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Die Effekte von Cannabis und Ecstasy auf die neurokognitive Hirnfunktion beim Menschen

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Zusammenfassung

Cannabis und Ecstasy (MDMA) sind die am häufigsten konsumierten illegalen Drogen, wobei sich besonders hohe Prävalenzraten unter jungen Erwachsenen finden. Tierexperimentelle Studien belegen das schädliche und potentiell neurotoxische Potential beider Substanzen. Bisherige Studien mit Konsumenten ergaben Hinweise auf Auffälligkeiten in der kognitiven Leistungsfähigkeit und der zugrundeliegenden kognitiven Hirnfunktion. Eine eindeutige Interpretation der Ergebnisse wird allerdings durch methodische Probleme bisheriger Studien erschwert. Ziel der hier vorgelegten Arbeit war es, mittels funktioneller Kernspintomographie (fMRT) zu untersuchen, ob der Konsum von Ecstasy und Cannabis zu nachhaltigen Veränderungen in der kognitiven Hirnfunktion beim Menschen führt.

In der ersten Studie wurde untersucht, ob der Konsum von Cannabis in der Adoleszenz zu Störungen in der normalen Hirnentwicklung führen kann. Dabei zeigten erwachsene Cannabis-Konsumenten mit adoleszentem Konsumbeginn relativ zu Konsumenten mit späterem Konsumbeginn erhöhte parietale Aktivierung während der Bearbeitung einer Arbeitsgedächtnisaufgabe. In einer zweiten Studie wurde untersucht, welchen spezifischen Einfluss das Einstiegsalter, die Konsumhäufigkeit und die Konsumdauer auf gedächtnisassoziierte hippokampale Funktion bei Cannabis Konsumenten haben. Hier zeigte sich ein spezifischer Zusammenhang zwischen höherer Konsumhäufigkeit und erhöhter parahippokampaler Aktivierung. In der dritten Studie wurde mittels eines prospektiven Längsschnitt-Designs der Effekt von Ecstasy auf die kognitive Hirnfunktion bei beginnenden Konsumenten untersucht. Hier zeigte sich bei Probanden, welche in den 12 Monaten zwischen den Messungen einen relevanten Konsum entwickelt hatten, eine relativ verminderte gedächtnisassoziierte parahippokampale Aktivierung.

Zusammenfassend ergaben sich bzgl. Cannabis: (1) Hinweise auf eine erhöhte Vulnerabilität des adoleszenten Gehirns für nachhaltige Effekte, (2) Hinweise auf spezifische Einflüsse der Konsumhäufigkeit auf hippokampale Auffälligkeiten bei Cannabis-Konsumenten. Bzgl. Ecstasy deuten die Ergebnisse (1) einen direkten Zusammenhang mit veränderter Hirnfunktion, (2) sowie eine erhöhte Vulnerabilität der Hippokampalen Formation an.

Summary

Cannabis and Ecstasy (MDMA) are among the most commonly used recreational illicit drugs in western industrial nations and prevalence rates are particularly high among young people. Findings from several studies with laboratory animals raised the concern that Cannabis and Ecstasy have the potential to induce neurotoxic effects. Previous studies with human recreational users suggest associations between the use of these substances and alterations in cognitive brain function. However, results have been inconsistent and most studies are confounded with methodological problems. The aim of this thesis was to investigate if the use of ecstasy and cannabis leads to sustained effects on cognitive brain function in humans by means of fMRI.

In the first study it was investigated whether the adolescent use of Cannabis leads to alterations in regular brain development. In comparison to users with a later onset, users who started to use cannabis during early adolescence showed increased superior parietal activity during working memory challenge. The second study addressed the question whether altered memory-related functioning within the hippocampal formation of cannabis users is associated with specific parameters of use (age of onset, duration of use, frequency of use). A selective association between a higher frequency of use and increased parahippocampal activity has been shown. The third study addressed the question whether the use of ecstasy leads to altered neurocognitive functioning in moderate recreational users by means of a prospective-longitudinal design. Participants who continued to use ecstasy in the 12 months between the baseline and follow-up measurement showed relative decreased memory-related parahippocampal activity at follow-up.

Summary: Regarding cannabis it was shown that (1) the developing brain might be more vulnerable to sustained effects, (2) the frequency of use has a particular critical impact on intact parahippocampal functioning in cannabis users. Regarding Ecstasy (1) a direct association with altered brain functioning and (2) a particular high vulnerability of the hippocampal formation was shown.

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1. Einleitung

Bereits seit Mitte der 1980er verdichten sich Hinweise aus tierexperimentellen Studien, welche auf ein neurotoxisches Potential von Ecstasy (3,4-Methylendioxymethamphetamin; MDMA und einige chemische Analoga) hinweisen (Capela et al., 2009). Wiederholt und in hoher Dosierung verabreicht führt MDMA zu einer selektiven und anhaltenden Veränderung serotonerger Systeme im ZNS von verschiedenen Spezies, einschließlich nicht-menschlicher Primaten (Green et al., 2003a, Capela et al., 2009). Histologische Untersuchungen zeigten, dass dieser Veränderung eine Degeneration und Zerstörung serotonerger Axonterminale zugrunde liegt. Die Relevanz der tierexperimentellen Daten für den menschlichen Wochenend-Konsumenten bleibt allerdings umstritten (De La Garza et al., 2007).

Aufgrund der potentiell schädlichen Auswirkungen von MDMA bleibt eine direkte experimentelle Überprüfung an menschlichen Versuchspersonen ethisch bedenklich, die Forschung hat sich daher auf mögliche Veränderungen serotoninassoziierter Funktionen bei Ecstasy-Konsumenten konzentriert. Der Neuromodulator Serotonin (5-HT) spielt eine wichtige Rolle bei der „Feinabstimmung“ vieler physiologischer, psychologischer und kognitiver Prozesse, wie bspw. der Regulation des Schlafes und zirkadianer Rhythmen, Vasokonstriktion, psychischem Wohlbefinden, Gedächtnis und Lernen. Als Folge serotonerger Schäden durch den Konsum von Ecstasy wären somit vielfältige funktionelle Störungen beim menschlichen Konsumenten denkbar. Eine in den letzten 15 Jahren stark angewachsene Zahl von Querschnittstudien ergab Hinweise auf spezifische kognitive Defizite bei Ecstasy-Konsumenten im Vergleich zu Kontrollprobanden (Gouzoulis-Mayfrank and Daumann, 2006a). Die konsistentesten Befunde zeigten sich dabei im Bereich Gedächtnis und Lernen, wohingegen die Befundlage bzgl. exekutiver Funktionen, wie bspw. Arbeitsgedächtnis und Planung deutlich uneinheitlicher ist (Gouzoulis-Mayfrank and Daumann, 2006a, Jager et al., 2007a, Schilt et al., 2008, Gouzoulis-Mayfrank and Daumann, 2009). Eine eindeutige Interpretation der Ergebnisse und somit ein Zusammenhang der Auffälligkeiten mit dem Konsum von Ecstasy wird allerdings durch methodische Probleme

bisheriger Studien erschwert (Lyvers, 2006, Kalechstein et al., 2007). Bei den bisherigen Untersuchungen handelt es sich größtenteils um Querschnittstudien, sodass die festgestellten Besonderheiten bereits vor dem Konsum bestanden haben könnten bzw. sogar eine Prädisposition für den Konsum darstellen könnten. Die wenigen Längsschnittstudien untersuchten zumeist starke Konsumenten, sodass auch hier Vergleichsdaten aus der Zeit vor dem Konsum fehlen. Zusätzlich ist in den letzten Jahren verstärkt ein Trend zum Mischkonsum festzustellen, so wird Ecstasy heutzutage zumeist in Kombination mit Alkohol, Cannabis und Amphetamin konsumiert (Smart and Ogborne, 2000, Gouzoulis-Mayfrank and Daumann, 2006a, EMCDDA, 2009). Bisher gelang es nur in wenigen Studien durch die Rekrutierung von „reinen" Ecstasy-Konsumenten (Daumann et al., 2003b) bzw. durch den Einsatz multipler Regressionsanalysen (Jager et al., 2008, Schilt et al., 2008) einen spezifischen Effekt von Ecstasy zu überprüfen. Gerade der weitverbreitete Begleitkonsum von Cannabis stellt eine unweigerliche Störvariable in vielen Studien zu möglichen Auswirkungen des Ecstasy-Konsums dar (Gouzoulis-Mayfrank and Daumann, 2006b, Parrott et al., 2007). Desweiteren bleibt die Generalisierbarkeit bisheriger Befunde ungeklärt. Probandenkollektive bisheriger Studien setzen sich zumeist aus starken Konsumenten zusammen, welche bereits mehrere hundert Ecstasy-Pillen über einen langen Zeitraum konsumiert hatten. Epidemiologische Studien deuten allerdings darauf hin, dass der typische Konsument einen wesentlich moderateren Konsum entwickelt und den Konsum spontan im Alter von Ende zwanzig einstellt (von Sydow et al., 2002). Schließlich bleibt anhand der vorliegenden Studien der Einfluss von weiteren, nahezu unweigerlich mit dem Ecstasy-Konsum einhergehenden Veränderungen im Gesundheitsverhalten, wie etwa veränderte Schlaf- und Ernährungsgewohnheiten, ungeklärt.

