses Cortisollevel adaptieren und unter diesem eine stärkere Konditionierbarkeit aufweisen. Probanden mit einem höheren chronischen Cortisollevel zeigten hier entsprechend erhöhte Konditionierbarkeit unter akut erhöhten Cortisollevels. Probanden, die an ein niedriges Cortisollevel adaptiert sind, zeigten bessere Konditionierbarkeit in Abwesenheit einer akuten Stressinduktion.

Die Ergebnisse unterstützen die sogenannte Mismatch-Hypothese, nach der Organismen sich an hohe Belastungen wie chronischen Stress anpassen und schließlich nur unter entsprechender Belastung optimal funktionieren (Nederhof & Schmidt, 2012). Während also Organismen, die nicht an hohe Belastung gewöhnt sind, unter Belastung weniger gut funktionieren, funktionieren an hohe Belastung adaptierte Organismen weniger gut, wenn keine Belastung vorliegt. Aktuell steht jedoch die Forschung, welche Faktoren sich im HCC widerspiegeln, noch am Anfang. So zeigen bisherige Befunde, dass HCC innerhalb einer Person stabil ist, was auf langfristige Einflussfaktoren hindeutet (Stalder et al., 2012). Andere Befunde zeigen aber auch Einflüsse von aktuellen Lebensereignissen, wie Arbeitslosigkeit (Dettenborn et al., 2010) und eine Veränderbarkeit durch Interventionen wie *Mindfulness Based Stress Reduction* (Goldberg et al., 2014). Andere denkbare Einflüsse wie genetische Komponenten oder frühkindliche Einflüsse sind zudem bislang noch kaum untersucht.

4.3. Fragestellung III: Welche neuronalen Korrelate stehen mit der Extinktion appetitiver Konditionierung in Zusammenhang? (Kruse et al., 2017)

Während die Untersuchung der Akquisition appetitiver Konditionierungsprozesse wichtige Beiträge zum Verständnis der Entstehung psychischer Störungen liefert, geben die

Untersuchungen der Extinktion im Bereich der Grundlagenforschung wichtige Hinweise für die Behandlung dieser Störungen. Insbesondere die neuronalen Korrelate des Extinktionslernens bei appetitiver Konditionierung wurden beim Menschen bisher nicht untersucht. Die Ergebnisse wurden 2017 in der Fachzeitschrift Social, Cognitive, and Affective Neuroscience veröffentlicht. Zur Untersuchung der Extinktion nahmen die Probanden (N=21 Männer) 24h nach der Akquisition an einem Extinktionstraining im MRT teil, bei dem weder CS+ noch CS- verstärkt wurden. Subjektive Ratings, die nach der Akquisition und wieder nach der Extinktion erfasst wurden, zeigten eine Abnahme der Bewertung des CS+ als positiv und aufregend. Auch die Wahrscheinlichkeit eine Belohnung erhalten zu können wurde nach der Extinktion als geringer eingeschätzt. Erneut wurde eine Aufteilung in eine frühe und eine späte Phase vorgenommen. In der frühen Phase findet sowohl Belohnungsabruf statt, als auch der Beginn des Extinktionslernens. Dies zeigte sich auch in der EDA. Die konditionierte EDA auf den CS+ nahm von der frühen Phase der Extinktion zur späten Phase signifikant ab.

Auf neuronaler Ebene wurden in der frühen Phase der Extinktion differentielle BOLD-Antworten im Kontrast CS+ - CS- im linken dACC, linken vACC, rechten Ncl. Caudatus und rechten Hippocampus gefunden. In der späten Phase kann der Abruf des Extinktionslernens untersucht werden. Hier wurde signifikante bilaterale Aktivierung des NAcc und der Amygdala gefunden. Insbesondere die neuronale Aktivierung der späten Phase kann dabei erfolgtem Extinktionslernen zugeschrieben werden. Die frühe Phase bildet vermutlich sowohl Abruf der Akquisitionsgedächtnisspur als auch den Beginn des Extinktionslernens ab. Zur besseren Einordnung der Ergebnisse wurden die neuronalen Korrelate auf Zusammenhänge mit den Veränderungen der subjektiven Ratings des CS+ von vor zu nach der Extinktion untersucht. Dafür wurde aus den subjektiven Ratings ein Extinktionsindex berechnet, für den die CS+-Ratings nach der Akquisition von den Ratings nach der Extinktion subtrahiert wurden. Größere Differenzen bedeuten dabei größeren Extinktionserfolg, da Probanden in diesem Fall ihre Bewertung des CS+ erfolgreich angepasst haben. Korrelationen mit der Peak-Voxel-Aktivierung zeigten geringeren Extinktionserfolg der UCS-Erwartung im Zusammenhang mit Aktivierung des dACC in der frühen Phase. Das dACC ist insbesondere in Furchtkonditionierungsstudien mit dem Abruf affektiven Lernens in Zusammenhang gebracht worden, wobei dieser wie in den aktuellen Ergebnissen insbesondere den Outcome enkodiert (Alexander & Brown, 2011; Etkin et al., 2011). Eine erfolgreiche Extinktion des CS+-Arousals hing insbesondere mit erhöhter vACC-Aktivierung in der frühen Phase zusammen. Erfolgreiche Extinktion der CS+-Valenz hing insbesondere mit erhöhter Aktivierung des NAcc in der späten Phase zusammen. Zusätzlich wurde eine Modulation durch das Persönlichkeitsmerkmal

Impulsivität untersucht, das mit der BIS-15 (Meule, Vögele & Kübler, 2011) erfasst wurde und als Risikofaktor für die Entwicklung von Suchterkrankungen gilt (Potenza & Taylor, 2009). Hier konnte sowohl für den NAcc als auch die Amygdala ein negativer Zusammenhang mit Impulsivität gezeigt werden. Die Ergebnisse deuten darauf hin, dass höhere Impulsivität mit reduzierter differentieller Aktivierung während der Extinktion assoziiert ist.

Insbesondere konnte die vorliegende Studie erstmalig zeigen, dass der NAcc und die Amygdala, beim Menschen die neu gebildete Extinktionsgedächtnisspur zu enkodieren scheinen und damit frühere Tierstudien bestätigen. Zusätzlich wurde mit dem vACC ein Areal identifiziert, das vor allem bei frühen Extinktionsprozessen eine Rolle spielt.

5. Diskussion und Ausblick

In der vorliegenden Dissertation wurden die neuronalen Korrelate appetitiver Konditionierungsprozesse im Zusammenhang mit akutem und chronischem Stress sowie appetitive Extinktion untersucht. Dabei lag ein besonderer Fokus auf akutem Stress, der als wichtiger Faktor bei der Modulation von Lernprozessen gilt. Darüber hinaus wurde der Einfluss von chronischem Stress, erfasst über das HCC, auf appetitive Konditionierungsprozesse untersucht. Da die neuronalen Korrelate appetitiver Konditionierung bislang kaum erforscht sind, wurde das Zusammenspiel dieser Faktoren zunächst in Bezug auf die Akquisition untersucht. Im Bereich der appetitiven Extinktion wurden die neuronalen Korrelate beim Menschen bislang nicht näher beforscht, weshalb hier zunächst die Grundlagen dieses Prozesses untersucht wurden.

Im ersten Schritt wurde für die berichteten Studien eine neue Variante der bislang in der Belohnungs- und appetitiven Konditionierungsforschung eingesetzten Paradigmen entwickelt, das besonders geeignet scheint die beabsichtigten Fragestellungen zu bearbeiten. Eine wichtige Eigenschaft, die den Einsatz von Furchtkonditionierungsparadigmen beim Menschen so fruchtbar gemacht hat, war und ist die Möglichkeit der Translation von Befunden der Tierforschung zum Menschen (Milad & Quirk, 2012). Klassische Furchtkonditionierung bietet somit ein Bindeglied zwischen Grundlagenforschung am Tier und anwendungsorientierter klinischer Forschung am Menschen. Das hier genutzte Paradigma scheint einen ähnlichen Nutzen für die Untersuchung appetitiver Lernvorgänge zu bieten. Wie in der Tierforschung wurden die Probanden nicht vorab über die expliziten Kontingenzen instruiert, sondern es wurde eine klassische Akquisitionsphase genutzt. Gleichzeitig mussten die Probanden im Dissertationsprojekt, vergleichbar zu den Tierstudien, eine einfache Verhaltensaufgabe ausführen, um die Belohnung zu erhalten. Diese Eigenschaften, die für eine erfolgreiche translationale Studie neuronaler Korrelate appetitiver Konditionierung zentral erscheinen, wurden in bisherigen Humanstudien im fMRT noch nicht gemeinsam realisiert. Unter diesen Bedingungen konnten mehrere zentrale Befunde aus Tierstudien zu appetitiver Konditionierung repliziert werden. Wie in den Tierstudien wurde ein Netzwerk identifiziert, das im Kern aus OFC, vACC und Hippocampus besteht, dem eine zentrale Rolle beim Erlernen einer spezifischen Belohnungs-Assoziation beim CS+ zugeschrieben wird (Xu & Sudhof, 2013). Die Inaktivierung des vACC führte auf behavioraler Ebene zu Annäherungsverhalten sowohl an den CS+ als auch an den CS- (Bussey et al., 1997). Die in der vorliegenden Arbeit unter akutem Stress beobachtete reduzierte differenzielle Aktivierung in diesen Arealen wurde parallel mit vergleichsweise stärkerem Annäherungsverhalten auf den CS- gefunden. Besonders zu beachten ist, dass die Ergebnisse unabhängig von der Cortisolreaktivität beobachtet wurden, was für eine dopaminerge Vermittlung spricht. Hier zeigen die behavioralen Daten, dass die geringere Differenzierung zwischen CS+ und CS- auf neuronaler Ebene in der Stressgruppe als Übergeneralisierung der Konditionierung auf behavioraler Ebene zu interpretieren ist. Die gewonnenen Erkenntnisse stellen einen wichtigen Schritt dar, bisherige widersprüchliche Befunde, die sowohl besseres als auch schlechteres Belohnungslernen unter Stress zeigten, zu vereinen. Übergeneralisierung von Lernvorgängen scheint in hohem Maße abhängig von spezifischen Eigenschaften des Paradigmas. Insbesondere, wenn mehrere Assoziationen mit Belohnung und Bestrafung gelernt werden, kann Übergeneralisierung sowohl als besseres oder schlechteres Belohnungslernen erscheinen.

Die in Tierstudien für die Enkodierung erfolgreicher Extinktion als zentral identifizierten Strukturen Amygdala und NAcc konnten auch in der späten Phase der Extinktion gefunden werden. Erstmalig wurden hier zudem Probanden, die am MID-Paradigma teilgenommen haben, 24 Stunden später erneut untersucht. Es konnte gezeigt werden, dass der langfristige Abruf der Belohnung insbesondere durch den dACC vermittelt wird. Dieses Ergebnis zeigt die deutliche Ähnlichkeit zwischen neuronalen Korrelaten appetitiver Konditionierung und Furchtkonditionierung auf. Unsere Ergebnisse lassen eher vermuten, dass in vielen Fällen, in denen bisher vom Belohnungsnetzwerk oder vom Furchtnetzwerk die Rede ist, tatsächlich vielmehr ein Verstärkerlernnetzwerk oder ein affektives Lernnetzwerk untersucht wird. Dies könnte insbesondere vor dem Hintergrund von häufigen Komorbiditäten zwischen affektiven Störungen, Angststörungen und Suchterkrankungen zu einem besseren Verständnis gemeinsamer Vulnerabilitätsfaktoren beitragen.

Die Nutzung eines innovativen Verfahrens zur Bestimmung der Haarcortisolkonzentration erlaubt ein besseres Verständnis der Modulation appetitiver Konditionierung durch Stress und insbesondere das Hormon Cortisol, das oft als Stresshormon interpretiert wird. Es konnte gezeigt werden, dass ein differenzieller Zusammenhang zwischen kumulativem Cortisol und Konditionierbarkeit in Abhängigkeit von akutem Stress besteht. Bessere Konditionierbarkeit konnte jeweils bei einer Passung zwischen kumulativem Cortisolstatus und akutem Stressstatus beobachtet werden. Damit konnten erste Befunde vorgelegt werden, die zeigen, dass die kumulative Haarcortisolkonzentration in einer gesunden Stichprobe assoziative Lernvorgänge und deren neuronale Korrelate signifikant moduliert.

Bedeutung für klinische Forschung

In Bezug auf Suchterkrankungen können aus den bearbeiteten Fragestellungen mehrere Schlussfolgerungen gezogen werden, die in anwendungsnäheren Arbeiten weiterverfolgt werden müssen. Übergeneralisierung appetitiver Konditionierung wurde in dem vorliegenden Dissertationsprojekt als möglicher Mechanismus identifiziert, durch den Stress das Risiko für die Entstehung psychischer Störungen erhöhen könnte. Die unter Stress mit einem CS+ assoziierte Konsumreaktion könnte im Alltag auf weitere neutrale Stimuli generalisieren. Selbst wenn diese nicht direkt mit einer Belohnung gepaart wurden, könnten diese also Craving verursachen, wodurch dieses potentiell häufiger auftritt. Die konditionierte Reaktion auf den CS+ wird unter akutem Stress durch vorangegangene erhöhte kumulative Ausschüttung von Cortisol noch verstärkt. Inwieweit diese Mechanismen in der Entstehung und Aufrechterhaltung von Suchterkrankungen eine Rolle spielen, sollte in klinischen Studien überprüft werden. Schließlich konnte gezeigt werden, dass die Amygdala und der Nucleus Accumbens die neu erworbene Extinktionsgedächtnisspur zu kodieren scheinen. Der dACC hingegen ist am Abruf der zuvor gelernten Belohnungsassoziation beteiligt, was Modelle des Abrufs der Furchtgedächtnisspur auf Abruf von konditionierter Belohnung generalisiert. Schließlich konnte Impulsivität als potentieller Faktor für negative Therapieprognosen identifiziert werden, da diese mit reduziertem differentiellen Extinktionslernen einherging. Dies erweitert die Rolle der Impulsivität vom Risikofaktor um eine Rolle in Bezug auf Prognose des Therapieerfolgs.

Ausblick

Vor dem Hintergrund einer Vielzahl verschiedener Befunde zum Einfluss von akutem Stress auf Belohnungsverarbeitung, ist weitere Forschung nötig, um die genauen Mechanismen zu klären. In Bezug auf die Grundlagenforschung ist festzuhalten, dass eine systematische Untersuchung von Generalisierungsprozessen bei emotionalem Lernen für eine Integration bislang widersprüchlicher Ergebnisse zentral sein könnte. Insbesondere Studien, die parallel Belohnung und Bestrafung einsetzen, beobachten möglicherweise im einen Fall Übergeneralisierung des Lernens aus Belohnung, im anderen Fall Übergeneralisierung des Lernens aus Bestrafung. In Bezug auf das Verständnis der Modulation von Belohnungslernen durch Stress und insbesondere Cortisol ist es nötig in weiteren Studien den Betrachtungsrahmen zu erweitern. Dazu gehört die Überprüfung der Generalisierbarkeit der Ergebnisse auf eine gemischtgeschlechtliche Stichprobe.