Um gesicherte Aussagen darüber zu treffen, ob die bei Ecstasy-Konsumenten festgestellten Auffälligkeiten nicht bereits vor Beginn des Drogenkonsums bestanden, oder ob begleitende Variablen des Gesundheitsverhaltens für einen Teil der Defizite verantwortlich gemacht werden können, sowie um die Entwicklung und den Verlauf der durch Ecstasy verursachten Veränderungen zu erfassen, wurde von der DFG die Prospektivstudie „Folgewirkungen von

Jugenddrogen beim Menschen“ (Studie 05-087) gefördert. Da der Begleitkonsum von Cannabis eine wichtige konfundierende Variable in Studien zu den Langzeitwirkungen von Ecstasy darstellt, sollten zusätzlich spezifische Auswirkungen von Cannabis auf Kognition und kognitive Hirnfunktion im Querschnitt-Design untersucht werden.

Die in dieser Arbeit vorgestellten Studien waren Teil dieses DFG-geförderten Projektes und befassen sich ausschließlich mit der Anwendung der funktionellen Magnetresonanztomographie (fMRT) zur Untersuchung möglicher Auswirkungen von Cannabis und Ecstasy auf kognitive Hirnfunktionen.

2. Hintergrund Cannabis und Ecstasy

2.1. Hintergrund Cannabis

Cannabis ist die in den westlichen Industrienationen und weltweit mit Abstand am häufigsten konsumierte illegale Droge (EMCDDA, 2007; OFDT, 2005; UNO WDR, 2006). Prävalenzraten für den Lebenszeit und aktuellen Konsum sind vor allem unter Jugendlichen und jungen Erwachsenen hoch, so gehen neuere epidemiologische Surveys in Deutschland von einer Lebenszeitprävalenz von 25% und einer 30-Tage Prävalenz von 8% unter Jugendlichen und jungen Erwachsenen im Alter von 12 bis 19 Jahren aus (BZgA 2007). Neben den potentiell negativen Auswirkungen des Gebrauchs als Rauschdroge wird in den letzten Jahren zunehmend (wieder) die therapeutische Anwendung von Cannabis bei einer Reihe von neurologischen und anderen Krankheitsbildern diskutiert (Wood, 2004, Ware and Beaulieu, 2005, Collin et al., 2007, Machado Rocha et al., 2008).

Die primär psychoaktiv wirksame Substanz in Cannabis ist Delta-9-Tetrahydrocannabinol (THC). Pharmakologisch akut wirkt THC als Ligand an den endogenen Cannabinoid 1 (CB1) Rezeptoren (Glass et al., 1997, Ameri, 1999). Die CB1 Rezeptoren sind heterogen im gesamten Gehirn verteilt, wobei insbesondere Cerebellum, Teile der Basalganglien, Hippokampus und viele Regionen des Neokortex eine besonders hohe Dichte an CB1

Rezeptoren aufweisen (Glass et al., 1997, Tsou et al., 1998). Die CB1 Rezeptoren und die assoziierten Endocannabinoid Transmitter scheinen eine wichtige Rolle bei der Modulation der Aktivität lokaler neuronaler Netzwerke zu spielen. Darüber hinaus belegt eine Reihe von Studien eine wichtige medierende Funktion des Cannabinoid Systems im Hippokampus bei der Begünstigung von Lernprozessen (Stella et al., 1997, Misner and Sullivan, 1999, Iversen, 2003).

Subjektiv geht der Konsum von Cannabis, v. a. bei erfahrenen Konsumenten, üblicherweise mit einem Gefühl der Euphorie, Entspannung und Depersonalisation einher (Johns, 2001, Green et al., 2003b, Iversen, 2003). Angst, Panik und psychotische Symptome werden teilweise, allerdings v. a. von unerfahrenen Konsumenten oder nach der Einnahme von hohen Dosen, berichtet (Green et al., 2003b).

2.2. Hintergrund Ecstasy

3,4-Methylendioxymethamphetamin (MDMA), besser bekannt als „Ecstasy“, ist die nach Cannabis am häufigsten konsumierte illegale Droge. Wie auch beim Cannabis finden sich besonders hohe Prävalenzraten für den Konsum von Ecstasy unter Jugendlichen und jungen. Wie beim Cannabis finden sich beim Ecstasy vor allem unter (EMCDDA, 2007; OFDT, 2005; UNO WDR, 2006). Epidemiologische Surveys in Deutschland gehen von einer Lebenszeitprävalenz von 5,4% und einer 12-Monats-Prävalenz von 1,9% unter Jugendlichen und jungen Erwachsenen im Alter zwischen 18 bis 24 Jahren aus (Reitox, 2008). In vielen westlichen Industrienationen ist der Konsum von Ecstasy eng mit Großveranstaltungen der Technoszene und der Disko- und Clubszene verbunden. In dieser Subpopulation finden sich entsprechend erhöhte Prävalenzraten, so wurde eine Lebenszeitprävalenz von 50% für Clubbesucher europäischer Metropolen (Tossmann et al., 2001) und eine 12-Monats-Prävalenz von 80% für Clubbesucher in Washington D.C. (USA) (Yacoubian et al., 2003) berichtet.

MDMA wurde 1912 erstmalig synthetisiert, 1914 durch die Firma Merck in Deutschland patentiert, allerdings niemals auf den Markt gebracht. Aufgrund seines entaktogenen (entaktogen: „das Innere berührend“) Wirkprofils wurde MDMA vom Chemiker Alexander Shulgin als ergänzendes Hilfsmittel in der Psychotherapie (Shulgin, 1986) vorgeschlagen und bis zu seinem Verbot 1985 von einigen wenigen Therapeuten genutzt (Greer and Tolbert, 1986, Grinspoon and Bakalar, 1986, Greer and Tolbert, 1998). Zu Beginn der 80er Jahre verbreitete sich der Gebrauch von MDMA als Rauschdroge durch die rasant wachsende Beliebtheit von Massentanzveranstaltungen mit elektronischer Musik (sog. Raves). Obschon die Popularität von Raves in den letzten Jahren stark abnimmt, hat sich der Gebrauch von Ecstasy zunehmend auch in die Diskotheken- und Club -kultur verbreitet, sodass sich Ecstasy auch heute noch ungebrochener Popularität erfreut. Auf der anderen Seite gibt es seit 2001 erste kontrollierte Studien zur therapeutischen Anwendungen von MDMA, zurzeit begrenzt auf die Indikationen einer posttraumatischen Belastungsstörung und schwerer Depression (Doblin, 2002, Check, 2004, Parrott, 2007).

Die pharmakologischen Akutwirkungen von MDMA sind gut untersucht. MDMA führt akut zu einer verstärkten Ausschüttung und Wiederaufnahmehemmung der endogenen Neurotransmitter Serotonin (5-HT) und, in weit geringerem Maße, Dopamin (DA) und Noradrenalin (NA) (de la Torre et al., 2000, Kalant, 2001, Green et al., 2003a). Die Zellkörper der zentralen serotonergen Neurone liegen eng beieinander in den Raphe Kernen des Mittelhirns und projizieren über ihre teils sehr langen Axone in nahezu alle Hirnregionen (Azmitia and Segal, 1978, Parent et al., 1981). Die dichteste serotonerge Innervation findet sich dabei im Hypothalamus, Septum, Striatum, Thalamus, Hippokampus und vielen Regionen des zerebralen Kortex (Jacobs and Azmitia, 1992, Leger et al., 2001). In zentralen Nervensystem (ZNS) ist Serotonin als Neuromodulator an vielen wichtigen funktionellen Systemen wie bspw. Schlaf, Schmerzempfinden, vegetativen Funktionen, neuroendokrinen Sekretionen sowie der Regulation kognitiver Prozesse beteiligt (Jouvet, 1967, Lucki, 1992).

Subjektiv geht der Konsum von Ecstasy zumeist mit positiv empfundener Nähe zu anderen Menschen, Angstfreiheit, Euphorie, Selbstakzeptanz und kommunikativer Offenheit einher (Cohen, 1995, Cami et al., 2000, Baylen and Rosenberg, 2006). Neben diesen emotionalen Effekten enthält das psychotrope Wirkspektrum von Ecstasy auch amphetamin-ähnliche und halluzinogene Effekte (Gouzoulis-Mayfrank et al., 1999). Allerdings werden auch vereinzelt negative Akuteffekte wie bspw. Angst, Unruhe und Verwirrung beschrieben (Greer and Tolbert, 1986).

3. Stand der Forschung

3.1. Cannabis und Kognition, kognitive Hirnfunktion

Eine steigende Anzahl wissenschaftlicher Publikationen berichtet von kognitiven Defiziten bei Cannabis-Konsumenten (Grant et al., 2003, Gonzalez, 2007, Solowij and Battisti, 2008). Im Vergleich zu Kontrollen zeigten chronische Konsumenten Beeinträchtigungen in den Bereichen episodisches Gedächtnis, Arbeitsgedächtnis, Aufmerksamkeit und exekutive Funktionen (Block and Ghoneim, 1993, Bolla et al., 2002, Solowij et al., 2002a, Solowij et al., 2002b, Messinis et al., 2006). Da die Hauptwirksubstanz des Cannabis, THC noch Tage bis Wochen nach dem letzten Konsum im Körper von chronischen Konsumenten nachweisbar ist (McGilveray, 2005), bleibt allerdings unklar, ob es bei den berichteten Defiziten um subakute und somit potentiell reversible oder um länger andauernde Folgewirkungen des Cannabis-Konsums handelt. Die Befundlage hierzu ist uneinheitlich, so berichten Studien mit einer Abstinenzzeit von bis zu 28-Tagen teils von einer Normalisierung der Leistungsfähigkeit (Schaeffer et al., 1981, Pope et al., 2001a), teils von andauernden Defiziten (Bolla et al., 2002, Solowij et al., 2002b). Eine Metaanalyse von Grant (Grant et al., 2003) und ein aktuelles Review von Solowij und Battisti (Solowij and Battisti, 2008) finden die konsistentesten Befunde für Langzeiteffekte vor allem im Bereich Gedächtnis. Die kognitiven Defizite konnten in mehreren Studien mit spezifischen Parametern des Cannabis-Konsums

assoziiert werden. Zusammenhänge zwischen einer schlechteren kognitiven Leistung zeigten sich dabei v.a. mit einem früheren Einstiegsalter (Ehrenreich et al., 1999, Pope et al., 2003), einer längeren Dauer des Konsums (Solowij et al., 2002a, Messinis et al., 2006) und einer höheren Konsumhäufigkeit (Pope and Yurgelun-Todd, 1996, Pope et al., 2001b).