Zusammenfassung

Die hier dargestellte Arbeit konnte insbesondere die Untersuchung neuronaler Korrelate appetitiver Konditionierung voranbringen. Insgesamt wurden 8 Artikel zum Gebiet des emotionalen Lernens in Fachzeitschriften veröffentlicht, davon 2 als Erstautor, ein Artikel in geteilter Erstautorenschaft und 5 Artikel als Ko-Autor. Es konnte gezeigt werden, dass akuter Stress wie erwartet die Akquisition der Konditionierung beeinflusst und Übergeneralisierung verstärkt. Die Moderation des Zusammenhangs kumulativer Cortisolausschüttungen mit konditionierten Reaktionen durch akuten Stress ist differenziert zu betrachten. Stärkere konditionierte Reaktionen waren unter akutem Stress mit höherer kumulativer Cortisolausschüttung assoziiert, in Abwesenheit von akutem Stress jedoch mit niedrigerer kumulativer Cortisolausschüttung. Schließlich konnten auch erstmals die neuronalen Korrelate der appetitiven Extinktion beim Menschen identifiziert werden, die den Wert der Integration von Befunden aus Tierstudien, Furchtkonditionierung und appetitiver Konditionierungsforschung aufzeigen. Zusammengefasst konnte damit der Zusammenhang von akutem und chronischem Stress mit appetitiver Konditionierung präzisiert werden und Grundlagen für ein neuronales Modell appetitiver Extinktion gelegt werden.

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Original article

Neural correlates of appetitive extinction in humans

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Abstract

Appetitive extinction receives attention as an important model for the treatment of psychiatric disorders. However, in humans, its underlying neural correlates remain unknown. To close this gap, we investigated appetitive acquisition and extinction with fMRI in a 2-day monetary incentive delay paradigm. During appetitive conditioning, one stimulus (CS+) was paired with monetary reward, while another stimulus (CS−) was never rewarded. Twenty-four hours later, subjects underwent extinction, in which neither CS was reinforced. Appetitive conditioning elicited stronger skin conductance responses to the CS+ as compared with the CS−. Regarding subjective ratings, the CS+ was rated more pleasant and arousing than the CS− after conditioning. Furthermore, fMRI-results (CS+ − CS−) showed activation of the reward circuitry including amygdala, midbrain and striatal areas. During extinction, conditioned responses were successfully extinguished. In the early phase of extinction, we found a significant activation of the caudate, the hippocampus, the dorsal and ventral anterior cingulate cortex (dACC and vACC). In the late phase, we found significant activation of the nucleus accumbens (NAcc) and the amygdala. Correlational analyses with subjective ratings linked extinction success to the vACC and the NAcc, while associating the dACC with reduced extinction. The results reveal neural correlates of appetitive extinction in humans and extend assumptions from models for human extinction learning.

Key words: fMRI; reward; conditioning; extinction; nucleus accumbens; amygdala

Introduction

Appetitive conditioning and extinction receive increasing attention as a model for psychiatric disorders (Martin-Soelch *et al.*, 2007). Especially, the extinction of reward associated cues plays an important role in the ability to flexibly interact with the environment and, in clinical cases, in the treatment of addiction-related disorders (Taylor *et al.*, 2009; Everitt, 2014). However, most research on the neural mechanisms of appetitive extinction is conducted in animals (Millan *et al.*, 2011). In humans, only the neural mechanisms of fear extinction have been extensively investigated (Quirk and Mueller, 2008; Sehlmeyer *et al.*, 2009).

Appetitive conditioning in humans can be studied using a classical (Pavlovian) conditioning paradigm or the monetary incentive delay paradigm (MID), which adds an operant conditioning component to the task. In both cases, a neutral stimulus (e.g. a colored rectangle; CS+) is paired with the chance to get a reward (UCS; e.g. money), whereas another neutral stimulus (CS−) is never paired with the chance to win. Additionally, in the MID paradigm, to gain the reward, subjects are required to press a button in reaction to an operant cue that appears in all trials with a varying delay after the CS. The repeated pairing of the CS+ with the UCS (reward) in appetitive conditioning paradigms results in conditioned responses (CRs). CRs comprise positive affective ratings of the CS+ and elevated skin conductance reactions (SCRs) as compared with the CS− (Kirsch *et al.*, 2003; Klucken *et al.*, 2013b, 2015; Andreatta and Pauli, 2015). The MID, however, has rarely been used as a learning task as subjects have in most cases been instructed about the CS-UCS contingencies beforehand.

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Brain areas involved in appetitive conditioning mainly include the nucleus accumbens (NAcc) in the ventral striatum, the amygdala, the orbitofrontal cortex (OFC), as well as the caudate nucleus in the dorsal striatum and the anterior cingulate cortex (ACC) (Martin-Soelch *et al.*, 2007; Chase *et al.*, 2015). The amygdala mediates the association between CS and UCS during acquisition of conditioning (Balleine *et al.*, 2003). The NAcc as part of the mesolimbic dopamine system is thought to encode the salience of the reward associated stimulus and plays an important part in mediating reward, while the dorsal striatum is involved in goal-directed behavior (dorsomedial striatum) and habit learning (dorsolateral striatum) (O'Doherty *et al.*, 2004; Burton *et al.*, 2015). The ventral ACC plays a role in early discriminative learning, while the dorsal ACC encodes the outcome of a CS+ (Gabriel *et al.*, 2003; Alexander and Brown, 2011). The roles of other areas like the OFC are still debated since different studies ascribed different roles to the OFC including the encoding of expected UCS-value, recall of reward and appetitive extinction (Cox *et al.*, 2005, Moorman and Aston-Jones, 2014; Stalnaker *et al.*, 2015).

In appetitive extinction, the CS+ is no longer paired with a reward. This leads to the formation of a new extinction memory able to inhibit the previously learned CS-UCS association but not deleting it (Bouton, 2002). In effect, extinction learning reduces the CRs (Andreatta and Pauli, 2015). With respect to its neural correlates, animal studies highlight the role of the basolateral amygdala (Tye *et al.*, 2010; Portero-Tresserra *et al.*, 2013) and the ventral striatum (Janak *et al.*, 2004; Millan *et al.*, 2010) in appetitive extinction learning. In these brain areas, different neural populations encode the previously learned reward association as well as the extinction memory. The only fMRI-study using an appetitive extinction paradigm was conducted by Tobler *et al.* (2007). However, the authors focused on the association of reward learning-related activations in the striatum and the midbrain and individual finances and found slower learning in richer as compared with poorer participants. Regarding fear extinction in humans, the reported neural correlates including the amygdala, the NAcc, hippocampus and ventromedial prefrontal cortex are comparable to those reported for appetitive extinction in animals (LaBar *et al.*, 1995; Schiller *et al.*, 2008; Sehlmeyer *et al.*, 2009). A more detailed insight in the neural correlates of human appetitive extinction can help to translate animal findings and give insight into similarities and differences to fear extinction.

The aim of this study was the identification of the neural correlates of human appetitive extinction using the MID-task. After an appetitive conditioning paradigm on the first day, an extinction training was conducted 24 h later without any reinforcement of the CS+ and the CS−. We were interested in examining, whether among others the amygdala or the NAcc are involved in appetitive extinction learning in humans as they are in animals. We further explored correlations between neural activations and changes in subjective ratings from post-acquisition to post-extinction.

Materials and methods

Subjects

Twenty-one male subjects ($M = 23.33$ years; s.d. $= 2.50$; range = 18–28 years) were recruited to take part in the study. All subjects were right-handed, native German speakers with European background and had normal or corrected-to-normal vision. Subjects reporting any current or past mental problems or a consumption of psychotropic drugs were excluded as well as subjects with chronic illnesses or treatments preventing them from entering the MRI scanner. Because the experiment was part of an ongoing project exploring endocrinological effects on appetitive conditioning, only male subjects were recruited. All subjects gave written informed consent and received the money they won during the acquisition phase in addition to monetary compensation or course credit for their time. The study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee. Subjects also filled out the Barratt Impulsiveness Scale (BIS-15, German version, Meule *et al.*, 2011). The mean score was 32.24 (s.d. $= 5.22$), similar to the evaluation sample ($M = 30.04$; s.d. $= 6.13$).

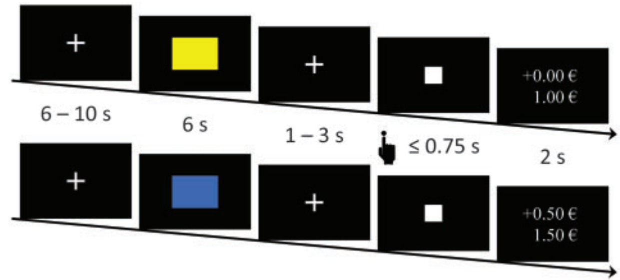

Fig. 1. MID-task. Subjects first saw a CS− or a CS+ (colored rectangle). After a variable delay, a target appeared for a short time. Subjects were instructed to push a button as soon as the target appeared. During the acquisition phase in trials that began with a CS+, fast reactions led to a win. After the target had vanished, feedback about the win and the current total was displayed. During extinction, the same procedure was used but without wins after the CS+.

Procedure

Acquisition and extinction of the appetitive conditioning paradigm took place on two consecutive days roughly 24 h apart and always between 1 and 6 p.m. in the afternoon. As part of a larger study, all subjects took part in a placebo version of the *trier social stress test* (TSST) on the day of the acquisition (Het *et al.*, 2009). This entails thinking of and subsequently talking about a pleasant topic (e.g. a favorite movie) and counting upwards in steps of five in an empty room for 15 min in sum. The placebo TSST was performed after the first subjective rating and a practice run of the experiment outside the scanner and directly before entering the scanner. Cortisol analyses confirmed that the placebo TSST did indeed not lead to a stress reaction. Moreover, PANAS (Positive and Negative Affect Schedule; Krohne *et al.*, 1996) ratings collected before and after the placebo TSST did not indicate any positive [$t(20) = 1.67$; $P = 0.11$] or negative [$t(20) = 0.99$; $P = 0.33$] mood change (for correlations of positive mood and acquisition of conditioning see the supplementary information).

Acquisition. A modified version of the MID paradigm (Knutson, 2000) was used as conditioning procedure and was conducted in the MRI (Figure 1). It consisted of 21 CS+ with partial reinforcement (~62% of trials followed by a reward) and 21 CS− trials, with a yellow or a blue rectangle serving as CS+ or CS−. No more than two CS trials of a kind were presented in succession and CS trials were distributed evenly in two blocks of 20 trials (10 CS+ and 10 CS−), with one CS+ and one CS− trial presented at the beginning. All trials were presented in succession; the blocks were not discernible to the subjects. The first trials of the CS+ and the CS− were later excluded from the analyses because learning could not have taken place yet (Phelps *et al.*, 2004; Klucken *et al.*, 2013a).

Every trial started with a variable intertrial interval ranging from 6 to 12 s followed by the presentation of a CS+ or a CS− for 6 s. Following the CS+ or CS−, after a variable (1–3 s) interstimulus interval displaying a fixation cross, the target (a white square) was presented for at least 16 ms up to a maximum of 750 ms (Figure 1). Subjects were instructed to press the button every time as soon as the target was presented regardless of the CS presented before, which they did (no significant differences in response frequency between CS or phases). Pressing the reaction button while the target was visible always resulted in a win of 0.50€, when a CS+ had been presented beforehand. To ensure a similar reinforcement for all subjects (aim: 6.50€ for wins in 62% of CS+ trials) distributed across the whole acquisition phase, the presentation time of the target was adjusted to individual reaction times (RT) in a multistep procedure (see later). Pressing the button during a target presentation that was preceded by the CS− never resulted in a win. Immediately after the target vanished, feedback on the win of money and the current balance was presented for 2.5 s.

Presentation time of the target was based on a training session with different stimuli and was longer in trials with a planned reward (win: $M_{RT} + 2 \times s.d._{RT}$ loss: $M_{RT} - 2 \times s.d._{RT}$; Dillon *et al.*, 2008; Balodis *et al.*, 2012). If subjects did not win as planned during the acquisition phase, the presentation time of the target was adapted online based on the subjects' performance (−20 ms if subjects won unplanned, +20 ms if subjects did not win as planned; Hahn *et al.*, 2009; Hermans *et al.*, 2010). CS+ trials that did not result in wins as planned or vice versa were adaptively repeated in scheduled CS+ trials with the according duration of target presentation. The adaptive online corrections of cue presentation times were necessary for only 4.1% of CS+ trials across all subjects or an average of <1 CS+ trial per subject. The target presentation times in CS− trials were taken from the CS+ trials and adapted accordingly.

Extinction. The extinction phase was conducted in the scanner 24 h later. It consisted of 40 trials with 20 CS+ and 20 CS− trials. Both early and late phase consisted of 10 CS+ and 10 CS− trials in pseudorandomized order with no more than two trials of the same condition presented directly after one another. As before, subjects were instructed to always press the button when they saw the target. In contrast to the acquisition phase, subjects could not win any money regardless how fast they reacted to the target.

Subjective ratings

Subjects were asked to rate the stimuli used as CS+ and CS− on the scales arousal, valence and UCS-expectancy prior to the acquisition phase, after the acquisition phase and after the extinction phase. To avoid reactivation of the memory trace before extinction (Schiller *et al.*, 2010, 2013; Agren *et al.*, 2012; Agren, 2014), ratings were not collected again right before extinction. For the affective ratings, 9-point self-assessment manikin scales were used (Bradley and Lang, 1994), while UCS-expectancy was rated in 10% steps from 0 to 100%. Subjective ratings were analyzed in 2 (CS) × 3 (Time) analyses of variance (ANOVA) using SPSS 22 (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL, USA). Significant interactions were followed up with paired *t*-tests and were corrected for multiple comparisons.

Skin conductance measuring

Skin conductance was measured during acquisition and extinction with reusable Ag/AgCl electrodes filled with isotonic (0.05M NaCl) electrolyte medium placed on the non-dominant left hand. Data were collected with a sampling rate of 1 kHz. Ledalab 3.4.4 was used for preprocessing and data analysis (Benedek and Kaernbach, 2010). For preprocessing, the data were downsampled to 100 Hz and smoothed with a 32 sample FWHM Gaussian kernel. All data were visually screened. Technical artifacts (e.g. short negative spikes likely caused by the fMRI) were interpolated with spline interpolation. Due to technical difficulties, three subjects had to be excluded from SCR analysis, leaving a sample of 18 subjects for SCR analysis. A time window of 1–6 s following the onset of CS+ and CS− was defined as analysis window. The extracted response was defined as the highest difference between a maximum and the minimum that directly preceded it. The preceding minimum had to be within the analysis window for the response to be counted. Responses smaller than 0.01 μS were considered zero responses. To screen for non-responders, we checked whether all participants showed at least two responses > 0.05 μS to the UCS, which was the case for all participants. All maximum responses were log (μS + 1) transformed to correct for violation of normal distribution of the data. Mean SCRs for CS+ and CS− were calculated for the acquisition phase as well as for the early and late phase of the extinction phase. Skin conductance data were then analyzed separately for the acquisition phase in a paired *t*-test (CS+ *vs* CS−) and for the extinction phase in a 2 (CS+ *vs* CS−) × 2 (time: early phase *vs* late phase) using SPSS 22 (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL, USA).

fMRI

All MRI images were acquired using a 3 Tesla whole-body tomograph (Siemens Prisma) with a 64-channel head coil. The structural images consisted of 176 T1-weighted sagittal slices (slice thickness 0.9 mm; FoV = 240 mm; TR = 1.58 s; TE = 2.3 s). For the functional images, a total of 432 images was acquired on the first day (acquisition phase), while 420 images were acquired on the second day (extinction phase). This difference in the number of images is caused by two additional trials (1 CS+/1 CS−) during the acquisition phase compared with the extinction phase. Images were acquired with a T2*-weighted gradient echo-planar imaging (EPI) with 36 slices covering the whole brain (voxel size = 3 × 3 × 3.5 mm; gap = 0.5 mm; descending slice acquisition; TR = 2 s; TE = 30 ms; flip angle = 75; FoV = 192 × 192 mm; matrix size = 64 × 64; GRAPPA = 2). The field of view was positioned automatically relative to the AC-PC line with an orientation of −40°. Preprocessing, first and second level analysis was done using Statistical Parametrical Mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK; 2008) implemented in Matlab 7.14 (Mathworks Inc., Sherbourn, MA). For preprocessing, all EPI images were coregistered to an EPI template, realigned and unwarped, slice time corrected, normalized to MNI standard space and smoothed with a Gaussian Kernel at 6 mm FWHM. Functional data were analyzed for outlying volumes using a distribution free approach for skewed data (Schweckendiek *et al.*, 2013). Each resulting outlying volume was later modeled within the general linear model as a regressor of no interest.

The experimental conditions entered during the acquisition phase were CS+, CS−, UCS+, NoUCS+ (no win feedback following a CS+) and UCS− (no win feedback following a CS−). Because the target was presented for a few milliseconds before every feedback and inseparable from the feedback itself, it was not separately modeled. The experimental conditions during the extinction phase were similar to the acquisition but without the UCS+ condition because the CS+ was no longer reinforced. During acquisition, the first CS+ and CS− trial was modeled separately as learning could not have taken place yet. CS regressors were split into an early phase ($CS+_{early}$/$CS-_{early}$) and a late phase ($CS+_{late}$/$CS-_{late}$), to more clearly discern between early and late effects (LaBar *et al.*, 1998; Phelps *et al.*, 2004; Milad *et al.*, 2007). CS and UCS events were modeled using the duration the respective stimulus was presented (CS = 6 s; UCS = 2 s). The maximum collinearity between a CS+ and a UCS regressor was <0.20. All regressors were convolved with the canonical hemodynamic response function. The six movement parameters were entered as covariates alongside regressors of no interest for the identified outlying volumes. The time series was then filtered with a high pass filter (time constant = 128 s). For acquisition, a CS+ − CS− contrast was calculated for every subject, while for extinction $CS+_{early} - CS-_{early}$ and $CS+_{late} - CS-_{late}$ were computed.

On the group level, one-sample *t*-tests were performed for the computed first level contrasts to examine neural differences in appetitive conditioning and extinction. Whole brain analyses were conducted with $P < 0.05$ family-wise-error (FWE) corrected and $k > 10$ voxels. Region of interest (ROI) analyses were conducted using the small volume correction in SPM8 with $P < 0.05$ (FWE) and $k > 5$ voxels. The ROI masks for NAcc, amygdala and caudate were taken from the 'Harvard-Oxford cortical and subcortical structural atlases' provided by the Harvard Center for Morphometric Analysis. The masks for OFC, ventral ACC and dorsal ACC were created in MARINA (Walter *et al.*, 2003). To explore associations of neural activations and subjective measures of extinction, difference scores of CS+ ratings from post-acquisition to post-extinction were calculated. These were correlated with the extracted β estimates for significant peak voxels from the early and late extinction phase.

Results

Subjective ratings

ANOVA of the subjective rating scales (Table 1) revealed main effects of CS and Time as well as significant CS × Time interactions in valence [CS: $F(1, 20) = 7.33$; $P = 0.014$; Time: $F(2, 40) = 7.87$; $P = 0.001$; CS × Time: $F(2, 40) = 5.15$; $P = 0.01$], arousal [CS: $F(1, 20) = 12.67$; $P = 0.002$; Time: $F(2, 40) = 5.49$; $P = 0.008$; CS × Time: $F(2, 40) = 8.40$; $P = 0.001$] and UCS-expectancy ratings [CS: $F(1, 20) = 78.10$; $P < 0.001$; Time: $F(2, 40) = 76.75$; $P < 0.001$; CS × Time: $F(2, 40) = 165.31$; $P < 0.001$]. CS+ and CS− differed significantly on all three rating scales [Valence: $t(20) = 3.63$; $P = 0.001$; Arousal: $t(20) = 4.13$; $P < 0.001$; UCS-expectancy: $t(20) = 19.80$; $P < 0.001$] after conditioning while they did not differ before the acquisition phase [Valence: $t(20) = 0$; $P = 1$; Arousal: $t(20) = -0.13$; $P = 0.900$; UCS-expectancy: $t(20) = 0.19$; $P < 0.851$].

After the extinction phase, ratings of the CS+ dropped [valence: $t(20) = 3.75$; $P < 0.001$; arousal: $t(20) = 4.02$; $P < 0.001$; UCS-expectancy: $t(20) = 16.49$; $P < 0.001$] while ratings of the CS− remained unchanged [valence: $t(20) = 1.34$; $P = 0.20$; arousal: $t(20) = 0$; $P = 1$; UCS-expectancy: $t(20) = -0.45$; $P = 0.658$]. In addition, CS+ and CS− did no longer differ significantly on ratings of UCS-expectancy, arousal and valence after the extinction phase [valence: $t(20) = 1.11$; $P = 0.280$; arousal: $t(20) = 1.12$; $P = 0.275$; UCS-expectancy: $t(20) = 2.03$; $P = 0.056$].

Table 1. Mean (s.d.) subjective ratings of CS+ and CS −

		Pre-acquisition	Post-acquisition	Post-extinction
Arousal	CS +	3.19 (1.86)	6.00 (1.92)*,†	3.95 (2.25)*
	CS −	3.24 (1.92)	3.52 (1.54)	3.52 (1.99)
Valence	CS +	5.43 (1.94)	6.76 (1.67)*,†	4.71 (1.98)*
	CS −	5.43 (1.60)	4.67 (1.62)	4.24 (2.05)
UCS-expectancy	CS +	5.62 (1.75)	8.19 (1.50)*,†	1.52 (2.02)*
	CS −	5.52 (1.57)	0.76 (0.83)*	0.90 (1.18)

*indicates a significant difference to the mean rating of the same CS at the previous time point ($P < 0.05$).
†indicates a significant difference to the mean rating of the CS- at the same time point ($P < 0.05$).

Table 2. ROI activations during the acquisition phase (CS+ − CS−)

structure	Side	k	x	y	z	z_{max}	r	P_{corr}
Amygdala	L	121	−24	−2	−12	3.24	0.65	0.038
	R	106	20	−2	−12	3.37	0.66	0.029
Caudate	L	493	−8	16	−2	4.86	0.84	<0.001
	R	517	10	10	4	5.05	0.85	<0.001
dACC	L	1299	−6	10	42	5.19	0.86	<0.001
	R	1426	4	18	38	5.38	0.88	<0.001
Midbrain		621	4	−30	−2	4.80	0.83	<0.001
NAcc	L	104	−8	16	−2	4.53	0.81	<0.001
	R	85	8	8	−4	4.67	0.78	<0.001
OFC	L	120	14	16	−12	3.56	0.69	0.050

Localization, cluster size (k), effect size (r) and statistics (FWE-corrected) of the peak voxel in the respective ROI.

Skin conductance responses

During the acquisition phase, SCRs showed a significant effect of conditioning [$t(17) = 3.01$; $P = 0.004$] (Figure 2). During the extinction phase, analysis of SCRs in a 2 × 2 ANOVA did neither show a main effect of CS [$F(1, 17) < 1$] nor a main effect of Time [$F(1, 17) = 1.90$; $P = 0.186$]. There was a significant CS × Time interaction [$F(1, 17) = 4.58$; $P = 0.047$]. The interaction was mainly driven by a significant reduction in mean SCRs toward the CS+ from the early to the late phase [$t(17) = 2.41$; $P = 0.015$] pointing to a successful extinction of conditioning.

Hemodynamic responses

Acquisition. The acquisition phase on the first day reliably activated the reward network implicated in the acquisition of appetitive conditioning, including NAcc, amygdala and OFC (Table 2). In addition, we computed a model separating CS + presentations, which were later reinforced, from those that were not. A comparison of reinforced and unreinforced CS+ trials showed no trends or significant differences (all $P > 0.31$). A separate analysis of the early and late phase of the acquisition can be found in the supplementary information.

Extinction. In the early extinction phase, activations were found in the left dorsal and ventral ACC as well as the right caudate nucleus and hippocampus (Table 3, Figure 3). In the late phase, bilateral activation of NAcc and amygdala reached significance (Figure 4). There were no significant differences in the contrast CS− − CS+ in the early or the late phase of extinction.

Table 3. ROI activations during the early phase and late phase of extinction (CS+ − CS−)

contrast	Structure	side	k	x	y	z	z_{max}	r	P_{corr}
CS + (early) − CS − (early)	dACC	L	616	−10	2	34	4.18	0.77	0.007
	vACC	L	330	16	38	20	3.58	0.69	0.034
	Caudate	R	189	8	16	8	3.52	0.69	0.033
	Hippocampus	L	194	−24	38	−4	3.33	0.66	0.073
		R	72	22	−38	6	3.53	0.69	0.041
CS + (late) − CS − (late)	Amygdala	L	85	−12	−6	−18	3.43	0.67	0.024
		R	130	16	−4	−18	3.36	0.66	0.032
	Nacc	L	71	−12	8	−8	3.24	0.64	0.020
		R	30	12	14	−6	2.99	0.61	0.033

Localization, cluster size (k), effect size (r) and statistics (FWE-corrected) of the peak voxel in the respective ROI.

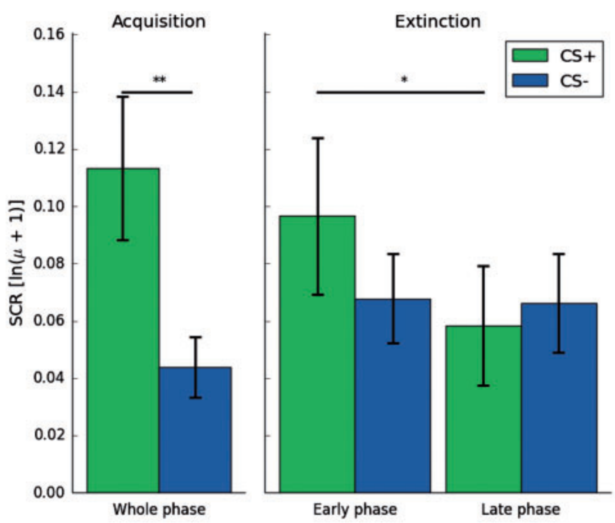

Fig. 2. SCRs (μS log-transformed) for the early and late acquisition phase and the early and late extinction phase. During acquisition, there is a main effect of CS with higher reactions to the CS+. During extinction, there is a CS × Time interaction with higher reactions to the CS+ during the early phase than the late phase. All error bars indicate SEM. *< 0.05; **< 0.01.

Correlations of BOLD responses during extinction with subjective ratings. Extinction indices were calculated separately for UCS-expectancy, valence and arousal by subtracting the post-acquisition ratings for the CS+ from the respective post-extinction ratings. These differences were then correlated with the extracted β estimates at the peak voxel coordinates of the significant neural activations from the early and late extinction phases.

We found activation of the dorsal ACC to be correlated with UCS-expectancy to the CS+ ($r = -0.64$; $P = 0.002$): increased activations of the dACC during early extinction were linked to less reduction of UCS-expectancy from post-acquisition to post-extinction (Figure 5). In contrast, higher activations of the ventral ACC during early extinction were correlated with greater reductions of CS+ arousal ratings ($r = 0.48$; $P = 0.029$). Moreover, activation of the left NAcc was positively correlated with a reduction in CS+ valence ratings ($r = 0.44$; $P = 0.048$).

Following a worthwhile suggestion from an anonymous reviewer, we also investigated the association of impulsivity with the extinction-related BOLD responses. It was hypothesized that higher impulsivity scores might impair extinction-related neural activation. Correlational analyses of BIS-15 scores with the BOLD responses in the late extinction phase did indeed reveal reduced neural activations in the left amygdala ($r = -0.37$; $P = 0.049$), the right amygdala ($r = -0.52$; $P = 0.008$), the NAcc ($r = -0.40$; $P = 0.038$) and a trend in the right NAcc ($r = -0.31$; $P = 0.083$) (see supplementary for further analyses).

Discussion

This study aimed to investigate the neural correlates of appetitive extinction in humans. Using the MID paradigm, neural, subjective and psychophysiological responses were measured on the first (acquisition phase) and second day (extinction phase). On the first day, subjects successfully acquired CRs to the CS+, which were reflected in increased SCRs to the CS+, increased subjective ratings and in an increased involvement of the neural circuit for appetitive conditioning as compared with the CS−. On the second day, CRs were extinguished as subjects did no longer receive a reward following the CS+. This led to a decline in SCRs and in subjective ratings of the CS+. fMRI-results showed activations of the dACC and vACC, as well as the caudate nucleus and the hippocampus during early extinction, while the NAcc and the amygdala were involved during late extinction. Moreover, activation of the dACC was associated with less extinction of subjective ratings, while activations of the vACC and the NAcc were associated with greater extinction of subjective ratings. Because there are still only few fMRI studies on the acquisition of appetitive conditioning, we will briefly discuss these results before discussing appetitive extinction in detail.

Acquisition phase

The results for the acquisition of appetitive conditioning are in line with previous studies on this topic, which also found conditioned SCRs and subjective ratings (Delgado *et al.*, 2008; Andreatta and Pauli, 2015). Neural activations mainly comprised the dorsal and ventral striatum, the amygdala, the midbrain, as well as the dorsal ACC and the OFC, which together form the reward circuitry and are considered to play key roles in appetitive conditioning (Haber and Knutson, 2010; Chase *et al.*, 2015). The striatum is thought to encode incentive salience as well as changes in valence (O'Doherty *et al.*, 2003; Berridge and Kringelbach, 2015). As this study used secondary reinforcement, i.e. money, this might have had an influence on the pattern of neural activations as well. It has previously been shown that different reinforcement types lead to the engagement of different but overlapping subregions in the reward network, especially the ventral striatum (Valentin *et al.*, 2007). In case of the

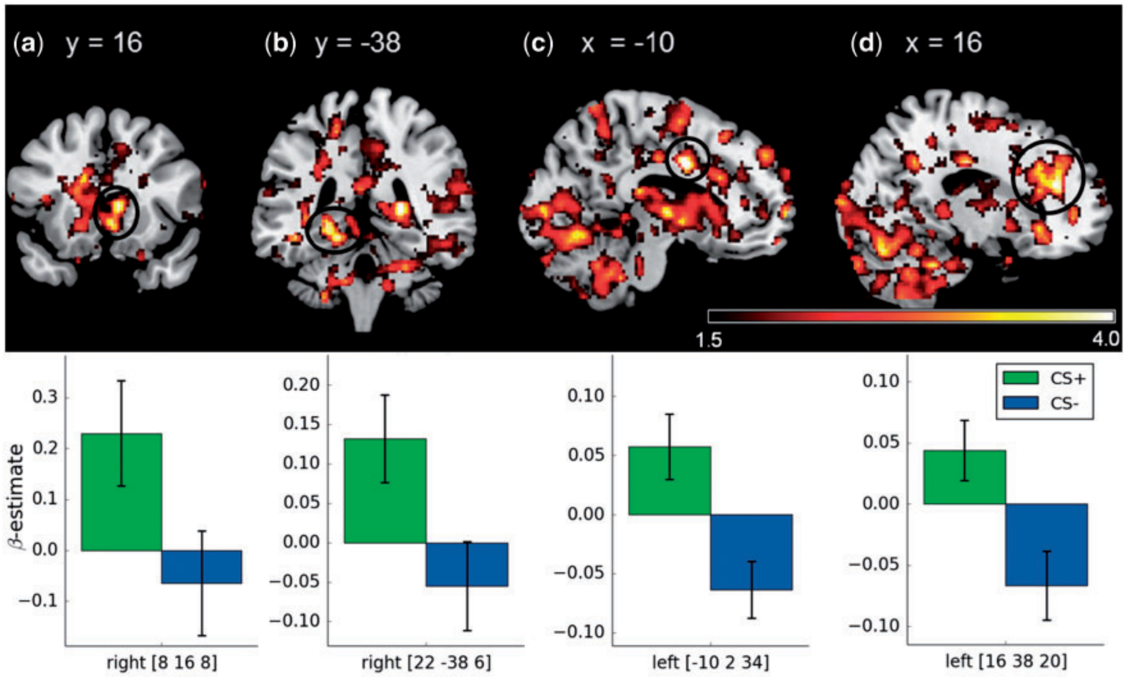

Fig. 3 Significant ROI activations during the early phase of extinction (CS$+_{early}$ − CS$-_{early}$) on voxel level $P < 0.05$ (FWE-corrected). (a) caudate nucleus, (b) hippocampus, (c) dACC (d) and vACC. Displayed t-values are thresholded at $t < 1.5$. (Lower row) Mean β-weights of the respective CS+ and CS− activations. All error bars indicate SEM.