In den letzten Jahren wurden verstärkt Untersuchungen mittels funktioneller Magnetresonanztomographie durchgeführt, um die neuronalen Grundlagen der kognitiven Auffälligkeiten bei Cannabis-Konsumenten zu untersuchen (Quickfall and Crockford, 2006, Chang and Chronicle, 2007, Martin-Santos et al., 2010). Im Vergleich zu Kontrollen zeigten abstinente chronische Konsumenten trotz unauffälliger Leistung veränderte Hirnaktivierung bei einer Reihe kognitiver Funktionen, wie etwa assoziatives Lernen (Jager et al., 2007b, Nestor et al., 2008), Aufmerksamkeit (Chang et al., 2006) und Arbeitsgedächtnis (Kanayama et al., 2004, Jager et al., 2006). Zumeist wurden stärkere neuronale Aktivierung innerhalb der beteiligten neuronalen Netzwerke und/oder nicht primär aufgabenrelevanten Hirnregionen bei den Konsumenten von Cannabis berichtet. Im aktuellsten Review über die Auswirkungen von Cannabis auf Hirnaktivierung interpretieren Martin-Santos und Mitarbeiter (Martin-Santos et al., 2010) diese Hyperaktivität bei unauffälliger kognitiver Leistung bei Cannabis-Konsumenten als kompensatorischen Mechanismus im Sinne der „cognitive efficiency hypothesis“ (Vernon, 1983, Rypma and D'Esposito, 2000). Demnach benötigen die Cannabis-Konsumenten höhere neurokognitive Ressourcen, um eine Leistungsfähigkeit im normalen Bereich aufrecht zu erhalten.

Zusammenfassend erlauben die Ergebnisse funktioneller MRT allerdings zurzeit keine gesicherte Aussage über mögliche langfristige Auswirkungen des Cannabis-Konsums auf neuronale Hirnaktivierung. Unterschiede in der Methodik und der untersuchten Hirnfunktion erschweren eine Vergleichbarkeit der Ergebnisse unterschiedlicher Studien. Zusätzlich wird eine eindeutige Interpretation der Befunde durch methodische Schwächen bisheriger Studien erschwert. Erhöhte Psychopathologiewerte und vermehrter Konsum weiterer legaler und

illegaler Droge innerhalb der Gruppe der Cannabis-Konsumenten, stellen wichtige konfundierende Variablen dar.

3.2. Ecstasy und Kognition, kognitive Hirnfunktion

Aufgrund von tierexperimentellen Studien, welche ein neurotoxisches Potential von MDMA belegen, wuchs mit der wachsenden Popularität von Ecstasy in den 1980er Jahren die Besorgnis, dass ähnliche Schäden auch bei den menschlichen Wochenend-Konsumenten von Ecstasy auftreten könnten. Wiederholte und hohe Gaben von MDMA führen im Tierversuch zu einer neurotoxischen Degeneration serotonerger Axonendigungen in verschiedenen Hirnregionen, wobei die Schäden im präfrontalen Kortex und Hippokampus am ausgeprägtesten erscheinen (Ricaurte et al., 1988a, Ricaurte et al., 1988b, Battaglia et al., 1991, Callahan et al., 2001). In Folge der selektiv serotonergen Neurotoxizität kommt es zu einer Abnahme der 5-HT Konzentration und der Anzahl der 5-HT Rezeptoren und 5-HT Transporter im Hirngewebe, welche noch Jahre nach der letzten MDMA Gabe nachweisbar waren (Hatzidimitriou et al., 1999, Green et al., 2003a). Obschon unklar bleibt, inwiefern diese tierexperimentellen Ergebnisse direkt auf die menschlichen Wochenend-Konsumenten übertragbar sind (De La Garza et al., 2007), legen Studien aus dem Humanbereich mittels Messung der 5-HT bzw. 5-HT Metaboliten-Konzentration im Liquor, Messungen der 5-HT Transporterdichte und der postsynaptischen 5-HT Rezeptoren mit nuklearmedizinischen PET und SPECT Methoden (McCann et al., 1998, Reneman et al., 2002, Green et al., 2003a) nahe, dass das neurotoxische Potential von Ecstasy auch für den menschlichen Wochenend-Konsumenten von Bedeutung sein dürfte. Ergebnisse anderer Studien deuten allerdings an, dass es sich dabei um vorübergehende und zumindest in einigen Hirnregionen reversible Auffälligkeiten im 5-HT System handeln könnte (Reneman et al., 2001, Thomasius et al., 2006).

Aufgrund der Beteiligung von 5-HT an vielen funktionellen Systemen wären als Konsequenz serotonerg-neurotoxischer Schädigungen durch den Konsum von Ecstasy eine Reihe von Störungen in den Bereichen Psychopathologie, Kognition, neuroendokrine Sekretion und

Schlafregulation denkbar (Gouzoulis-Mayfrank and Daumann, 2006a). Allerdings weisen die Ergebnisse bisheriger Studien zumeist methodische Schwächen auf, welche die Interpretierbarkeit der Ergebnisse deutlich einschränken (Lyvers, 2006, Kalechstein et al., 2007). Die meisten Untersuchungen gibt es zu den Bereichen Psychopathologie und Kognition, wobei die Literatur insbesondere für den Bereich Psychopathologie inkonsistent ist. Konsistenter sind die Befunde hinsichtlich kognitiver Funktionen (Rogers et al., 2009). Zahlreiche Querschnitts- (Morgan, 1999, Gouzoulis-Mayfrank et al., 2000, Fisk et al., 2004, Wareing et al., 2004, Quednow et al., 2007) und einige Longitudinalstudien (Zakzanis and Young, 2001, Zakzanis et al., 2003, Schilt et al., 2007) berichten von subtilen kognitiven Defiziten bei moderaten bis starken Ecstasy-Konsumenten. Im Einklang mit aktuellen quantitativen und qualitativen Überblicksarbeiten (Kalechstein et al., 2007, Zakzanis et al., 2007, Gouzoulis-Mayfrank and Daumann, 2009) finden neuere prospektive Langzeitstudien des niederländischen NEXT Projektes mit hohen methodischen Standards (De Win et al., 2005) Ecstasy-spezifische kognitive Defizite primär im Bereich Gedächtnis und Lernen (Schilt et al., 2007, Schilt et al., 2008).

Ähnlich wie beim Cannabis wurden in den letzten Jahren zunehmend fMRT-Studien zur Erforschung der neuronalen Basis kognitiver Defizite und zur Klärung der potentiell neurotoxischen Auswirkungen des Ecstasy-Konsums durchgeführt. Die meisten Studien untersuchten dabei neuronale Aktivierungsmuster von Ecstasy-Konsumenten bei der Bearbeitung von Arbeitsgedächtnis- oder assoziativen Lernparadigmen (Daumann et al., 2003a, Daumann et al., 2004, Jacobsen et al., 2004, Moeller et al., 2004, Daumann et al., 2005, Jager et al., 2007a, Jager et al., 2008, Roberts et al., 2009). Während der Bearbeitung von Arbeitsgedächtnisaufgaben zeigten Ecstasy-Konsumenten im Vergleich zu Kontrollen dabei ein uneinheitliches Muster von teils stärkerer, teils schwächerer Aktivierung in diversen Hirnregionen einschließlich parietaler, frontaler, limbischer und temporaler Regionen (Daumann et al., 2003a, Daumann et al., 2003b, Jacobsen et al., 2004, Moeller et al., 2004). Allerdings fand eine aktuelle Untersuchung keine Effekte von Ecstasy auf arbeitsgedächtnisassoziierte neuronale Aktivierung unter Berücksichtigung des Konsums

anderer illegaler Substanzen wie bspw. Amphetamin und Kokain (Jager et al., 2008). Studien mit assoziativen Lernparadigmen erzielten etwas konsistentere Befunde, so zeigten Ecstasy-Konsumenten zumeist schwächere Aktivierung in (para-)hippokampalen Regionen (Daumann et al., 2005, Roberts et al., 2009), allerdings konnten diese Ergebnisse unter Berücksichtigung der Effekte anderer illegaler Rauschdrogen nicht bestätigt werden (Jager et al., 2008).

Zusammenfassend lässt sich die aktuelle Untersuchungslage zur Nachweisbarkeit neurotoxischer Effekte von Ecstasy mittels fMRT beim Menschen als lückenhaft und teils widersprüchlich bezeichnen. Die bisherigen Ergebnisse weisen zum großen Teil methodische Schwächen wie bspw. nicht kontrollierte präexistente Auffälligkeiten, polytoxikomane Konsummuster der Probanden und die Fokussierung auf die Subgruppe relativ starker Konsumenten und somit mangelnder Generalisierbarkeit, auf.