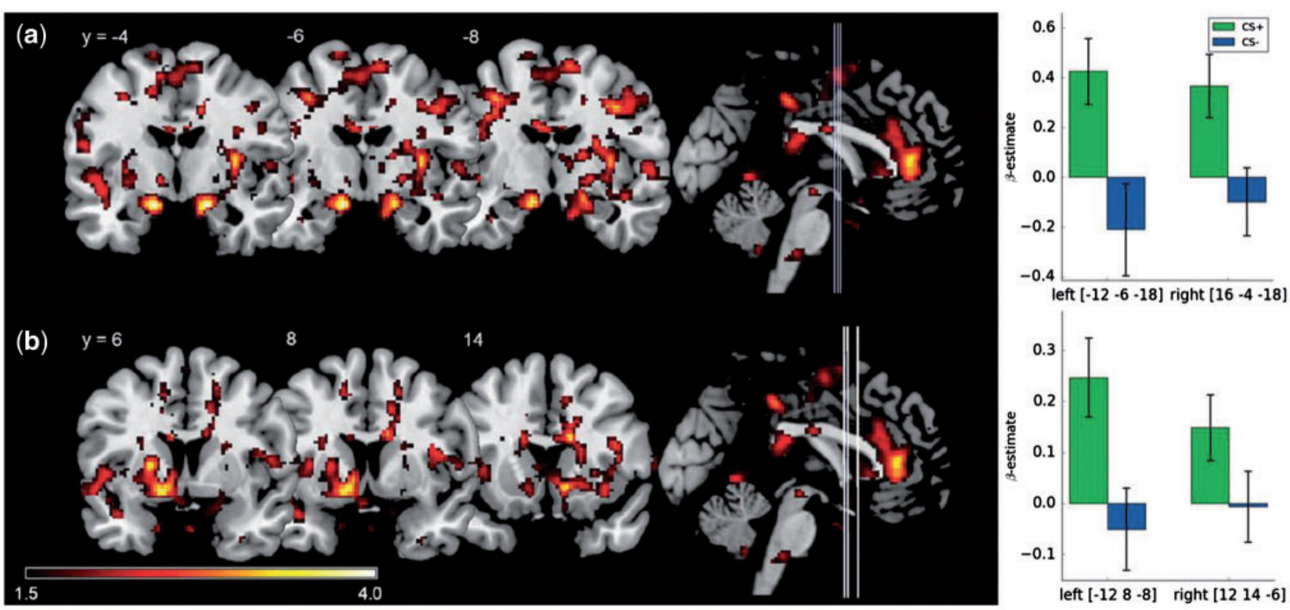

Fig. 4 Significant ROI activations during the late phase of extinction (CS$+_{late}$ − CS$-_{late}$) on voxel level $P < 0.05$ (FWE-corrected). (a) Bilateral activation of the amygdala and (b) bilateral activation of the NAcc. Lines on the sagittal slices on the right side indicate the coronal slices depicted on the left. Displayed t-values are thresholded at $t < 1.5$. (Right) Mean β-weights of the respective CS+ and CS− activations. All error bars indicate SEM.

amygdala, its involvement in conditioning with primary reinforcement has consistently been shown, while its involvement in appetitive conditioning with secondary reinforcers is still under debate (Martin-Soelch et al., 2007). Some studies that used money as reinforcer did not find activation of the amygdala (Kirsch et al., 2003, Cox et al., 2005), while others did (Jiang et al., 2014; Kumar et al., 2014). The findings of the current study support the involvement of the amygdala in classical conditioning with secondary reinforcers. In general, the amygdala is thought to encode the association of CS and unconditioned reaction facilitating the conditioned reaction (Martin-Soelch et al., 2007).

The present paradigm could be regarded as a more 'active conditioning design' because subjects had to press a button in each trial. In contrast, previous studies also used completely 'passive conditioning designs', in which subjects received rewards (e.g. money, food and pleasant pictures) without any personal effort (Delgado et al., 2008; Andreatta and Pauli, 2015; Schweckendiek et al., 2016). This active learning design could

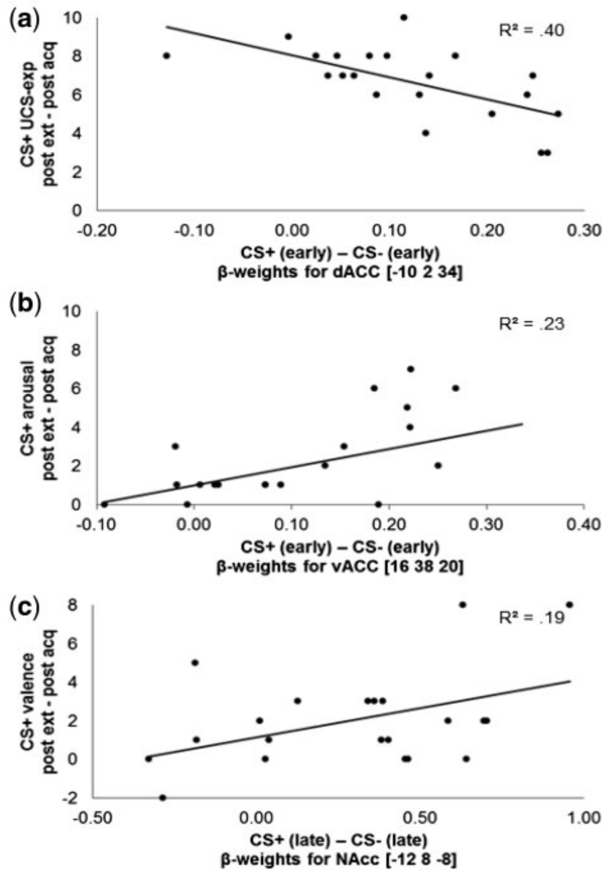

Fig. 5 Significant correlations of extracted β-weights in significant ROIs during early and late extinction (CS+ – CS–) with differences in subjective ratings to the CS+ (post-extinction – post-acquisition). Higher differences indicate extinction success. (a) Negative correlation of neural activations in the dACC with extinction of UCS-expectancy to the CS+. (b) Positive correlation of neural activations in the vACC with extinction of CS+ arousal. (c) Positive correlation of neural activations in the NAcc with extinction of CS+ valence.

also have impacted the CRs. It could be assumed that an active learning paradigm may lead to a stronger CS+/CS– differentiation. Nevertheless, it would be interesting to directly compare active and passive conditioning designs and compare CRs and other relevant features like contingency awareness.

Appetitive extinction

The main research aim was to investigate the extinction of appetitive conditioning and to explore possible mechanisms through its neural correlates. Subjective ratings of UCS-expectancy, valence and arousal showed successful extinction of appetitive conditioning. This is in line with a recent study by Andreatta and Pauli (2015), who were also able to successfully extinguish appetitive conditioning in subjective ratings of valence and arousal. However, other studies on appetitive extinction found resistance to appetitive extinction especially in subjective ratings of valence (Baeyens *et al.*, 2005; Dwyer *et al.*, 2007). In contrast to this study, these two studies used a classical conditioning paradigm, without requiring the subjects to perform a task to obtain the reward. This may have increased the importance in addition to the effects on subjective ratings, the observed appetitive extinction also extended to psychophysiological recordings of skin conductance. This supports the study by Andreatta and Pauli (2015), who also report successful appetitive extinction in SCRs and startle responses.

Early phase of appetitive extinction

During the early phase of extinction, the contrast CS+ – CS– revealed significant activations in the caudate, the hippocampus, as well as the dorsal and the ventral ACC. At this point, SCRs to the CS+ were still higher than in the late phase and the reported neural correlates might also be part of a recall of the consolidated appetitive conditioning (Milad *et al.*, 2007). Although a few fear conditioning studies report increased activation to the CS– as compared with the CS+ (Hermann *et al.*, 2012; Merz *et al.*, 2014), no increased activation to the CS– was found in this study. As one of the main areas implicated in goal-directed behavior (O'Doherty *et al.*, 2004; Burton *et al.*, 2015), activation of the caudate/dorsomedial striatum during the early phase of extinction can be viewed as part of the recall of appetitive conditioning 24 h after acquisition. Previous experiments in rodents found that the inactivation of the dorsomedial striatum after appetitive conditioning mimicked the effect of appetitive extinction (Yin *et al.*, 2005). Activation of the hippocampus has also been associated with the recall of (reward) associative learning (Wolosin *et al.*, 2012; Hattori *et al.*, 2015). Studies on fear extinction, however, discuss a role for the hippocampus in encoding contextual information in extinction learning (Abraham *et al.*, 2014). Moreover, we found activations of the dorsal and ventral ACC. In a computational model based on a range of studies, the dorsal ACC has been proposed to encode predictions of appetitive and aversive outcomes based on previous experiences (Alexander and Brown, 2011). Increased activation of dorsal ACC in substance dependent subjects has been shown to reflect reduced sensitivity to omission of reward (Alexander *et al.*, 2015). Activation of the ventral ACC on the other hand is assumed to play a role in early discriminative learning or the updating of previously learned associations and might therefore be a part of the extinction learning process (Gabriel *et al.*, 2003; Schiller *et al.*, 2008). This functional difference between the dorsal and the ventral ACC has already been discussed for fear extinction (Etkin *et al.*, 2011). The authors review converging evidence showing correlations between dACC and expression of fear conditioning and vACC and inhibition of fear conditioning. This pattern is reflected in the presented correlations of activity in dACC and vACC with changes in subjective ratings and therefore seems to extend to appetitive extinction. Although higher activation of the dorsal ACC was associated with lasting UCS-expectancy following the CS+, higher activation of the ventral ACC was associated with reductions of arousal ratings of the CS+. Taken together with previous studies on fear conditioning, the present findings suggest that for both appetitive and aversive conditioning dACC encodes previously acquired conditioning while the vACC is part of early extinction processes.

Late phase of appetitive extinction

In the late phase, SCRs showed a decline in reactions to the CS+ indicating successful appetitive extinction. Neural correlates in the late phase can therefore be interpreted as part of the processing of extinction (Milad *et al.*, 2007). On a neural level, the amygdala and the NAcc were significantly activated during the late phase of extinction (CS+ – CS–). Based on animal studies, Quirk and Mueller (2008) already ascribed the (basolateral) amygdala an important role in human appetitive extinction. Studies with monkeys and rats showed that after functional inactivation of the (basolateral) amygdala, animals continued to show CRs throughout extinction. It has been hypothesized that the amygdala encodes the changing CS-UCS association during extinction (Martin-Soelch *et al.*, 2007). Tye *et al.* (2010) reported neuron populations in the basolateral amygdala that became active only once extinction learning had begun. Notably, the neurons encoding extinction did not react to omission of reward during a partial reinforcement schedule or to the absence of reward at the beginning of extinction. Similarly, this study also employed a partial reinforcement schedule and found an activation of the amygdala only in the late phase of extinction. Therefore, the activation of the amygdala does not seem to reflect an early reaction to each omission of a possible reward but rather the formation of (new) extinction memory. Moreover, one study on fear extinction that investigated the late phase of extinction separately also found activation of the amygdala in the late phase (Milad *et al.*, 2007) while other studies report mixed results (for a systematic review see: Sehlmeyer *et al.*, 2009).

In addition to the amygdala, we found increased activation of the NAcc, which is one of the main research foci in animal studies on appetitive extinction (Janak *et al.*, 2004; Millan *et al.*, 2010; for a review see: Millan *et al.*, 2011). These studies show that the NAcc core mainly plays a role in acquisition of conditioning while the NAcc shell is involved in extinction of appetitive conditioning. Moreover, in human studies the NAcc has also been associated with the acquisition of new or changing contingencies (Klucken *et al.*, 2009a; Sehlmeyer *et al.*, 2009). Interestingly, activations of the NAcc were only found during the late phase of extinction but not in the early phase. Continued involvement of the NAcc in the MID paradigm, however, has not yet been explored after a 24-h period of consolidation. Animal studies already showed inactivation of the NAcc after the acquisition phase only impaired the reversal of contingencies and extinction of conditioning but not the expression of the consolidated CS-UCS association (Lorens, 1971). Thus, extinction of appetitive conditioning was no longer possible without the NAcc (Reading and Dunnett, 1991). The explored associations of neural activations and changes in subjective ratings also support the involvement of the NAcc in mediating extinction and valence: activation of NAcc was associated with reductions of valence ratings of the CS+ from post-acquisition to post-extinction. This supports the involvement of the NAcc in appetitive extinction and is further in line with reports on the NAcc mediating stimulus valence (Cooper and Knutson, 2008; Berridge and Kringelbach, 2015). In sum, the present results support a role of the NAcc in encoding the update of conditioned valence during acquisition and extinction.

Several authors assume that conditioning is an important model for the development and maintenance of addiction, while extinction processes mirror the treatment of addiction. A central element in the treatment of addiction is therefore the extinction of drug-related cues (Millan *et al.*, 2011). We found increased activation of the NAcc during extinction. The NAcc has repeatedly been associated with changes in the relationship between CS and UCS (Schiller *et al.*, 2008; Klucken *et al.*, 2009b), which is an important element in exposure-based therapy. Another central element of exposure-based therapy is the inhibition of conditioned reactions, which is thought to be mediated by the amygdala (Quirk and Mueller, 2008).

Moreover, in line with current models of addiction, activation of the NAcc and the amygdala during the late phase of extinction was negatively correlated with the participants' impulsivity. This supports assumptions that impulsivity is a risk factor for addiction (Potenza and Taylor, 2009; Gullo and Potenza, 2014; Potvin *et al.*, 2015). Although previous studies showed associations of impulsivity with reward anticipation (Beck *et al.*, 2009; Balodis *et al.*, 2012), this study indicates that impulsivity is also associated with lower activations related to the extinction of previously learned reward associations. Because appetitive extinction serves as a model for the treatment of addiction, these findings indicate that low impulsivity might be a predictor for the success of exposure-based interventions or that these interventions might benefit from impulse control trainings.