4. Funktionelle Magnetresonanztomographie

Seit den 1990er Jahren wurden neben den etablierten Verfahren (PET, SPECT, MEG, EEG) eine ganze Reihe von Verfahren zur Abbildung neuronaler Korrelate spezifischer Hirnfunktionen mittels funktioneller Magnetresonanztomographie (fMRT) entwickelt. Die am häufigsten angewandte und wohl einflussreichste Technik basiert auf einer hämodynamischen Reaktion als Epiphänomen neuronaler Aktivierung (Villringer and Dirnagl, 1995), auch bekannt als blood oxygenation level dependent oder BOLD Effekt (Kwong et al., 1992, Ogawa et al., 1992). Der Begriff fMRT wird daher häufig synonym mit dem Begriff BOLD Bildgebung verwendet (so auch im Weiteren der vorliegenden Arbeit).

4.1. Der BOLD Effekt

Der BOLD Effekt basiert auf den unterschiedlichen magnetischen Eigenschaften von oxygenierten und desoxygenierten Hämoglobin (Ogawa et al., 1990, Ogawa et al., 1992) und

erlaubt es, Blut selbst als endogenes Kontrastmittel für die funktionelle Bildgebung zu nutzen. Verantwortlich dafür ist das an Hämoglobin gebundene Eisen: oxygeniertes Hämoglobin besitzt diamagnetische Eigenschaften, wohingegen desoxygeniertes Hämoglobin weitestgehend paramagnetisch ist und zu lokalen Inhomogenitäten im lokalen Magnetfeld führt. Eine lokale Verstärkung der neuronalen Aktivität führt zu einer Steigerung der Metabolismusrate im betroffenen Gewebe, dadurch kommt es zu einer unmittelbaren lokalen Verringerung der relativen Konzentration oxygenierten Hämoglobins (initialer Abfall/ initial dip) und nach einer kurzen Latenz zu einer deutlichen lokalen Überversorgung mit oxygenierten Hämoglobin (hämodynamische Reaktion). Da der initiale Abfall zurzeit noch nicht reliabel zu erfassen ist, basieren fMRT-Studien auf der etwas verzögert eintretenden, ausgeprägteren und länger andauernden Überversorgung (der sog. Positive BOLD-Antwort). Diese eigentliche BOLD Antwort erreicht ihren Maximalwert dabei erst mit einer Verzögerung von 4-6 Sekunden, um nach weiteren 10-12 Sekunden auf das Ausgangsniveau zurückzukehren. Trotz der zeitlichen Ausdehnung scheint das BOLD Signal zeitlich aber der neuronalen Aktivierung gut zu folgen (Menon and Kim, 1999). Im Hinblick auf die Antwortlatenz besitzt die fMRT eine Auflösung von deutlich unter einer Sekunde (Formisano and Goebel, 2003). Zusammen mit der Fähigkeit der fMRT, die Aktivität von Neuronenpopulationen in der Größe von wenigen Kubikmillimetern abzubilden, erweist sich die fMRT somit hinsichtlich zeitlicher und räumlicher Auflösung der PET überlegen.

4.2. Anwendung der fMRT

Die Anwendung der fMRT erlaubt die Abbildung von neuronaler Aktivierung bei spezifischen induzierten Emotionen oder kognitiven Funktionen. Bei der Abbildung von Hirnaktivierung bestimmter kognitiver Funktionen wie bspw. Aufmerksamkeit, Lernen und Gedächtnis hat sich die fMRT in den letzten Jahren zur Hauptuntersuchungsmethode entwickelt (Raichle, 2001, Ramsey et al., 2002). Um die neuronale Aktivität zu ermitteln, wird zumeist die kognitive Subtraktionsmethode angewandt. Dabei wird der Unterschied zwischen einer Aktivierungsbedingung (Aufgabe) und einer Kontrollbedingung, welche der Ruhezustand

sein kann, verglichen. Häufig wird anstatt des Ruhezustands in der Kontrollbedingung eine zusätzliche Aufgabe gestellt, welche die gleichen sensomotorischen Aspekte (bspw. visuelle Wahrnehmung, motorische Aktivierungen) wie die Aktivierungsaufgabe enthält. Durch die anschließende Subtraktion der Kontrollbedingung können somit aufgaben-irrelevante Aktivierungen kontrolliert werden (Beutel, 2006). Zumeist wird die so ermittelte aufgaben-spezifische BOLD Antwort als neuronale Aktivierung interpretiert. Allerdings ist der tatsächliche Zusammenhang zwischen neuronaler Aktivität und dem fMRT Signal (der sog. Neurovaskuläreren Kopplung) wesentlich komplexer, sie umfasst zahlreiche vaskuläre, metabolische und neuronale Prozesse, von denen einige zum Teil wenig verstanden sind (Logothetis, 2002). Ergebnisse einiger eleganter Studien aus der Grundlagenforschung weisen darauf hin, dass die durch fMRT gemessene Hirnaktivierung dem lokalen Feldpotential und somit dem input in ein Neuronenensemble eher entspricht als den schnelleren Aktionspotentialen und somit dem Output eines Neuronenensembles (Logothetis et al., 2001, Logothetis, 2003).

4.3. fMRT in der Untersuchung substanzbedingter Folgewirkungen

Die fMRT erscheint für den Einsatz zur Untersuchung möglicher langfristiger Auswirkungen des Drogenkonsums sehr vielversprechend. Die fMRT ist nicht-invasiv und benötigt keine radioaktiven Substanzen und ist somit besonders für wiederholte Messungen geeignet. Bereits in einer Reihe vorheriger Studien wurden Unterschiede in neuronalen Aktivierungsmustern zwischen Kontrollen und Konsumenten verschiedener Drogen wie bspw. Cannabis (Jager et al., 2006, Jager et al., 2007b, Nestor et al., 2008), Ecstasy (Daumann et al., 2003a, Jacobsen et al., 2004, Daumann et al., 2005), Kokain (Hanlon et al., 2010, Moeller et al., 2010) und Methamphetamin (Salo et al., 2009, Kim et al., 2010) nachgewiesen. Somit erscheint die fMRT als geeignetes Verfahren, um drogenassoziierte Veränderungen in der Gehirnfunktion zu erfassen. Darüber hinaus ermöglicht die fMRT, Prozesse der Reorganisation und Kompensation in neuronalen Netzwerken darzustellen und könnte somit in der Lage sein, bereits subtile Auswirkungen des Konsums zu erfassen, bevor

messbare Defizite in der kognitiven Leistung offensichtlich werden. Ergebnisse aus Untersuchungen an klinischen Populationen mit neurodegenerativen Erkrankungen sprechen für eine höhere Sensitivität der fMRT zur Erfassung subtiler neurokognitiver Veränderungen im Vergleich zu traditionellen Leistungstests; so zeigten sich etwa veränderte neuronale Aktivierungen bei HIV Patienten bereits vor messbaren Leistungsdefiziten (Chang et al., 2001).

Beim Einsatz der fMRT ist allerdings auch eine Reihe von Einschränkungen zu berücksichtigen. Um Bewegungsartefakte in den fMRT-Daten zu minimieren, dürfen die untersuchten Personen weder sprechen noch zu starke Bewegungen ausführen. Zusammen mit der hohen Lautstärke des MRT-Scanners führt dies zu einer Begrenzung der Kommunikation und möglicher Reaktionen der Probanden während der Untersuchung. Darüber hinaus sind aus Sicherheitsgründen Personen von der Untersuchung ausgeschlossen, welche Kontraindikationen für eine MR Untersuchung aufweisen (Schwangerschaft, ferromagnetische Gegenstände im oder am Körper).

5. Fragegestellungen der vorgelegten Studien

Ziel der in dieser Dissertation vorgelegten Studien war es, potentielle Effekte der weitverbreiteten Jugenddrogen Cannabis und Ecstasy auf die kognitive Hirnfunktion mittels fMRT zu untersuchen.

Zunächst wurde untersucht, ob das Einstiegsalter in den Cannabis-Konsum zu spezifischen Auffälligkeiten in der arbeitsgedächtnisassoziierten Hirnfunktion bei erwachsenen Cannabis-Konsumenten führt. Weiterführend wurde untersucht, ob die individuellen Cannabis-Konsumparameter Einstiegsalter, aktuelle Häufigkeit des Konsums und Dauer des regelmäßigen Konsums spezifische Auswirkungen auf die bei Cannabis-Konsumenten berichteten Auffälligkeiten in der gedächtnisassoziierten hippokampalen Aktivierung haben.

Abschließend wurde in einer prospektiven Longitudinalstudie untersucht, ob der Konsum von Ecstasy zu Veränderungen in der arbeitsgedächtnis- und gedächtnisassoziierten Hirnfunktion führt.

6. Durchgeführte Studien

Die vollständigen veröffentlichten Studien bzw. das zur Veröffentlichung eingereichte Manuskript sind im Anhang dieser Arbeit eingebunden.