A few limitations need to be addressed. First, the sample of this study consisted only of male subjects. Studies of the neural correlates of human fear conditioning showed effects of sex hormones on fear extinction (Merz *et al.*, 2012). Therefore, further studies need to include female subjects to allow for a generalization of the present findings. Second, in the early extinction phase, effects of recall of appetitive conditioning and aspects of early extinction learning probably overlap. This study cannot entirely disentangle these two processes during the early extinction phase. Based on animal findings on appetitive extinction, regions involved in the expression of conditioning and extinction were expected to overlap. Although animal studies are able to disentangle these neuron populations in multicell recordings, the spatial resolution of fMRI does not allow this. Therefore, differences between acquisition, early extinction phase, and late extinction phase on a neural level could not be studied by direct comparison but its neural correlates had to be analyzed separately.

In conclusion, this study aimed to investigate extinction of appetitive conditioning and its neural correlates. Processes during the early phase of extinction can reflect the recall of the acquired appetitive conditioning and early processes of extinction learning. These differing processes were most pronounced in the ACC. Although the dACC was associated with stable expectance of rewards, the vACC was associated with extinction of subjective ratings. This extends previous aversive conditioning results to appetitive conditioning leading to the assumption that the ACC is involved in emotional learning regardless of the specific valence. The activation of amygdala and NAcc during the late phase of extinction supports their role in encoding the new extinction memory, which is in line with animal studies on appetitive extinction. Thus, our study provides a first insight into the neural correlates of appetitive extinction in humans.

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Supplementary data

Supplementary data are available at *SCAN* online.

Conflict of interest. None declared.

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Appendix II: Kruse, O., Tapia León, I., Stark, R. & Klucken, T. (2017). Neural correlates of appetitive extinction in humans. Social Cognitive and Affective Neuroscience, 12 (1), 106–115.

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Altered reward learning and hippocampal connectivity following psychosocial stress

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ABSTRACT

Acute stress has a profound influence on learning, as has been demonstrated in verbal learning or fear conditioning. However, its effect on appetitive conditioning is still unclear. Fear conditioning research suggests the possibility of overgeneralization of conditioning to the CS- under acute stress due to its effect on prefrontal and hippocampal processing.

In this study, participants (N = 56 males) were subjected to the *Trier Social Stress Test* or a placebo version. After that, all participants underwent an appetitive conditioning paradigm in the fMRI, in which one neutral cue (CS+) was repeatedly paired with reward, while another (CS-) was not. Importantly, the stress-group revealed overgeneralization of conditioning to the CS- on the behavioral level. On the neural level, stressed participants showed increased connectivity between the hippocampus and amygdala, vACC, and OFC, which maintain specificity of conditioning and also showed reduced differential activation. The results indicate overgeneralization of appetitive conditioning promoted by maladaptive balancing of pattern separation and pattern completion in the hippocampus under acute stress and are discussed with respect to clinical implications.

Introduction

Learning about cues that signal reward is a key element in interactions with our environment. If we repeatedly take a tasty snack out of a blue box, we will soon prefer this blue box over other boxes and our mouth will begin to water as soon as we see it. Previous research showed that this reward learning is altered by acute stress, however, the precise effect of acute stress on reward learning is still unclear (Berker et al., 2016; Lighthall et al., 2013). Reward learning processes can be conceptualized as an appetitive conditioning paradigm, in which a neutral cue (CS+) is repeatedly paired with the chance to win a reward (UCS; e.g. money). Another neutral cue (CS-) is never paired with the UCS. After few pairings, the participants show increased responses to the CS + as compared to the CS- like increased valence and arousal ratings, elevated skin conductance responses (SCRs), and an activation of the reward circuit (Klucken et al., 2015). However, while it is clear that acute stress exerts a profound influence on the reward circuit (Gold et al., 2015; Montoya et al., 2014; Pruessner et al., 2008), its precise effect on appetitive conditioning is still unclear. Studies examining the topic face several difficulties. This includes type and timing of the stressor, as the sympatho-adrenergic response occurs rapidly, while the hypothalamic-pituitary axis (HPA) takes more time to effect the secretion of cortisol (Hermans et al., 2014). Moreover, stress hormones interact with sex hormones and oral contraceptives in females, which often confound effects of gender on emotional learning (Merz et al., 2010; Merz and Wolf, 2017).

The neural circuit underlying reward learning includes the amygdala, the dorsal and ventral striatum, the orbitofrontal cortex (OFC), the anterior insula, as well as the dorsal and ventral anterior cingulate cortex (dACC/vACC) (Haber and Knutson, 2010; Martin-Soelch et al., 2007). Within this circuit, the amygdala is thought to encode the learned CS/UCS-association (Chase et al., 2015). The ventral striatum is assumed to be a key element in the reward circuit, encoding the acquired motivational salience of the cue and CS/UCS contingencies (Klucken et al., 2009). The anterior insula is thought to integrate interoception of emotional reactions with information about the emotional event in reward learning or processing of psychosocial stressors (Kogler et al., 2015; Sescousse et al., 2013). The vACC is thought to play a key role in

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differential conditioning, with a focus on early differentiation between the CS+ and the CS- (Gabriel et al., 2003), while the dACC on the other hand is thought to encode the expected outcome of the CS+ (Alexander and Brown, 2011; Etkin et al., 2011). In addition, the anterior insula has been identified as part of the salience network and is assumed to reflect increased attention towards stimuli associated with reward as well as encoding affective and psychophysiological responses (Chase et al., 2015; Hermans et al., 2014; Kogler et al., 2015).

Previous research on acute stress and conditioning in animals revealed increased generalization of conditioning and impaired goal-directed action under acute stress. In humans, overgeneralization of conditioning from the CS+ to the CS- under stress has previously been discussed in post-traumatic stress disorder (Besnard and Sahay, 2016). In fear conditioning, a network of hippocampus, OFC, and vACC is thought to balance generalization and specificity of conditioning (Xu and Sudhof, 2013). Moreover, this circuit has links to the striatum, amygdala, and midbrain to influence the expression of conditioning. This is in line with research reporting altered functional connectivity of the hippocampus to prefrontal areas as well as the amygdala in fear generalization (Lissek et al., 2014). In this network stress is argued to be an important factor tipping the scales toward generalization of learning (Pedraza et al., 2016). Reduced activation of the vACC, as has also been observed in humans under acute stress (Born et al., 2010; Pruessner et al., 2008), can induce overgeneralization of conditioned responses from the CS+ to the CS- (Cardinal et al., 2003). For the hippocampus, preliminary research suggests that stress impairs pattern separation of different stimuli (Besnard and Sahay, 2016). Structures with ties to the core network mediating specificity of conditioning like amygdala and striatum, which themselves are highly susceptible to stress, are central to the acquisition and expression of conditioning. In a study by Born et al. (2010) participants under acute stress chose more food under stress, while showing reduced activation of amygdala, striatum, hippocampus, and cingulate gyrus toward food cues.

Although the effects of conditioning develop over time, previous research on the effects of acute stress on learning has not taken into account the development of learning in the beginning and in later phases of learning. However, it has been observed that the effects of stress on learning become more pronounced in the late phase. In the dorsal striatum a shift from dorso-medial (caudate) to dorso-lateral (putamen) activation that occurs over time and promotes a shift from goal-directed to habit learning is facilitated under acute stress (Schwabe and Wolf, 2011). It has been suggested that this effect is induced by a deactivation of prefrontal areas, especially the OFC, and a resulting impairment of the executive network (Hermans et al., 2014; Schwabe et al., 2012). In general, under acute stress reduced differential activation of prefrontal and limbic areas has been observed (Dagher et al., 2009; Pruessner et al., 2008).

In the present study, we investigated the altered neural correlates of appetitive conditioning in the fMRI under stress. First, we expected both stressed and non-stressed participants to acquire appetitive conditioning. Second, we expected the stress-group to overgeneralize the acquired conditioning, showing reduced differential neural responses in areas regarding specificity of learning, hippocampus, vACC, OFC, and areas associated with the acquisition and expression of appetitive conditioning, namely the amygdala and the ventral striatum and increased functional connectivity between these structures. In addition, we investigated possible stronger goal-directed activation of the caudate in the control-group during the late phase as compared to the stress-group.

Materials and methods

Participants

A total of 60 male participants (mean age = 23.77 years; SD = 3.03 years) took part in the study. All participants had normal or corrected-to-normal vision and were right-handed, German native speakers with a European background. Exclusion criteria were past or current mental illness, consumption of psychotropic drugs, working in the night shift or travelling across time zones in the past two weeks, and any treatment preventing from entering the magnetic resonance imaging (MRI) scanner. After completion of the experiment, all subjects filled out the German versions of BDI-II (Beck Depression Inventory: Hautzinger, Keller and Kühner, 2009), BIS-15 (Barratt Impulsiveness Scale: Meule, Vögele and Kübler, 2011), and PSS (Perceived Stress Scale: Klein et al., 2016). Prior to the experiment, participants gave written informed consent. After the conclusion of their participation, they received monetary compensation or course credit for their time. Any money they won during the experimental run was paid out directly after participants left the MRI scanner. The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee. Due to technical difficulties data of four participants were excluded from the analysis (3 in the stress-group, 1 in the control-group), leaving 56 participants in the final sample (Table 1).

Procedure

To ensure similar baseline cortisol levels, data acquisition always took place in the afternoon between 1 p.m. and 6 p.m. and participants came into the lab at least 30 min before giving the first saliva sample (Fig. 1). After giving written informed consent, participants performed a training version of the paradigm consisting of different stimuli to familiarize themselves with the task and calculate the speed of their responses in order to adapt the difficulty of the MRI task. Next, participants were prepared for the MRI. Then they gave the first of a total of four saliva samples (Salivette, Sarstedt, Nürnbrecht, Germany), filled out the Positive and Negative Affect Scale (PANAS, Krohne et al., 1996), and were led to a separate room. Here, half the participants (stress-group) took part in the *Trier Social Stress Test* (TSST; Kirschbaum et al., 1993), while the other half (control-group) took part in the placebo version of the TSST (Het et al., 2009). After 20 min, they were led to the MRI, where they gave the second saliva sample and filled out the PANAS a second time. 10 min later they gave a third saliva sample, while in the MRI. The appetitive conditioning paradigm started 10 min after the third sample and a total of 40 min after the beginning of the TSST or Placebo-TSST. This ensured that the appetitive conditioning paradigm would take place after cortisol had been released by the pituitary and reached the brain, which takes about 20 min (Droste et al., 2008). 15 min later the subjects left the MRI, gave the last saliva sample and filled out the PANAS a third time.

TSST/placebo-TSST

The TSST was conducted according to Kirschbaum et al. (1993) in a room with two confederates (one male, one female) in white coats sitting at the head of a conference table. The participants of the stress-group were led in and received written instructions explaining the first task. After 5 min of preparation, one of the confederates instructed the participant to begin. After 5 min, they were given the second task, which again lasted 5 min after which participants could leave the room.

The placebo version was conducted as described by Het et al. (2009) with similar tasks with the same duration as in the TSST, but without elements of uncontrollability or social evaluation. Participants were

Table 1
Descriptive mean (SD) data of stress- and control-group, including age, depression (BDI-II), impulsivity (BIS-15), chronic stress (PSS), sleep duration (hours last night), and total win during the experiment. *p*-values of two-sample-*t*-tests (rightmost column).

	stress-group (n = 27)	control-group (n = 29)	*p*
Age [y]	23.48 (3.30)	23.83 (2.80)	.67
BDI-II	4.59 (4.89)	4.93 (4.50)	.79
BIS-15	33.77 (5.50)	32.51 (5.32)	.40
PSS	13.04 (5.58)	13.19 (4.47)	.92
sleep [h]	7.97 (1.02)	8.05 (0.88)	.76
Win [€]	6.46 (0.13)	6.43 (0.22)	.49

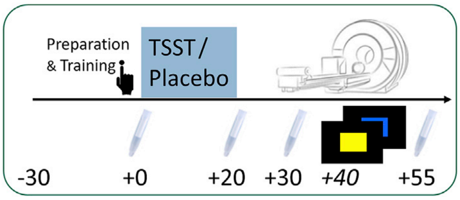

Fig. 1. Schedule of the experiment. Participants arrived about 30 min [-30] before the first saliva sample, and were prepared for scanning and familiarized themselves with the task in the meantime. Directly before the TSST/Placebo-TSST, they gave the first saliva sample [+0], the second sample directly after [+20]. The third sample was given in the MRI before the beginning of MRI measurements [+30], 10 min before the acquisition training [+40]. The fourth and last saliva sample was given after the participants left the MRI [+55].

alone in a room, which the experimenter only entered between tasks to give instructions for the next task.

To assess the stress-inducing effect of the TSST a total of four salivary cortisol samples was collected. Salivette sampling devices (Sarstedt, Nürnbrecht, Germany) were used and then stored at –20 °C until analysis. Samples were assayed using commercially available enzyme immunoassays (IBL International, Hamburg, Germany). Overall cortisol reactivity corrected for baseline cortisol (AUC_i; Pruessner, Kirschbaum, Meinlschmid and Hellhammer, 2003) was then computed. AUC_i differences between groups were analyzed in a two-sample *t*-test using SPSS 22 (SPSS 22.0 for Windows, IBM Inc., Chicago, IL, USA).

Acquisition training

The appetitive conditioning paradigm took place in the MRI scanner and was a modified version of the well-established Monetary Incentive Delay (MID) paradigm (Knutson et al., 2000), adapted as an uninstructed classical conditioning paradigm as previously described in Kruse et al. (2017). A total of 42 trials were presented, 21 CS+ trials and 21 CS- trials. A blue and a yellow rectangle randomly served as CS+ or CS- counterbalanced across groups. 13 of 21 CS+ trials were followed by a win of 0.50€ (reinforcement rate ≈ 62%). The number of trials was designed to exclude the first trial for both CS when learning could not yet have occurred. In addition, the same number of reinforced trials occurred during the early phase (trial 2-11) and the late phase (trial 12-21) to have similar halves for later analysis. Splitting the acquisition in half allows to analyze different phases of the learning process, while minimizing the loss of statistical power (Kolada et al., 2017; Lonsdorf et al., 2017; Tabbert et al., 2005).

Each trial lasted for about 20s with 12s of inter-trial-interval distributed randomly to the beginning and end of the trial (Fig. 2). The CS+ or CS- was then presented for 6s followed by a variable inter-stimulus-interval (1-3s), during which a fixation cross was displayed. This delay was followed by a presentation of the target, a white square, to which the participants had to try to react to by pressing a button while it was visible. While the participants were instructed to always react as fast as possible, a fast enough reaction only led to a win (0.50€) in CS+ trials. To ensure a similar total reinforcement across all participants (aim: 6.50€ ≈ 62% of CS+ -trials) as well as an equal distribution of wins across the whole acquisition phase, the presentation time of the target was varied between 16ms and 750ms. Based on the training session, the mean reaction time (RT_M) and its standard deviation (RT_{SD}) was calculated. The presentation time of the target was longer, when a win was scheduled ($RT_M + 2 \times RT_{SD}$), and shorter, when a loss was scheduled ($RT_M + 2 \times RT_{SD}$). Target presentation times during CS- trials varied accordingly. The target was always directly followed by a presentation of feedback on the win of money and the current balance for 2s.

In the rare case (4.1% of CS+ trials and less than one trial per subject) of an unscheduled win (or loss) because a subject reacted faster (or slower) as established by the training session, a later trial was scheduled to compensate and future response windows were adapted (+20ms for unscheduled losses, -20ms for unscheduled wins).

Subjective ratings

The stimuli serving as CS+ and CS- were rated on the scales arousal, valence and UCS-expectancy before the first training session and the TSST/Placebo-TSST as well as after the acquisition phase. Valence and arousal were rated on a 9-point-Likert scale using the Self Assessment Manikin (SAM, Bradley and Lang, 1994). UCS-expectancy was rated in 10% steps from 0% to 100%. Subjective ratings were analyzed in 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Pre vs Post) analyses of variance (ANOVA) using SPSS 22. Significant interactions were followed up with paired *t*-tests.

Reaction times

During the experiment, button presses in reaction to the target were recorded. Median reaction time data were then analyzed in a 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA using SPSS 22. Significant interactions were followed up by *t*-tests. Moreover, subjects left out targets in 0.06% of trials, with one participant in the control-group not reacting to targets following a CS- throughout the late phase. As group differences with respect to leave-out might skew the analysis of reaction times the number of leave-outs in the respective conditions were analyzed for group differences in a non-parametric Mann-Whitney-U-test using SPSS 22. Post-hoc comparisons were corrected for multiple testing with Bonferroni correction.

Skin conductance measuring

Skin conductance was continuously measured during the acquisition

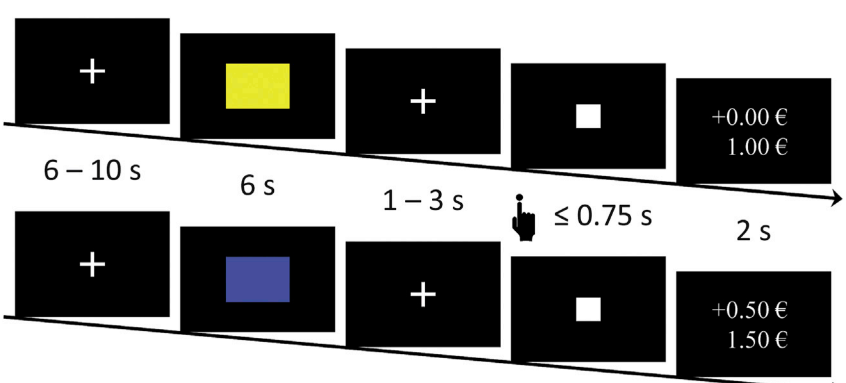

Fig. 2. MID task as in Kruse et al. (2017). Subjects first saw a CS- or a CS+ (colored rectangle). After a variable delay a target appeared for a short time. Subjects were instructed to push a button as soon as the target appeared. In trials that began with a CS+ fast reactions led to a win. After the target vanished, feedback about the win and the current total was displayed.

phase with reusable Ag/AgCl electrodes filled with isotonic (0.05M NaCl) electrolyte medium placed on the non-dominant left hand. Data were collected with a sampling rate of 1 kHz. Ledalab 3.4.4 was used for preprocessing and data analysis (Benedek and Kaernbach, 2010). For preprocessing the data were downsampled to 100 Hz and smoothed with a 32 sample FWHM Gaussian kernel. All data were visually screened. Technical artifacts were spline interpolated. A time window of 1-6s following the onset of CS+ and CS- was defined as analysis window and reflects the CS presentation time. A continuous decomposition analysis (CDA) was performed to separate the underlying tonic response from the specific responding to the stimulus, which was then extracted for analysis. Responding was measured as area under the curve per analysis window after subtraction of the tonic response. All extracted responses were log (μS + 1) transformed to correct for violation of normal distribution of the data. Mean SCRs for CS+ and CS- were calculated for the early and late acquisition phase. Skin conductance data were then analyzed in a 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA using SPSS 22.

fMRI

All MRI images were acquired using a 3 T whole-body tomograph (Siemens Prisma) with a 64-channel head coil. Structural images consisted of 176 T1-weighted sagittal slices (slice thickness 0.9 mm; FoV = 240 mm; TR = 1.58s; TE 2.3s) and were acquired one day later, when participants returned to take part in an appetitive extinction paradigm. This allowed to acquire salivary samples before and after the fMRI sequence as soon as possible. During structural image acquisition a static television test screen was shown. Functional images were acquired with a T2*-weighted gradient echo-planar imaging (EPI) with 36 slices covering the whole brain (voxel size = 3 × 3 × 3.5 mm; gap = 0.5 mm; descending slice acquisition; TR = 2s; TE = 30ms; flip angle = 75; FoV = 192 × 192 mm; matrix size = 64 × 64; GRAPPA = 2). The field of view was positioned automatically relative to the AC-PC line with an orientation of -40°. A total of 432 images were acquired. Statistical Parametrical Mapping (SPM12, Wellcome Department of Cognitive Neurology, London, UK; 2014) implemented in Matlab 7.14 (Mathworks Inc., Sherbourn, MA) was used for preprocessing the raw data, as well as first and second level analysis. Preprocessing of the EPI images comprised coregistration to the EPI-template provided by SPM, realignment and unwarping (including motion correction), slice time correction, normalization to MNI standard space (resampling to 2 mm isovoxel with trilinear interpolation) as well as smoothing with a 6 mm FWHM Gaussian kernel. Functional data were analyzed for outlying volumes using a distribution free approach for skewed data (Schweckendiek et al., 2013). Each resulting outlying volume was later modeled within the first-level general linear model (GLM) as a regressor of no interest.

On the first level, single subject data were modeled within the GLM with the experimental conditions $CS+_{early}$, $CS+_{late}$, $CS-_{early}$, $CS-_{late}$, UCS+, NoUCS+ and NoUCS-. NoUCS+ and NoUCS- modeled the feedback that no money was won either after presentation of the CS+ or the CS- respectively. The first presentation of the CS+ and the CS- was modeled separately on regressors of no interest as learning could not have taken place yet. CS+ and CS- regressors were split in early (trial 3-22) and late (trial 23-42) phase to assess correlates of early and late learning separately (Davis et al., 2010; Kruse et al., 2017; LaBar et al., 1998; Lonsdorf et al., 2017; Phelps et al., 2004). All regressors were stick functions convolved with the canonical hemodynamic response function. Six movement parameters estimated in the motion correction step of preprocessing were entered as covariates. Additionally, regressors of no interest were entered for the identified outlying volumes, e.g. due to motion during acquisition of a volume which cannot be corrected for by realignment. The time series was filtered with a high-pass-filter (time constant = 128s). For group analysis, the contrasts $CS+_{early} – CS-_{early}$ and $CS+_{late} – CS-_{late}$ were computed.

On the second level, to assess the neural correlates of appetitive conditioning across groups, one-sample *t*-tests were carried out analyzing the neural correlates of acquisition of appetitive conditioning across groups for the contrasts $CS+_{early} – CS-_{early}$ and $CS+_{late} – CS-_{late}$. Secondly, to assess group differences between stress-group and control-group, two-sample *t*-tests were carried out comparing the differential BOLD-response in early and late phase separately ($CS+_{early}$ – $CS-_{early}$ and $CS+_{late}$ – $CS-_{late}$). Cortisol reactivity (AUC_i) was entered as a covariate on separate regressors per group to control for possible interaction effects. Please see the supplemental results for interactions with cortisol reactivity between the groups. Region of interest (ROI) analyses were conducted using small volume correction in SPM12 with $p < .05$ (FWE) and $k > 5$ voxels. Amygdala, anterior cingulate cortex (ACC), nucleus accumbens (NAcc), caudate, orbitofrontal cortex, anterior insula, and hippocampus were selected as regions of interest. If possible, masks were taken from the "Harvard-Oxford cortical and subcortical structural atlases" (probability threshold 0.25) provided by the Harvard Center for Morphometric Analysis. The ACC was separated in the ventral and dorsal part reflecting their differing functions in appetitive conditioning (Etkin et al., 2011; Martin-Soelch et al., 2007). The masks for OFC, ventral, and dorsal ACC were created in MARINA (Walter et al., 2003). In addition, we conducted an exploratory whole-brain analysis for differences between groups, with $p < .05$ (FWE) and $k > 5$ voxels. This analysis yielded no additional results and is therefore not reported.

Functional connectivity (psycho-physiological interaction)

Further, we identified regions changing their functional connectivity in correlation with the acquisition of appetitive conditioning using psycho-physiological interaction analysis as implemented in SPM12 adapting the protocol of Bosch et al. (2017). The chosen seed regions were the bilateral hippocampi. For these regions, the first eigenvariate time series (physiological regressor) were extracted for the whole region of interest and entered into additional first level models alongside the contrast of interest (CS+ - CS-; psychological regressor) and their interaction (psycho-physiological interaction regressor). First level models were otherwise as described above. Separate models were set up for analysis of left and right hippocampal connectivity. On the second level, two-sample *t*-tests were carried out to assess group differences. Cortisol reactivity (AUC_i) was entered as a covariate on separate regressors per group to control for possible interaction effects.

Results

Acute stress reactivity

Salivary cortisol concentrations were analyzed to test the successful induction of acute stress by the TSST (Fig. 3). The experimental groups displayed significantly differing cortisol reactivity in AUC_i ($t(54) = 4.55$; $p < .001$; d = -1.22) with higher values in the stress-group (M = 11.20; SD = 10.41) than the control-group (M = 1.08; SD = 5.74). Critically, the salivary cortisol concentration in the third sample, shortly before the beginning of the experiment, was significantly greater in the stress-group ($t(54) = 5.49$; $p < .001$; d = 1.47).

Subjective ratings

Effects of conditioning were assessed by analyzing subjective ratings of the conditioned stimuli (Table 2). Three 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Pre vs Post) repeated measures analyses of variance (ANOVA) were conducted for ratings of arousal, valence and UCS-expectancy (Table 3); all revealing a main effect of CS as well as a significant CS × Time interaction (all $p < .001$). ANOVA for arousal and UCS-expectancy additionally showed a main effect of Time (both $p < .001$). There was no main effect of Stress exposure (all $p > .32$). Taken together, all rating scales confirm successful acquisition of conditioning.

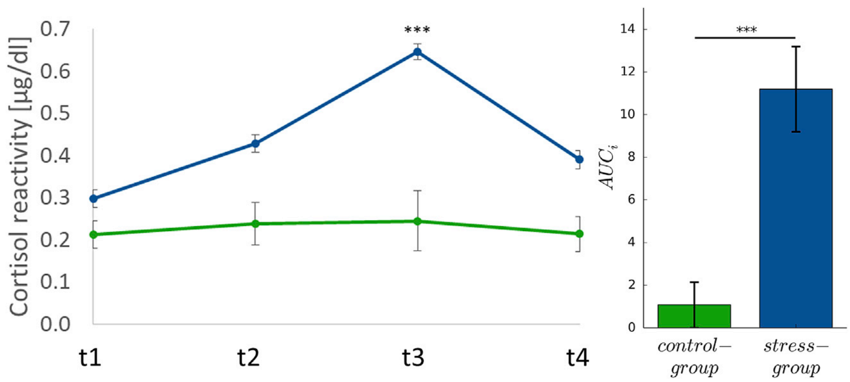

Figure 3. (Left panel) Raw mean salivary cortisol levels at the four sampling points. Significantly increased cortisol level in stress-group at the critical time point t3 shortly before the fMRI paradigm. (right panel) Cortisol reactivity calculated as Area under the Curve corrected for baseline cortisol (AUC_i) plotted for stress- and control-group. Error bars denote standard errors of the mean (SEM). Participants of the stress-group display significantly higher cortisol secretion. *** = $p < .001$.

Table 2
Subjective ratings of the stimuli (CS+ & CS-) with respect to arousal, valence, and UCS-expectancy. Mean (SD) values prior to stress induction and experiment and following the acquisition phase separately for stress- and control-group.

		pre-acquisition		post-acquisition	
		stress-group	control-group	stress-group	control-group
arousal	CS+	3.31 (2.07)	3.21 (1.66)	5.65 (2.31)	5.61 (2.08)
	CS-	3.12 (1.89)	3.57 (1.89)	3.00 (1.77)	3.25 (1.53)
valence	CS+	5.96 (1.46)	5.50 (1.80)	6.77 (1.58)	6.75 (1.71)
	CS-	5.46 (1.79)	5.57 (1.40)	4.42 (1.68)	4.75 (1.69)
UCS-expectancy	CS+	6.27 (1.56)	5.36 (1.87)	7.58 (2.76)	8.18 (1.41)
	CS-	5.92 (1.52)	5.50 (1.84)	0.96 (1.18)	0.82 (0.82)

Table 3
Main effects and interaction effects from 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA for subjective ratings of arousal, valence, and UCS-expectancy with *F*-value, *p*-value, and effect size partial η^2. † = *p* < *.10;* *** = $p < .001$.

	effect	*F*-value	*p-value*		η^2_{part}
arousal	CS	53.22	<.001	***	.51
	Time	18.79	<.001	***	.27
	Stress exposure	0.17	.685		<.01
	CS × Time	27.13	<.001	***	.34
	CS × Stress exposure	1.62	.208		.03
	Time × Stress exposure	0.03	.873		<.01
	CS × Time × Stress exposure	0.07	.800		<.01
valence	CS	32.01	<.001	***	.38
	Time	0.07	.796		<.01
	Stress exposure	0.002	.968		<.01
	CS × Time	20.18	<.001	***	.28
	CS × Stress exposure	1.18	.282		.02
	Time × Stress exposure	0.75	.391		.01
	CS × Time × Stress exposure	0.07	.797		<.01
UCS-expectancy	CS	219.54	<.001	***	.81
	Time	32.69	<.001	***	.39
	Stress exposure	1.02	.317		.02
	CS × Time	225.23	<.001	***	.81
	CS × Stress exposure	0.07	.793		<.01
	Time × Stress exposure	3.48	.068	†	.06
	CS × Time × Stress exposure	1.80	.186		.03

Reaction times

Investigating reaction times, 2 (Stress exposure: Stress vs Control) × 2 (CS: CS+ vs CS-) × 2 (Time: Early phase vs Late phase) ANOVA revealed a main effect of CS ($F(1, 53) = 68.47$; $p < .001$; $\eta^2_{part} = 0.56$) and a main effect of Stress exposure ($F(1, 53) = 5.31$; $p = .025$; $\eta^2_{part} = 0.09$) but not a main effect of Time ($F(1, 53) = 2.74$; $p = .104$; $\eta^2_{part} = 0.50$), which was qualified by a CS × Time interaction ($F(1, 53) = 10.76$; $p = .002$; $\eta^2_{part} = 0.17$) (Fig. 4). As expected, reactions following a CS+ were faster overall and did not improve significantly between the early and late phase ($t(55) = 1.24$; $p = .222$; $d = 0.14$), while reactions to the CS- slowed from the early to the late phase ($t(54) = -2.74$; $p = .008$; $d = -.33$). Importantly, there was a CS × Time × Stress exposure interaction ($F(1, 53) = 6.53$; $p = .014$; $\eta^2_{part} = 0.11$). There was no significant Time × Stress exposure ($F(1, 53) = 1.70$; $p = .198$; $\eta^2_{part} = 0.03$) or CS × Stress exposure ($F(1, 53) = 2.14$; $p = .149$; $\eta^2_{part} = 0.04$) interaction. The three-way interaction was driven by a decrease in reaction time in the control-group to targets following the CS- from the early to the late phase ($t(27) = -2.87$; $p = .008$; $d = -0.56$). The reaction times to targets following a CS- in the stress-group did not differ significantly from early to late phase ($t(26) = -0.64$; $p = .527$; $d = -.08$). Neither group displayed a