6.1. Spezifische Effekte eines frühen Einstiegsalters auf hirnfunktionelle Korrelate einer Arbeitsgedächtnisaufgabe bei erwachsenen Cannabis Konsumenten

Studien zu kognitiven Langzeiteffekten des Cannabis-Konsums berichten von spezifischen Defiziten in den Bereichen Arbeitsgedächtnis, assoziatives Lernen, Aufmerksamkeit und exekutive Funktionen (Bolla et al., 2002, Solowij et al., 2002b, Messinis et al., 2006). Ergebnisse einiger neurokognitiver Studien legen nahe, dass die Entwicklung und Persistenz dieser Leistungsdefizite vor allem bei Konsumenten auftreten, welche den Konsum im frühen und mittleren Jugendalter beginnen (Ehrenreich et al., 1999, Pope et al., 2003). Während der Adoleszenz führen progressive und regressive Veränderungen an den Nervenverbindungen im Gehirn zu einer Optimierung der kognitiven Leistungsfähigkeit (Spear, 2000, Gogtay et al., 2004, Sowell et al., 2004). Da das endocannabinoid System während dieser Periode fundamentale Prozesse der Gehirnreifung reguliert (Harkany et al., 2007), ist es denkbar, dass die Zufuhr exogener Cannabinoide durch den Konsum von Cannabis in dieser Periode zu Störungen in der normalen Gehirnentwicklung führen könnte und das jugendliche Gehirn während dieser Phase besonders vulnerabel für mögliche negative Folgen des Cannabis-Konsums ist.

Bisher gibt es keine funktionellen Bildgebungsstudien zu den Auswirkungen des jugendlichen Cannabis-Konsums auf kognitive Hirnaktivierung im Erwachsenenalter,

allerdings wurden in einer fMRT-Studie Zusammenhänge zwischen einem früheren Einstiegsalter und stärkerer aufmerksamkeitsassoziierter neuronaler Aktivierung berichtet (Chang et al., 2006).

Mit dem Ziel, mögliche Auswirkungen des jugendlichen Cannabis-Konsums auf neuronale Hirnaktivierung im Erwachsenenalter zu untersuchen, wurden in der hier vorgestellten Studie 26 erwachsene Cannabis-Konsumenten mit einem Einstiegsalter von unter 16 Jahren mittels einer fMRT Arbeitsgedächtnisaufgabe untersucht. Als Vergleichsgruppe in dieser Studie dienten 17 erwachsene Cannabis-Konsumenten mit einem späteren Einstiegsalter, allerdings ansonsten vergleichbaren Cannabis-Konsummustern. Die Wahl dieser Vergleichsgruppe ermöglichte es, direkt mit dem Konsum verbundene Störvariablen (wie bspw. subakute Effekte), als auch indirekt mit dem Konsum verbundene Störvariablen (bspw. Besonderheiten in Schlaf- und Ernährungsgewohnheiten) zu kontrollieren.

Trotz vergleichbarer Arbeitsgedächtnisleistung zeigte die Gruppe der Cannabis-Konsumenten mit einem frühen Einstiegsalter relativ zur Gruppe der Cannabis-Konsumenten mit einem späteren Einstiegsalter eine stärkere neuronale Aktivierung innerhalb des Arbeitsgedächtnisnetzwerkes. Eine weiterführende korrelative Analyse für das gesamte Studienkollektiv bestätigte den Zusammenhang zwischen einem jüngeren Einstiegsalter und einer stärkeren neuronalen Aktivierung im superioren Parietalkortex. Interessanterweise wiesen andere Cannabis-Konsumparameter wie bspw. die kumulativ konsumierte Gesamtmenge, aktuelle Konsumhäufigkeit oder die Abstinenzzeit keinen Zusammenhang mit der neuronalen Aktivierung auf.

Die Ergebnisse dieser Studie weisen darauf hin, dass das Gehirn während der Adoleszenz besonders vulnerabel für Auswirkungen des Cannabis-Konsums ist. Da die Konsumenten mit einem frühen Einstiegsalter erhöhte neuronale Aktivierung bei gleicher Leistung zeigten, können die Ergebnisse im Sinne einer suboptimalen kognitiven Effizienz als Folge des frühen Einstiegsalters gedeutet werden. Allerdings konnten alternative Erklärungen, wie etwa

die Möglichkeit, dass die festgestellten Unterschiede bereits vor dem Konsum bestanden, anhand der vorliegenden Daten nicht ausgeschlossen werden.

6.2. Gedächtnisassoziierte (para-)hippokampale Auffälligkeiten bei Cannabis-Konsumenten: spezifischer Zusammenhang mit der Konsumhäufigkeit

In der zweiten Studie sollte untersucht werden, ob die in früheren Studien berichteten funktionellen Auffälligkeiten im Hippokampus und Parahippokampus von Cannabis-Konsumenten bei Lern- und Gedächtnisaufgaben mit spezifischen Parametern des Cannabis-Konsums in Zusammenhang stehen.

Neurokognitive Studien berichten einen Zusammenhang zwischen kognitiven Leistungsdefiziten bei den Konsumenten von Cannabis und einem jüngeren Einstiegsalter (Ehrenreich et al., 1999, Pope et al., 2003), einer höheren Konsumhäufigkeit (Pope and Yurgelun-Todd, 1996, Pope et al., 2001b) und einer längeren Konsumdauer (Solowij et al., 2002b, Messinis et al., 2006). Nachdem in der ersten Studie ein Zusammenhang mit dem Einstiegsalter und arbeitsgedächtnisassoziierter neuronaler Aktivierung nachgewiesen werden konnte, sollte in der zweiten hier vorgelegten Studie untersucht werden, ob die individuellen Cannabis-Konsumparameter (1) Einstiegsalter, (2) aktuelle Häufigkeit des Konsums und (3) Dauer des regelmäßigen Konsums spezifische Auswirkungen auf die bei Cannabis-Konsumenten berichteten Auffälligkeiten in der gedächtnisassoziierten hippokampalen Aktivierung (Jager et al., 2007b, Nestor et al., 2008) haben. Die Klärung möglicher Zusammenhänge könnte es ermöglichen, besonders riskante Konsummuster und somit Populationen mit einem hohen Risiko für kognitive Folgewirkungen des Cannabis-Konsums zu identifizieren. Darüber hinaus können Zusammenhänge mit spezifischen Parametern des Konsums wichtige Informationen über die Persistenz der Folgewirkungen beinhalten. So schlagen bspw. Solowij und Battisti in ihrem aktuellen Review über die Folgewirkungen des Cannabis-Konsums auf Gedächtnisleistung (Solowij and Battisti, 2008) vor, Zusammenhänge mit der aktuellen Häufigkeit des Konsums als eher subakute und somit

reversible Auffälligkeiten zu interpretieren. Im Gegensatz dazu schlagen die Autoren vor, Zusammenhänge mit der Dauer des Konsums und der konsumierten Gesamtmenge als eher langandauernde und somit persistente Auffälligkeiten zu interpretieren.

Um die Frage nach möglichen Zusammenhängen zwischen gedächtnisassoziierten neuronalen Auffälligkeiten bei Cannabis-Konsumenten und spezifischen Konsumparametern zu klären, wurden in der zweiten Studie 42 Cannabis-Konsumenten mit einer hohen Variabilität in Einstiegsalter, Konsumhäufigkeit und Konsumdauer mittels eines assoziativen fMRT-Lernparadigmas untersucht. Auf Basis eines Mediansplits der Gesamtstichprobe wurden zunächst in drei Einzelvergleichen Konsumenten (1) mit längerer und kürzerer Konsumdauer, (2) einer größeren und geringeren Konsumhäufigkeit und (3) einem früheren und späteren Einstieg hinsichtlich Lernleistung und (para-)hippokampaler Aktivierung gegenübergestellt. Unterschiede in der (para-)hippokampalen Aktivierung zeigten sich dabei nur in der nach Konsumhäufigkeit gesplitteten Gruppe: Konsumenten mit einer höheren Konsumhäufigkeit zeigten im Vergleich zu Konsumenten mit einer geringeren Konsumhäufigkeit stärkere parahippokampale Aktivierung. Um die Effekte der drei Konsumparameter weiter zu separieren, wurde anschließend ein lineares Regressionsmodel mit den Konsumparametern als unabhängigen und Gedächtnisleistung und (para-) hippokampaler Aktivierung als abhängigen Variablen aufgestellt. Die Ergebnisse dieser Analyse bestätigten den spezifischen Zusammenhang zwischen einer höheren Konsumhäufigkeit und erhöhter parahippokampaler Aktivität, auch unter Berücksichtigung des Einstiegsalters und der Konsumdauer.

Zusammenfassend legen die Ergebnisse dieser Studie nahe, dass vor allem die aktuelle Konsumhäufigkeit einen spezifischen Einfluss auf die in vorherigen Studien berichteten parahippokampalen Auffälligkeiten bei Cannabis-Konsumenten hat. Die erhöhte Aktivierung kann im Sinne einer funktionellen Kompensation gedeutet werden, welche mit erhöhter Konsumhäufigkeit steigt, um die kognitive Leistung aufrechtzuerhalten. Bezüglich der Persistenz der Auffälligkeiten weist der Zusammenhang mit der aktuellen Konsumhäufigkeit

eher in Richtung subakute und somit wahrscheinlich reversible Effekte des Cannabis-Konsums auf parahippokampale Auffälligkeiten hin.