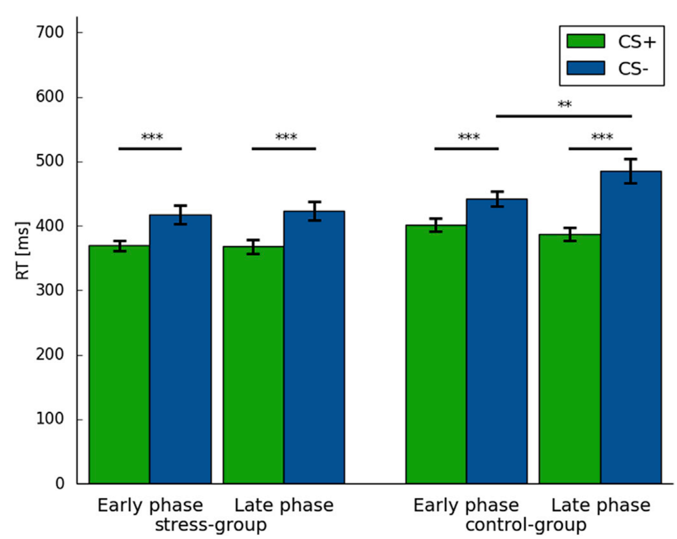

Fig. 4. Mean reaction times [RT] to the targets following the CS+ (green) and the CS- (blue) in milliseconds, separately for stress-group (left) and control-group (right) in early and late phase. Error bars denote standard errors of the mean (SEM). Both stress- and control-group react significantly faster to targets following a CS+. The stress-group shows generally faster reaction times, mainly driven by faster reactions to the CS-. In the late phase, reaction times to targets following a CS- differ significantly between groups, due to slower reactions of the control-group. Reaction times to targets following a CS+ do not differ between groups. $p < .001$ = ***; $p < .01$ = **.

significant change in reaction time to CS+ -targets from early to late phase (stress-group: $t(26) = 0.17$; $p = .866$; $d = 0.03$; control-group: $t(28) = 1.52$; $p = .140$; $d = 0.25$). In addition, participants of the control-group also left out reactions to targets more often than the stress-group ($U = 251.5$; $p = .012$; $d = 0.60$). To test for a CS × Time × Stress exposure interaction in the number of leave-outs as observed in reaction times, Wilcoxon Signed-Ranks tests were performed. In a similar pattern to median reaction times, Leave-outs of a target following the CS- occurred significantly more often in the late as compared to the early phase in the control-group ($z = -2.58$; $p = .01$; $d = -1.09$), while there was no such difference in the stress-group ($z = -1.10$; $p = .272$; $d = -.43$). There was no difference in leave-outs to targets following the CS+ from early to late phase in either group (stress-group: $z = 0.58$; $p = .564$; $d = 0.22$; control-group: $z = -1.00$; $p = .317$; $d = -.38$).

Skin conductance responses

On the level of psychophysiological responses, 2 (Stress vs Control) × 2 (CS+ vs CS-) × 2 (Early vs Late) ANOVA was carried out to assess the effect of conditioning in skin conductance responses (SCRs) assessed by continuous decomposition analysis (CDA). There were significant main effects of CS ($F(1, 54) = 37.76$; $p < .001$; $\eta^2_{part} = 0.41$) and Time ($F(1, 54) = 7.47$; $p = .008$; $\eta^2_{part} = 0.12$), indicating higher responses to the CS+ and a general habituation of responses in the late phase. There was no main effect of Stress exposure ($F(1, 54) = 1.56$; $p = .218$; $\eta^2_{part} = 0.03$) and no interaction effects (CS × Stress exposure: $F(1, 54) = 0.21$; $p = .650$; $\eta^2_{part} < 0.01$; CS × Time: $F(1, 54) = 0.002$; $p = .968$; $\eta^2_{part} < 0.01$; Time × Stress exposure: $F(1, 54) = 2.01$; $p = .162$; $\eta^2_{part} = .04$; CS × Time × Stress exposure: $F(1, 54) = 0.36$; $p = .551$; $\eta^2_{part} < 0.01$).

Hemodynamic responses

Main effect of conditioning

Analysis of the pre-defined ROIs across both experimental groups confirmed wide-spread BOLD-responses to the CS+ as compared to the CS- throughout structures of the reward circuit and in both early and late phase (Table 4). Furthermore, we exploratively correlated the differential BOLD responses with the number of leave-outs to both stimuli for both groups separately. While there were no significant correlations in the stress-group, several significant correlations emerged in the control-group. In the early phase, higher differential BOLD to the CS+ as compared to the CS- was associated with increased leave-outs to the CS- in the left ($r = 0.52$, $p = .004$) and right ($r = 0.58$, $p = .001$) amygdala, left dACC ($r = 0.42$, $p = .025$), left hippocampus ($r = 0.40$, $p = .033$), and left OFC ($r = 0.48$, $p = .004$). However, only the right amygdala survives the significance threshold corrected for the number of tests.

Effect of stress exposure (stress vs control)

On the group level, CS+ - CS- were compared separately for the early and the late phase. During both phases, the control-group, as opposed to the stress-group showed overall stronger responses in the contrast CS+ - CS- (Fig. 5). During the early phase, there were stronger differential responses in the right amygdala, the left OFC and the right vACC (Table 5).

In the late phase, higher differential responses were even more pronounced. Significant differences emerged in the left amygdala, left caudate, left hippocampus, bilateral insula left OFC, and the right vACC. Further analyses showed that the stress-group displayed relatively greater activation towards the CS- in the early phase as compared to the control-group for the left OFC ($t(54) = 2.58$; $p = .013$; $d = 0.69$), and in the late phase for the left caudate ($t(54) = 2.38$; $p = .021$; $d = 0.64$) and the right vACC ($t(54) = 2.43$; $p = .023$; $d = 0.65$). Trends were visible in

Table 4
Region of Interest (ROI) results in the contrast CS+ - CS- across groups for early and late phase. Indicating structure, hemisphere (side), cluster size (k), x-/y-/z-coordinates, z-value, family wise error (FWE) corrected p-value (p_{corr}), and effect size (r). † = $p < .10$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

structure	side	k	x	y	z	z_{max}	p_{corr}		r
$CS+_{early} - CS-_{early}$									
amygdala	L	171	−24	−2	−12	4.70	<.001	***	.58
	R	92	28	0	−12	3.57	.014	*	.46
caudate	L	493	−16	−6	20	6.83	<.001	***	.73
	R	517	10	10	0	6.51	<.001	***	.74
dACC	L	1334	−6	10	40	6.55	<.001	***	.74
	R	1494	10	12	38	7.14	<.001	***	.78
hippocampus	L	430	−28	−14	−14	4.42	.001	**	.55
	R	500	30	−34	−2	5.14	<.001	***	.62
insula	L	1162	−32	3	14	6.18	<.001	***	.71
	R	1018	34	26	2	6.12	<.001	***	.71
NAcc	L	107	−6	12	−2	5.80	<.001	***	.68
	R	85	10	10	−4	6.24	<.001	***	.71
OFC	L	484	−14	16	−14	4.93	<.001	***	.60
	R	341	14	50	−6	5.53	<.001	***	.66
vACC	L	416	−10	36	18	4.51	<.001	***	.56
	R	666	16	38	22	4.54	<.001	***	.56
$CS+_{late} - CS-_{late}$									
amygdala	L	31	−26	0	−28	3.33	.024	*	.43
	R	25	20	−4	−10	2.93	.077	†	.38
caudate	L	491	−8	4	4	5.75	<.001	***	.67
	R	514	12	12	−2	5.50	<.001	***	.65
dACC	L	1291	−10	12	38	7.24	<.001	***	.79
	R	1381	12	12	42	7.39	<.001	***	.80
hippocampus	L	45	−28	−38	0	3.02	.122		.39
	R	118	34	−30	−6	3.33	.053	†	.43
insula	L	853	−28	24	2	6.58	<.001	***	.74
	R	622	40	12	2	5.88	<.001	***	.68
NAcc	L	107	−14	14	−6	5.71	<.001	***	.67
	R	85	14	16	−6	5.29	<.001	***	.63
OFC	L	205	−14	16	−12	4.79	<.001	***	.59
	R	134	16	16	−12	5.03	<.001	***	.61
vACC	L	35	−12	34	28	3.42	.042	*	.44
	R	131	10	36	32	3.61	.022	*	.46

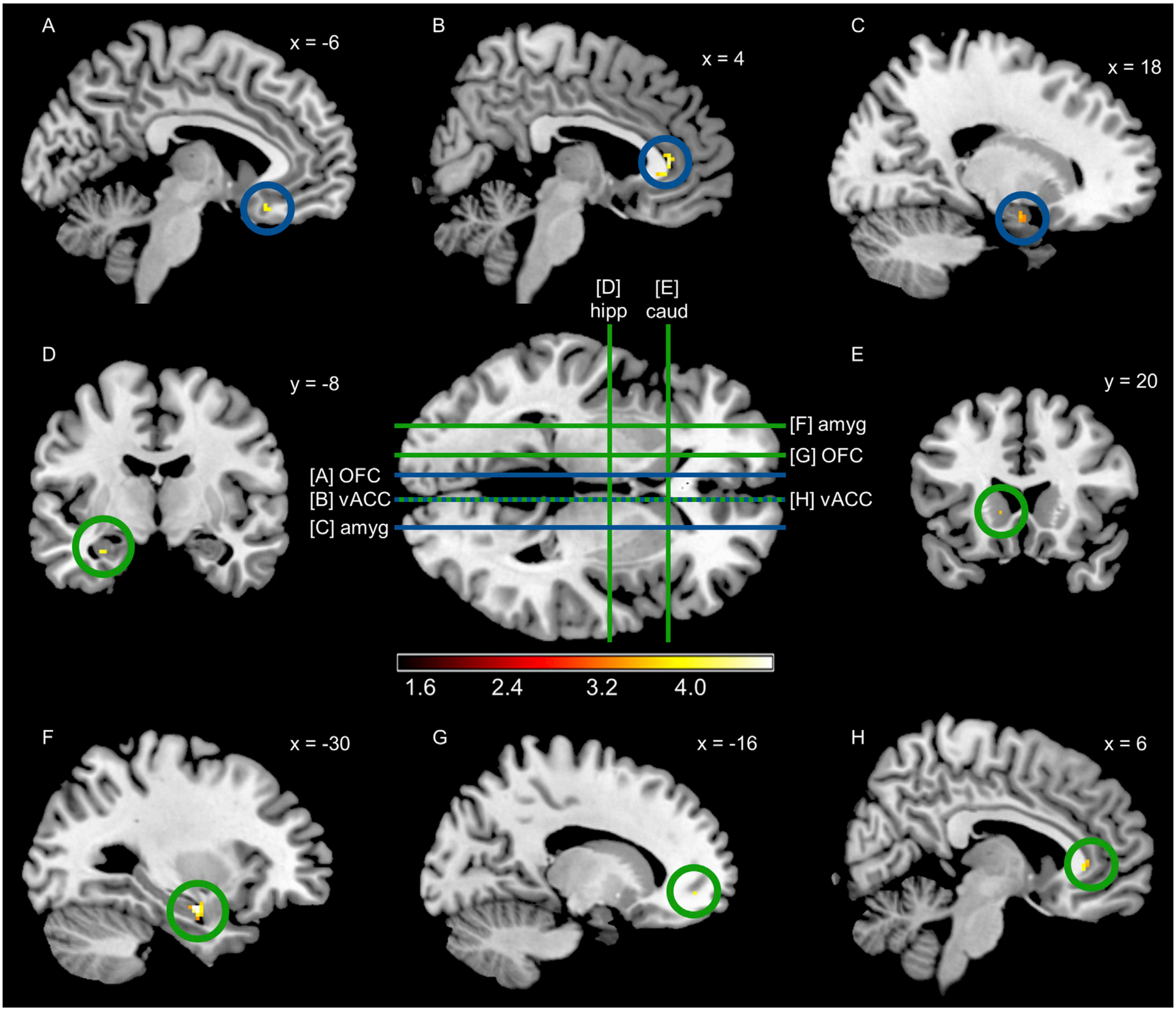

Fig. 5. Significantly increased differential BOLD in the control-group as compared to the stress-group. Upper row [A-C]: results for the early phase include left OFC, right vACC, and right amygdala (amyg) (peak voxels circled in blue). Middle and lower row [D-H]: results for the late phase include left hippocampus (hipp), left caudate (caud), left amygdala, left OFC, and right vACC (peak voxels circled in green). Center image: transversal plane (z = 0) denoting the intersecting planes of the results for the early phase (blue lines) and late phase (green lines). Display threshold is the respective $p < .05$ (FWE corrected) threshold per ROI.

Table 5
Region of Interest (ROI) results for the contrast control-group - stress-group in the contrast CS+ - CS- for early and late phase indicating structure, hemisphere (side), cluster size (k), x-/y-/z-coordinates, z-value, family wise error (FWE) corrected *p*-value (p_{corr}), and effect size (r). † = $p < .10$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

Control-group – Stress-group										
contrast	structure	side	k	x	y	z	z_{max}	p_{corr}		*r*
CS+ (early) – CS- (early)	amygdala	R	180	18	−2	−18	3.36	.027	*	.44
	OFC	L	341	−6	24	−16	3.62	.037	*	.47
	vACC	R	515	4	36	6	3.98	.007	**	.51
CS+ (late) – CS- (late)	amygdala	L	165	−30	−6	−18	4.28	<.001	***	.54
	caudate	L	379	−12	20	0	3.35	.038	*	.44
	hippocampus	L	242	−30	−8	−22	3.80	.012	*	.49
	insula	L	919	−42	4	−8	4.20	.005	**	.53
		R	81	42	−14	12	4.00	.010	**	.51
	OFC	L	191	−16	48	−6	3.55	.041	*	.46
	vACC	R	239	6	34	0	3.77	.013	*	.48

the early phase for right amygdala ($t(54) = 1.79$; $p = .079$; d = 0.48), as well as vACC left ($t(54) = 1.96$; $p = .055$; d = 0.52) and right ($t(54) = 1.88$; $p = .066$; d = 0.50).

Group differences in functional connectivity

In order to identify possible mechanisms behind the effect of stress psycho-physiological interactions with respect to the contrast of interest (CS+ - CS-) were performed with the left and right hippocampus as seed regions. Analyses revealed stronger connectivity in the stress-group between the left hippocampus and the left amygdala, the right OFC, and the left vACC (Fig. 6). Trends emerged in right amygdala, and the bilateral NAcc and dACC Table 6).

The right hippocampus also showed higher connectivity in the stress-group, with a significant difference in the right dACC, with trends in the bilateral NAcc. These results indicate overall higher hippocampal connectivity in the stress-group.

Table 6
Region of Interest (ROI) results for comparison of stress- and control-group in functional connectivity of the left and right hippocampus for the contrast CS+ - CS-, indicating structure, hemisphere (side), cluster size (k), x-/y-/z-coordinates, z-value, family wise error (FWE) corrected *p*-value (p_{corr}), and effect size (r). † = *p*<.*10;* * = *p* < .05; ** = *p* < .01; *** = *p* < .001.

structure	side	k	x	y	z	z_{max}	p_{corr}		*r*
stress-group – control-group (left hippocampus)									
amygdala	L	33	−18	−4	−12	3.21	.045	*	.42
	R	86	20	−4	−10	3.16	.056	†	.41
dACC	L	619	0	−8	50	3.51	.055	†	.45
	R	364	10	6	34	3.42	.077	†	.44
NAcc	L	22	−6	12	−2	2.78	.067	†	.37
	R	17	6	14	−2	2.71	.068	†	.36
OFC	R	501	12	34	−14	3.73	.031	*	.48
vACC	L	301	−8	36	8	4.42	.002	**	.56
stress-group – control-group (right hippocampus)									
dACC	R	71	10	2	34	3.68	.034	*	.47
NAcc	L	12	−6	12	−2	2.88	.051	†	.37
	R	12	6	14	−2	2.65	.077	†	.36

Discussion

The aim of this study was the investigation of the effect of an acute social stressor on appetitive conditioning. Its main focus were altered neural correlates and the identification of possible mechanisms by analyzing functional connectivity. Our analyses first established the success of the experimental stress induction (increased cortisol reactivity in the stress-group) and that acquisition of appetitive conditioning was successful. On the level of subjective ratings both groups rated the CS+ as more positive and arousing and had significantly higher expectancy of reward following the CS+ compared to the CS-. Moreover, in both groups there were increased SCRs to the CS+ as compared to the CS-. Importantly, widespread neural activation to the CS+ as compared to the CS- was visible throughout the reward network, including amygdala, striatum, ACC and OFC, again in the stress-group as well as the control-group.

Effect on the behavioral level

In general, both groups successfully acquired conditioned responses to the CS+. Interestingly, although both groups displayed faster reactions to targets following the CS+, there were group differences in overall responding as well as responding to targets following the CS-. Specifically, the stress-group showed overall faster responses to targets, while both groups were fast enough to win an equal amount of times, which was ensured by the adaptive nature of the paradigm. Most importantly, over time the control-group selectively reduced its effort in responding to targets following the CS-. This was visible in slower reactions and more leave-outs to targets following the CS- during the late phase in the control-group. As the CS- was never paired with a reward, this can be seen as a flexible adaptation of the control-group to the circumstances of the task, which was reduced under acute stress. The results are similar to animal studies of appetitive conditioning under stress. Association of a CS+ with approach behavior led stressed animals to also approach a CS-, that was never paired with a reward (Bussey et al., 1997). In humans, however, previous studies mostly did not include a CS- or had no behavioral element, which the subjects were able to approach without punishment and which was not instructed beforehand as a cue never followed by a reward. In a Go-NoGo-task, participants under acute stress therefore failed to approach a Go-option, while showing no differences to the control-group with respect to inhibiting a response to the punished NoGo-option (Berker et al., 2016). The results are consistent in showing that the stress-group chose to exhibit the same behavior across cues, while the control-group adapted more flexibly. This is in line with theories assuming more generalization on a behavioral level as well as an increase of habitual behavior under acute stress (Pedraza et al., 2016; Schwabe and Wolf, 2011).

From a network perspective, it is assumed that acute stress leads to reduced activation of the executive control network, including prefrontal regions, in favor of increased engagement of the salience network (Hermans et al., 2014). In a Stroop-like task, this pattern of network-activation was observed in subjects showing faster responding

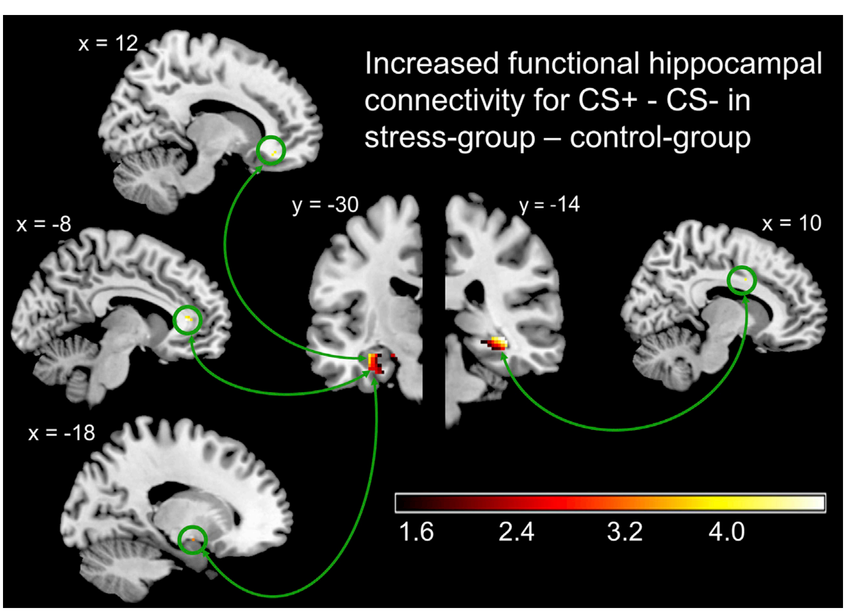

Fig. 6. Significantly increased functional connectivity for the contrast CS+ - CS- in the stress-group as compared to the control-group for the left hippocampus (left side) and the right hippocampus (right side). Functional connectivity of the left hippocampus in the stress-group is increased to right OFC, left vACC, and left amygdala. Functional connectivity of the right hippocampus in the stress-group is increased to right dACC. Display threshold is the respective *p* < .05 (FWE corrected) threshold per ROI.

with reduced accuracy (Kohn et al., 2017).

Effect on neural correlates of appetitive conditioning

On the neural level, greater differential activation in the contrast CS+ - CS- emerged in the control-group in both early and late phase of appetitive conditioning. Namely, there were differences in the amygdala, vACC, and OFC in both phases. Additional analyses showed that the stress-group showed increased responding to the CS- as compared to the control-group in the left OFC in the early phase with trends in right amygdala as well as bilateral vACC, and the left caudate, and right vACC in the late phase. Acute stress increases dopamine release as part of the sympatho-adrengergic stress response in the prefrontal cortex as well as the hippocampus, which presents a possible mechanism in shifting towards overgeneralization of learning (Grace, 2012; Kahnt and Tobler, 2016). Crucially, the group differences were also observed while controlling for cortisol reactivity. This underlines the possibility that it is not the cortisol effect that plays the main role in altered appetitive conditioning but rather early stress effects (e.g. dopamine release). For the associations with stress-induced cortisol-reactivity please see the supplement. Future studies therefore should assess the sympatho-adrenergic response in addition to cortisol reactivity and its effect on dopamine release.

The amygdala has repeatedly been identified to be a key region in classical conditioning (Chase et al., 2011). Within the framework of appetitive conditioning, it seems to encode the association of the CS+ with the UCR (Martin-Soelch et al., 2007). Reduced differential activation under acute stress might indicate impaired differential expression of the conditioned reaction. Viewed by itself, the reduced differential neural activation in the stress-group might also be explained by reduced acquisition of conditioning. Especially in humans, CS- or other additional cues provide context for the perception of the reward indicated by the CS+ (Nieuwenhuis et al., 2005), overgeneralization would possibly alter neural activation towards the CS- as well as the CS+. However, neither in subjective ratings, SCRs, nor reaction times the stress-group showed indicators of reduced acquisition of conditioning to the CS+. Behaviorally, the stress-group only differed in maintained responding to the CS-, a pattern which in animal studies has been identified as overgeneralization. In addition, in the control-group, but not in the stress-group, higher differential BOLD responses to the CS+ as compared to the CS- in the early phase was associated with increased leave-outs to the CS-, underlining the important role of the amygdala in the expression of appetitive conditioning.

In appetitive conditioning, the vACC is thought to be involved in discriminative processes (Gabriel et al., 2003; Parkinson et al., 2000), indicating impaired differential learning under acute stress. Animal findings show that a lack of differential vACC activation in appetitive conditioning leads to continued approach behavior to the CS- in addition to the CS+ (Bussey et al., 1997; Cardinal et al., 2003), which is in line with reduced differential responding as well as reduced differential activation of the vACC in the stress-group.

Under acute stress functioning of the OFC seems to be impaired (Born et al., 2010; Porcelli et al., 2012), resulting in increased habit learning independent of reward value (Schwabe et al., 2012). Besides the reduced differential OFC activation in the stress-group, during the late phase the caudate also showed a reduced differential BOLD response. As a key region in goal directed learning (O'Doherty et al., 2004), this underlines the possibility of reduced goal-oriented learning in the stress-group.

In the late phase, additional group differences emerged in the bilateral insula and the hippocampus with reduced differential activation in the stress-group. In contrast to the early phase, it can be assumed, that subjects have by now formed CS/UCS-associations. A spread of differential activation is in line with more pronounced behavioral differences in the late phase and indicates that the effect of stress does not slow learning but might rather set it on a different path that diverges increasingly.

For a long time, the insula has been thought to mainly play a role in aversive events. However, in line with a recent meta-analysis the insula seems to be involved in emotional processing in general (Sescousse et al., 2013). Moreover, we found reduced differential responding in the stress-group as compared to control-group in the late phase. This might indicate that CS+ and CS- were associated with more similar motivational salience in the stress-group as compared to control-group.

Importantly, a greater differential activation of the hippocampus emerged in the control-group during the late phase. The hippocampus is known as a key region in regulating the stress response via the HPA-axis but is also regarded as an important brain area for learning and memory (Wolf, 2003, 2009). It is thought to serve as a comparator of stimuli, performing pattern separation of CS+ and CS- and pattern completion to adaptively generalize associative learning (McHugh et al., 2007; Treves and Rolls, 1994). In line with the present results, relatively reduced hippocampal activity seems to impair the specificity of learning and foster generalization (Kahnt and Tobler, 2016). Acute stress increases dopamine release in the prefrontal cortex as well as the hippocampus, which presents a possible mechanism in shifting towards overgeneralization of learning (Grace, 2012; Kahnt and Tobler, 2016). One of the few animal studies using a differential conditioning paradigm showed generalization of learning across conditioned stimuli under inhibition of the hippocampus (Yang et al., 2011). But although reduced neural differentiation is in line with overgeneralization of conditioning, it could also be interpreted as reduced learning that is only detected on a neural level, while conditioned reactions are still clearly visible in subjective ratings and SCRs. The neural results do, however, support the assumption of overgeneralization based on the behavioral and translate neural correlates of overgeneralization, that have been identified in animal studies.

Effect on functional connectivity

In addition to significant differences between groups in differential BOLD responses in the hippocampus, functional connectivity analyses with the hippocampus as seed region revealed increased connectivity under acute stress. The pattern of increased hippocampal connectivity includes the amygdala, dACC, vACC, OFC, as well as trend in the NAcc. As several of these areas show reduced differential responding in the stress-group, increased connectivity might point toward inhibitory signaling under acute stress (Giustino and Maren, 2015; Li et al., 2015). Increased functional connectivity between prefrontal and limbic regions has under stress has previously been associated with reduced goal-directed-choice (Maier et al., 2015). Previous studies identified changes in connectivity between the hippocampus and several of these regions as relevant for differential learning and, most importantly, generalization of learning (Dunsmoor and Paz, 2015; Lopresto et al., 2016; Hermann et al., 2016). In animal studies the amygdalo-hippocampal pathway has been implicated in the generalization of fear learning from the CS+ to the CS- (Bergado-Acosta et al., 2008; Sangha et al., 2009). In humans, fear generalization was also associated with increased connectivity between amygdala and hippocampus (Lissek et al., 2014). Regarding prefrontal/hippocampal connectivity, Xu and Sudhof (2013) investigated connectivity in fear generalization. They identified a pathway between the hippocampus and the medial prefrontal cortex in rats (which in humans corresponds to, among others, vACC and OFC), as central for generalization of fear learning. From this central loop, additional pathways influence expression of associative learning via the amygdala, the caudate, and the midbrain.

Future directions and clinical implications

The effect of acute stress on neural networks involved in reward learning has received increasing attention in the recent years with studies reporting reduced as well as increased reward learning (Dillon et al., 2014; Morris and Rottenberg, 2015). While previous research already highlighted the importance of the timing of the stressor due to changes in the relative involvement of the salience network and the executive control network (Hermans et al., 2014), the present study highlights the possible overgeneralization of learning as a result of reduced prefrontal and hippocampal involvement. Learning took place shortly after the early sympatho-adrenergic stress response. Dopamine, whose release is increased under acute stress, has previously been reported as promoting overgeneralization (Hermans et al., 2014; Kahnt and Tobler, 2016; Lupien et al., 2007). For a better understanding of these effects, however, further studies are necessary that focus on possible molecular mechanisms of different parts of the stress response on these networks to integrate findings on altered network processing with findings on the effect of stress on specific regions.

Generalization of conditioning has long been a focus of clinically motivated research investigating the underlying mechanisms of psychiatric disorders. For example, in addiction, neutral context cues, that have never been directly paired with the consumption of e.g. the drug, lead to craving and induce consumption (Torregrossa et al., 2011). This overgeneralization seems to be related to altered dopamine levels, which have also been a main focus of schizophrenia research (Grace, 2012). Alterations in the reward circuit, possibly induced by stress hormones, have been in the focus of research investigating the development of various disorders such as addiction, depression, or schizophrenia (Bogdan and Pizzagalli, 2006; Grace, 2012; Sinha, 2008). This assumption, however, requires further investigation and transfer of fear generalization paradigms to appetitive conditioning. Moreover, future research should disentangle effects of generalization and habit learning in appetitive conditioning under acute stress.

Limitations

The present study induced acute stress using the well-established TSST. However, in contrast to pharmacological studies investigating the effect of cortisol, the subject cannot be blinded to the condition. Participants were instructed that the TSST was a separate experiment from the learning task, that were both designed to investigate effects of various traits on different tasks. The observed effects may however still be influenced by participants' expectancies about the experiment. Moreover, although the experimenters were not involved in administrating the TSST and did not know the research goals, they were not blinded to the participants' condition. Experimenter bias can therefore not be excluded as a contributing factor to the results. In addition, although men are at a higher risk of developing an addiction, further studies should include women to allow for a generalization of results and investigation of possible sex differences.

Conclusion

In sum, acute stress prior to the acquisition of appetitive conditioning did not impair the acquisition in general. However, it promoted overgeneralization of learning to the CS- on a behavioral level. From a network perspective, an important role comes to increased connectivity under acute stress between the hippocampus and other structures implicated in maintaining to specifity of conditioning, namely vACC and OFC, which show reduced differential activation under acute stress hinting at reduced activation of an executive control network. Moreover, the control-group showed increased differential activation of areas like the caudate, encoding goal-directed action, and amygdala, which was directly associated with reduction of effort towards the CS-. The present study suggests that acute stress promotes the pursuit of reward in the form of generalization to unrewarded stimuli. In the absence of negative consequences it might indeed be adaptive under acute stress to generalize appetitive conditioning instead of optimizing accuracy of learning. The present study suggests generalization of conditioning as a possible mechanism influenced by acute stress that can add to further understand its role in the development of psychiatric disorders.

Conflicts of interest

The authors declared that they had no conflict of interest with respect to their authorship or the publication of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2017.12.076.

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