6.3. Folgewirkungen von Ecstasy auf kognitive Hirnfunktion: Hinweise auf eine erhöhte Vulnerabilität der hippokampalen Formation aus einer prospektiven fMRT-Langzeitstudie mit beginnenden Konsumenten

Tierexperimentelle Studien belegen ein neurotoxisches Potential von 3,4-Methylendioxymethamphetamin („Ecstasy“) (Green et al., 2003a, Capela et al., 2009). Bisherige Querschnitts- (Fox et al., 2002, Gouzoulis-Mayfrank et al., 2003) und neuere Longitudinalstudien (Schilt et al., 2007) mit menschlichen Konsumenten berichten von subtilen kognitiven Defiziten, vor allem im Bereich Lernen und Gedächtnis, als mögliche Folge des Ecstasy-Konsums. Zusätzlich konnten in den letzten Jahren in einer Reihe von fMRT-Studien Auffälligkeiten in der neurokognitiven Hirnfunktion von Ecstasy-Konsumenten nachgewiesen werden (Daumann et al., 2003a, Daumann et al., 2003b, Daumann et al., 2004, Jacobsen et al., 2004, Moeller et al., 2004, Daumann et al., 2005, Roberts et al., 2009). Allerdings lässt sich aufgrund des Querschnitts-Designs und methodischer Schwächen bisheriger Studien nicht ausschließen, dass die festgestellten hirnfunktionellen Auffälligkeiten nicht schon vor Beginn des Konsums bestanden oder ob der Begleitkonsum anderer Drogen, vor allem von Amphetamin und Cannabis, für einen Teil der Auffälligkeiten verantwortlich ist.

Um diesbezüglich gesicherte Aussagen zu treffen, wurden in der dritten hier vorgelegten Studie 43 junge Erwachsene aus einer Hochrisikogruppe für zukünftigen Ecstasy-Konsum, vor der Entwicklung eines relevanten Konsums (t1) und erneut nach einem 12-monatigen Follow-up (t2) mittels einer fMRT-Arbeitsgedächtnisaufgabe und einer fMRT-Lernaufgabe untersucht. Um konfundierende Effekte eines Begleitkonsum, psychopathologischer Auffälligkeiten und prä-existenter Unterschiede in der allgemeinen Intelligenz zu kontrollieren wurden zu beiden Messzeitpunkten die folgenden Störvariablen erhoben: (1) Aspekte des Cannabis-, Alkohol- und Nikotin-Konsums, diese wurden mittels eines strukturierten

Interviews erfasst, (2) die subjektiv empfundene Beeinträchtigung durch körperliche und psychische Symptome, diese wurden mittels der Symptom Checkliste SCL-90-R (Derogatis et al., 1973) erhoben und (3) die allgemeine Intelligenz, diese wurde mittels der nicht-sprachlichen Standard Matrizen von Raven (Raven, 2000) erfasst.

Zur Auswertung der Daten wurden die Probanden auf Basis des zu t2 berichteten Konsums in eine Experimental- und eine Kontrollgruppe eingeteilt. Probanden, welche während der Follow-up Periode einen regelmäßigen Konsum von Ecstasy und/oder Amphetamin entwickelt hatten (Kumulativkonsum im Follow-up Zeitraum > 5 Ecstasy Tabletten oder 5 Gramm Amphetamin) wurden der Experimentalgruppe zugewiesen. Probanden welche im Follow-up Zeitraum mit Ausnahme von Cannabis keine illegalen Drogen konsumiert hatten bildeten die Kontrollgruppe. In Bezug auf soziodemographische Faktoren, Psychopathologie sowie Alkohol- und Nikotinkonsum zeigten sich keine signifikanten Unterschiede zwischen den Gruppen. Zudem zeigten sich keine signifikanten Gruppenunterschiede bzgl. des Cannabis-Konsums während der Follow-up Periode.

Die Auswertung der fMRT-Daten erfolgte mittels eines Designs für wiederholte Messungen, wobei sich mögliche Effekte des zwischenzeitlichen Ecstasy-Konsums auf die funktionelle Hirnaktivierung in einem Interaktionseffekt zwischen Zeitpunkt (Baseline / Follow-up) und Gruppe (Konsumenten / Kontrollen) zeigen sollten. Ein solcher Interaktionseffekt zeigte sich im linken Parahippokampus während der Bearbeitung der assoziativen Lernaufgabe. Eine weiterführende Analyse der individuellen prozentualen Veränderungen des fMRT-Signals im linken Parahippokampus ergab, dass sich dieser Interaktionseffekt aus einer ansteigenden Aktivierung in der Kontrollgruppe und einer abfallenden Aktivierung in der Konsumentengruppe zum zweiten Messzeitpunkt zusammensetzte. Da ein Großteil der Probanden aus der Konsumentengruppe in der Zwischenzeit sowohl Ecstasy als auch Speed konsumiert hatte, wurde eine weiterführende Korrelationsanalyse mit verschiedenen Parametern des zwischenzeitlichen Ecstasy- und Amphetamin-Konsums (Gesamtmenge, höchste und durchschnittliche Einzeldosis) und Veränderungen in der parahippokampalen Aktivierung durchgeführt. Ein signifikanter Zusammenhang zeigte sich dabei ausschließlich

mit der Kumulativdosis Ecstasy: eine höhere zwischenzeitlich konsumierte Dosis war mit einer stärkeren Verminderung assoziiert.

Zusammenfassend legen die Ergebnisse dieser Studie nahe, dass es sich bei den in früheren Querschnittstudien berichteten hippokampal gefundenen Auffälligkeiten in der neurokognitiven Hirnfunktion von Ecstasy-Konsumenten (Daumann et al., 2005, Roberts et al., 2009) mit hoher Wahrscheinlichkeit um eine Folge des Ecstasy-Konsums handelt. Im Gegensatz dazu konnten Hinweise auf Auffälligkeiten im fronto-parietallen Arbeitsgedächtnisnetzwerk als mögliche Folge des Ecstasy-Konsums aus früheren Querschnittsstudien (Daumann et al., 2003a, Daumann et al., 2003b, Daumann et al., 2004, Moeller et al., 2004) nicht bestätigt werden. Möglicherweise handelt es sich bei diesen zuvor festgestellten Auffälligkeiten eher um Effekte des unter Ecstasy-Konsumenten weitverbreiteten Cannabis Co-Konsums, so zeigten etwa Cannabis-Konsumenten im Vergleich zu nicht konsumierenden Kontrollen abweichende neuronale Aktivierungsmuster in frontalen und parietalen Regionen während der Bearbeitung von Arbeitsgedächtnisaufgaben (Kanayama et al., 2004, Jager et al., 2006). Andererseits hatten die Konsumenten in der hier vorgestellten Studie im Vergleich zu bisherigen Studien mit Ecstasy-Konsumenten sehr wenig Ecstasy konsumiert. Somit ist durchaus denkbar, dass sich Auffälligkeiten in fronto-parietalen Regionen erst mit fortgesetztem Konsum manifestieren. In diesem Sinne würden die Ergebnisse der vorgelegten Studie erste hirnfunktionelle Belege für die aus neurokognitiven Studien (Fox et al., 2002, Gouzoulis-Mayfrank et al., 2003, Schilt et al., 2007) abgeleitete Hypothese einer höheren Vulnerabilität der Hippokampalen Formation für die neurotoxischen Schäden durch Ecstasy beim Menschen darstellen.

7. Zusammenfassende Diskussion

7.1. Zusammenfassende Diskussion Cannabis

In der ersten hier vorgelegten Studie wurde untersucht, ob der Konsum von Cannabis im frühen und mittleren Jugendalter zu Störungen in der normalen Hirnentwicklung führen kann, welche noch bei erwachsenen Konsumenten nachzuweisen sind. Dabei zeigten erwachsene Konsumenten mit einem frühen Einstiegsalter (vor dem 16 Lebensjahr) stärkere und ausgedehntere Aktivierung im superioren Parietalkortex im Vergleich zu Konsumenten mit einem späteren Einstiegsalter bei der Bearbeitung einer Arbeitsgedächtnisaufgabe. Dadurch, dass sich beide Gruppen in anderen Parametern des Cannabis-Konsums glichen, konnten in dieser Studie selektive Auswirkungen eines frühen Einstiegs in den Cannabis-Konsum auf kognitive Hirnfunktion untersucht werden. Im Rahmen von etablierten Befunden zur Rolle der endogenen Cannabinoide bei der adoleszenten Neuroentwicklung und –reifung deuten die Ergebnisse dieser Studie auf eine besonders hohe Vulnerabilität des adoleszenten Gehirns für die negativen Auswirkungen des Cannabis-Konsums hin. Konsumenten mit einem frühen Einstiegsalter scheinen bei arbeitsgedächtnisassoziierten kognitiven Prozessen auf ontogenetisch frühere und somit suboptimale Aktivierungsmuster zurückzugreifen. Die interferierenden Auswirkungen des adoleszenten Cannabis-Konsums auf die normale Hirnentwicklung und –reifung erscheinen dabei vor allem vor dem Hintergrund eines immer jüngeren Einstiegsalters in den Cannabis-Konsum besorgniserregend. Des Weiteren wurde in einer zweiten Studie untersucht, ob neben dem Einfluss des Einstiegsalters auch andere Parameter des Konsums einen spezifischen Einfluss auf die bei Cannabis-Konsumenten berichteten hirnfunktionellen Auffälligkeiten haben. Zum einen wurden in neurokognitiven Studien neben dem Einstiegsalter wiederholt Zusammenhänge zwischen der aktuellen Konsumhäufigkeit und der Dauer des Konsums sowie kognitiven Leistungsdefiziten berichtet. Zum anderen berichten einige neuere fMRT-Studien konsistent abweichende gedächtnisassoziierte hippokampale Aktivierungsmustern bei Cannabis-Konsumenten. Um den spezifischen Einfluss der Parameter (1) Einstiegsalter, (2) aktuelle Konsumhäufigkeit und (3) Dauer des Konsums auf abweichende gedächtnisassoziierte hippokampale

Aktivierung zu untersuchen, wurden 43 Cannabis-Konsumenten mit einer hohen Variabilität in den Konsumparametern mittels einer fMRT-Lern- und Gedächtnisaufgabe untersucht. In dem anschließend aufgestellten Regressionsmodell konnte ein selektiver und spezifischer Einfluss einer höheren aktuellen Konsumhäufigkeit auf erhöhte hippokampale Aktivierung nachgewiesen werden. Da keine Zusammenhänge mit der Lern- und Gedächtnisleistung gefunden wurden, können die Ergebnisse im Sinne einer funktionellen Kompensation gedeutet werden: mit steigender Konsumhäufigkeit muss gleichzeitig die funktionelle Kompensation steigen, um die kognitive Leistungsfähigkeit aufrechtzuerhalten. Gleichzeitig lassen sich die hippokampalen Auffälligkeiten aufgrund ihres selektiven Zusammenhangs mit der aktuellen Konsumhäufigkeit in Anlehnung an Solowij und Battisi (Solowij and Battisti, 2008) als eher subakute und somit reversible Effekte des Cannabis-Konsums interpretieren.

Zusammenfassend wurden in diesen beiden Studien erstmals spezifische Auswirkungen adoleszenten Cannabis-Konsums auf neurokognitive Hirnfunktion bei erwachsenen Konsumenten nachgewiesen. Darüber hinaus ergaben sich Hinweise darauf, dass spezifische Parameter des Cannabis-Konsums unterschiedliche Auswirkungen auf spezifische kognitive Hirnfunktionen und –regionen haben.

7.2. Zusammenfassende Diskussion Ecstasy

Aufgrund methodischer Schwächen und des Querschnitts-Designs bisheriger Studien war es bisher nicht möglich, gefundene Auffälligkeiten in der neurokognitiven Hirnfunktion bei Konsumenten von Ecstasy eindeutig als Folge des Ecstasy-Konsums zu interpretieren. So konnte bisher nicht eindeutig ausgeschlossen werden, dass die gefundenen Auffälligkeiten nicht bereits vor dem Konsum vorlagen und nicht auf den Begleitkonsum von Amphetamin oder Cannabis zurückzuführen sind. Um diese Fragen zu klären, wurden in der dritten hier vorgestellten Studie insgesamt 43 junge Erwachsene mit einer hohen Wahrscheinlichkeit für zukünftigen Ecstasy- und/oder Amphetamin-Konsum mittels eines Prospektivdesigns zunächst vor der Entwicklung eines relevanten Ecstasy-Konsums und erneut nach 12 Monaten mittels einer fMRT-Arbeitsgedächtnisaufgabe und einer fMRT-Lernaufgabe

untersucht. Im Laufe der Zwischenzeit entwickelten 17 Probanden dieser Hochrisikogruppe einen relevanten Ecstasy- und/oder Amphetamin-Konsum, 12 Probanden konsumierten keine illegalen Drogen mit Ausnahme von Cannabis und dienten als Kontrollgruppe. Dabei zeigte sich, dass die Probanden, welche einen relevanten Konsum in der Zwischenzeit entwickelt hatten, relativ zu den Kontrollen eine verringerte Aktivierung in der Hippokampalen Formation zum zweiten Messzeitpunkt zeigten. Mögliche Effekte des Cannabis Begleit-Konsums auf diesen Befund konnten nahezu ausgeschlossen werden, da beide Gruppen in der Zwischenzeit vergleichbare Cannabis-Konsummuster zeigten. Durch weiterführende korrelative Analysen konnte der Einfluss von Ecstasy und Amphetamin weiter separiert werden: lediglich für die Anzahl der zwischenzeitlich konsumierten Ecstasy Pillen zeigte sich ein signifikanter Zusammenhang mit der Veränderung in der hippokampalen Aktivierung.

Zusammenfassend deuten die Ergebnisse dieser Studie auf Ecstasy-spezifische Effekte auf die gedächtnisassoziierte hippokampale Aktivierung hin. Die Tatsache, dass Veränderungen nur im Bereich der hippokampelen Formation, nicht jedoch in neokortikalen Arealen auftraten, unterstützt die aus Studien zu kognitiven Leistungsdefiziten von Ecstasy-Konsumenten abgeleitete Hypothese einer höheren Vulnerabilität der hippokampalen Formation im Vergleich zu neokortikalen Regionen für die neurotoxischen Auswirkungen von Ecstasy.

Einschränkend muss erwähnt werden, dass aufgrund der Tatsache, dass in dieser Studie keine Leistungsdefizite festgestellt werden konnten und die Studie einen relativ kurzen Zeitraum von nur 12 Monaten umfasste, keine Aussagen über die Bedeutsamkeit dieser Veränderungen im Alltag der Konsumenten oder über die Reversibilität der Effekte getroffen werden können.

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9. Veröffentlichungen

Nachfolgen sind die Veröffentlichungen aufgeführt, auf denen diese Arbeit basiert.

Die darin zitierte Literatur ist im Anhang der jeweiligen Arbeit aufgeführt.

Titel:

The impact of early-onset cannabis use on functional brain correlates of working memory.

Autoren:

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Eigener Anteil an der Arbeit:

Die Untersuchung war Teil des DFG-Projektes „Folgewirkungen von Jugenddrogen beim Menschen“ und wurde von mir durchgeführt, ausgewertet und publiziert. PD Dr. Daumann erhob die fMRT-Daten, betreute die Auswertung der fMRT-Daten und unterstützte die Optimierung des Manuskript.

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The impact of early-onset cannabis use on functional brain correlates of working memory

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ABSTRACT

Cannabis is the most commonly used illicit drug. Prevalence rates are particularly high among adolescents. Neuropsychological studies have identified cannabis-associated memory deficits, particularly linked to an early onset of use. However, it remains unclear, whether the age of onset accounts for altered cortical activation patterns usually observed in cannabis users. Functional magnetic resonance imaging was used to examine cortical activation during verbal working memory challenge in (1) early-onset (onset before the age of sixteen; $n=26$) and (2) late-onset cannabis users (age at onset at least sixteen; $n=17$). Early-onset users showed increased activation in the left superior parietal lobe. Correlational analyses confirmed the association between an earlier start of use and increased activity. Contrariwise neither cumulative dose, frequency nor time since last use was significantly associated with cortical activity. Our findings suggest that an early start of cannabis use is associated with increased cortical activation in adult cannabis users, possibly reflecting suboptimal cortical efficiency during cognitive challenge. The maturing brain might be more vulnerable to the harmful effects of cannabis use. However, due to a lack of a non-using control group we cannot exclude alternative interpretations.

1. Introduction

Cannabis is the most commonly used drug in western industrial nations and prevalence rates are particularly high among adolescents and young adults (EMCDDA, 2007; Monshouwer et al., 2005; OFDT, 2005; UNO WDR, 2006). An increasing number of studies reported various cognitive impairments in heavy cannabis users. In particular, deficits were found in working and episodic memory, as well as in executive and attentional functions (Block and Ghoneim, 1993; Bolla et al., 2002; Messinis et al., 2006; Solowij et al., 2002). However, if these adverse effects persist with prolonged abstinence or are merely transient remains controversial. Some studies reported recovery with prolonged abstinence (Pope et al., 2001; Schaeffer et al., 1981), while others reported persisting impairments in several cognitive domains such as attention, working memory and executive functioning (Bolla et al., 2002; Grant et al., 2003; Solowij et al., 2002).

Given these contrasting results, several studies have addressed the question whether specific characteristics of use might account for a varying degree of the observed deficits (Bolla et al., 2002; Ehrenreich et al., 1999; Pope et al., 2003; Schwartz et al., 1989; Solowij et al., 2002). Converging lines of evidence suggest that the development and persistence of these deficits are particularly linked to the onset of use. It has been shown, that the initiation of use prior to the age of 16 or 17 leads to enduring deficits on specific attentional functions (Ehrenreich et al., 1999) and short-term memory (Schwartz et al., 1989). Furthermore, only users who initiated use before the age of 17 showed persisting impairments in several neuropsychological measures after 28 days of monitored abstinence (Pope et al., 2003). Together with a report on reduced cortical grey matter in early-onset users (Wilson et al., 2000) it might be hypothesized that, if regular cannabis use starts during early and middle adolescence, it might produce permanent or at least long lasting alterations in neurocognitive functioning.

Although fundamental cognitive abilities evolve during childhood, existing cognitive abilities refine throughout adolescence (Spear, 2000). Findings from longitudinal studies suggest that neurodevelopment accompanies cognitive development with parietal and prefrontal associative cortices, involved in higher-order cognitive functioning, maturing last (Gogtay et al., 2004; Sowell et al., 2004).

Abbreviations: ADHD, attention hyperactivity disorder; ANCOVA, Analysis of covariance; BA, Brodmann area; BOLD, blood oxygenation level-dependent; DLPFC, dorsolateral prefrontal cortex; EOU, early-onset cannabis users; FDR, False discovery rate; fMRI, functional magnetic resonance imaging; FWE, Family Wise Error; LOU, late-onset cannabis users; MDMA, 3,4-Methylenedioxymethamphetamine; SOA, stimulus onset asynchrony; SPL, superior parietal lobe; ROI, region of interest; THC, Δ9-Tetrahydrocannabinol; WURS, Wender Utah Rating Scale.

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Brain dynamic changes during adolescence are basically driven by complex interactions between the nervous system and gonadal steroid hormones (Sisk and Foster, 2004) and continue throughout adolescence and young adulthood (Casey et al., 2005; Gogtay et al., 2004; Sowell et al., 2004). Given that the endocannabinoid system regulates fundamental neuromaturational processes (Harkany et al., 2007) and cannabis affects multiple endocrine systems (Brown and Dobs, 2002), cannabis use during this vulnerable period might interfere with regular brain development.

To investigate the neural correlates of cannabis-associated cognitive impairments, a growing number of studies employed functional magnetic resonance imaging (fMRI). Chronic users usually display altered activation of brain networks associated with the specific cognitive domain despite normal task performance (Chang et al., 2006; Jager et al., 2006; Kanayama et al., 2004). In terms of spatial and verbal working memory, increased activation in the superior parietal cortex, prefrontal cortex and anterior cingulate regions, have been reported (Jager et al., 2006; Kanayama et al., 2004).

Bearing in mind adolescent neuromaturational processes and the suggested associations between an early initiation of cannabis use and the development of cannabis-associated cognitive deficits, it, hence, might be of special interest if the age of onset accounts for altered cortical activation patterns in cannabis users. The purpose of this study was to clarify whether an early onset of cannabis use is linked to altered neural activity during working memory challenge.

2. Methods

2.1. Participants

Subjects in the present study were part of a larger study on the effects of drug use on neurocognition. Participants were examined with an extensive neurocognitive test battery and fMRI. Findings from the neurocognitive test battery for the entire sample will be published in a separate report. For the fMRI study, subjects were included if they reported a minimum cannabis lifetime usage of 10 g. All subjects were required to be at least 18 years old and right-handed. Exclusion criteria were: (1) any current or previous axis I psychiatric diagnosis (except for cannabis abuse), (2) childhood diagnosis of attention hyperactivity disorder (ADHD), (3) regular use of all other illicit substances except for cannabis (more than five occasions), (4) history of alcohol abuse and/or dependence (according to DSM-IV criteria, APA, 1994), (5) regular intake of any medication, (6) intake of any legal or illegal psychotropic substances or medication except for cannabis seven days prior to testing, (7) consumption of cannabis on the day of the examination, (8) pregnancy, or (9) other known contraindications for MRI scanning. All subjects gave written informed consent and received remuneration.

Forty-three cannabis users were enrolled in the present study. To obtain information about the impact of the age of onset of cannabis use on cortical activation, we median-split the entire sample into two groups according to the age of onset of cannabis use: (1) early-onset users (EOU), who first used cannabis before the age of sixteen ($n=26$) and (2) late-onset users (LOU), whose age at first cannabis use was at least sixteen ($n=17$). Previous studies used a comparable age at first use to distinguish between early- and late-onset users (Ehrenreich et al., 1999; Pope et al., 2003; Schwartz et al., 1989).

2.2. Cognitive task

Subjects performed three verbal n-back tasks with increasing memory load. N-back tasks have been shown to reliably initiate working memory activation in healthy subjects and drug using populations (Daumann et al., 2003a,b, 2004; Owen et al., 2005). A blocked periodic design was used incorporating alternating active and control conditions. Six alternating control and active blocks (duration each: 30 s) were presented per n-back task. In each block a sequence of twelve single capital letters was visually presented, each for 2100 ms (SOA 2500 ms) by means of a prismatic mirror. The switch between control and active conditions was indicated by a shift in the colour of the presented letters. In all conditions participants were asked to respond by button press when the target letter appeared. In the control (0-back) condition the target letter was designated ("G"). In the three active conditions 1-, 2- and 3-back, the target letter was defined as any letter that was identical to the one presented in the preceding 1, 2, or 3 trials, respectively. Each of the three n-back tasks comprised the same quantity of correct responses. Total scanning time per n-back task was 3:09 min, total experiment time was 9:27 min. The three n-back tasks were separately introduced by a verbal instruction.

2.3. Procedure

All subjects underwent a structured interview according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). To exclude participants with childhood ADHD all participants completed the German version of the Wender Utah Rating Scale (WURS). The WURS retrospectively assesses childhood ADHD by self-reported symptom severity between the age of 8 and 10 (Ward et al., 1993). Participants were excluded if they exceeded the recommended cut-off score of 46 (Ward et al., 1993). In addition, we took a medical history and detailed history of drug use including the following parameters of cannabis use: (1) age of first use, (2) time since the last use in days, (3) average frequency of use measured by average days of use per month, (4) maximum days of use per month ever, (5) estimated cumulative lifetime dose, (6) average and (7) highest daily dose ever used, as well as (8) duration of regular use in months. Studies validating self-reported voluntary substance use found a high reliability of the reported drug quantity (Martin et al., 1988; Rothe et al., 1997). Randomly taken hair samples by the Institute of Legal Medicine of the University of Cologne confirmed the self-reported substance use. In addition, qualitative drug screens were performed on the day of the examination with urine samples for amphetamines, benzodiazepines, cocaine, methadone, MDMA and cannabis (enzyme-multiplied immunoassay, von Minden GmbH). Participants were allowed to smoke cigarettes ad libitum before MRI acquisition. Participants were part of a larger study and underwent an extensive neurocognitive test battery. For an overview, measures of verbal working memory, mental flexibility and motor speed for the fMRI subsample will be reported in the present paper. Verbal working memory performance was assessed using the Digit Span Backwards test (from the WAIS-R, German version; Tewes, 1991). To measure mental flexibility and motor speed the Trail Making test (Trails A and Trails B; Reitan, 1955) was administered. This test is frequently used for screening for cognitive impairments in substance abusing populations (see e.g. Roberts and Horton, 2001). In order to control for confounding variables intellectual functioning and the use of alcohol and nicotine were assessed. Current intellectual functioning was assessed by the Raven Standard Progressive Matrices (Raven, 2000). In addition the use of alcohol (frequency of alcoholic drinks per week during the previous year) and nicotine (cigarettes per week) was assessed by means of separate questions within the cannabis use interview. The study was in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee of the Medical Faculty of the University of Cologne.

2.4. Imaging parameters

MRI employing blood oxygenation level-dependent (BOLD) contrast was performed on a clinical 1.5 T Philips ACS NT Gyroscan (Philips, Eindhoven, The Netherlands) using a singleshot multislice T2* weighted gradient echo EPI sequence (imaging parameters:

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60 volumes, TR: 3000 ms, TE: 50 ms, flip angle: 90°, matrix: 64×64, field of view: 192×192 mm, 30 contiguous slices parallel to the AC–PC line covering the whole brain, voxel size: 4×4×7 mm, no interslice gap). For each n-back task 60 dynamic scans were recorded. For anatomic reference and to exclude subjects with apparent brain pathologies, we obtained a T1-weighted Fast Field Echo sequence (imaging parameters: TR: 25 ms, TE: 4.6 ms, TI: 400 ms, flip angle: 30°, matrix 256×256, slice thickness: 2 mm). Images were acquired using a standard head coil.

2.5. Data analyses

Group differences for age, cannabis use and performance were analyzed by means of Student *t*-tests. In case the normality assumption was violated group differences were analyzed by means of non-parametric Mann–Whitney-*U*-test. Gender distribution was analyzed by means of χ^2 Fischer's Exact Test, differences in response latencies between the three n-back tasks were analyzed by means of repeated measures ANOVA using SPSS15 (SPSS Inc., Chicago, Ill). Imaging data were preprocessed and analyzed using SPM2 software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab7 (The Mathworks Inc., Sherborn, MA). To correct for motion-related variance components the scans obtained for each subject and each condition were initially realigned to the first image of each scan. All mean images were subsequently normalized with the SPM2 MNI template (resampled to $2\times2\times2$ mm^3 voxels), and smoothed with a Gaussian kernel (triple voxel size). Raw time series were detrended by the application of a high-pass filter (cut-off period: 128 s). For the following parameter estimation, an appropriate design matrix was specified using a boxcar function as reference waveform. Condition-specific effects in the three n-back tasks were assessed in a first level analysis by comparing the active condition (1-, 2- or 3-back) with their respective control condition (0-back) for each subject. To examine whether the paradigm led to significant cortical activation in the working memory specific fronto-parietal network, activation maps were obtained for each n-back task and examined using separate one sample *t*-tests. Minimum cluster size was set to 20 voxels, activations were considered significant if $p<.05$ (Family Wise Error (FWE) corrected for multiple comparisons). To examine whether cortical activity increased with increasing task difficulty, paired *t*-tests were computed to compare activity during the 1-, 2- and 3-back task ($p<.05$, FWE-corrected, cluster size>20 voxels). To explore differences between the groups, SPM2 group maps were generated using a random-effects model. Individual contrast images computed in the first level analysis were used to perform a two-sample *t*-test for each of the three n-back tasks. Given the exploratory nature of the present study and to improve statistical power, analyses were restricted to anatomically defined regions of interest (ROI). Based on findings from previous studies reporting differences in cortical activity during working memory challenge between cannabis users and controls (Jager et al., 2006; Kanayama et al., 2004), the analyses were restricted to the dorsolateral prefrontal cortex (DLPFC, anatomically defined as Brodmann areas 9 and 46) and the superior parietal lobe (SPL, anatomically defined as Brodmann area 7). Both the DLPFC and the SPL have consistently been associated to working memory processing (D'Esposito et al., 1998) and were reliably activated by a very similar verbal n-back paradigm in previous studies from our research team (Daumann et al., 2003a,b, 2004). ROIs were anatomically defined by use of the WFU Pickatlas (Version 2.4) which provides a method for generating ROI masks based on the Talairach Daemon database (see Maldjian et al., 2004; Maldjian et al., 2003; Tzourio-Mazoyer et al., 2002). The implemented atlases are in MNI space with dimensions of 91×109×91 sampled at 2 mm intervals, corresponding to the SPM MNI templates. ROI-based two-sample *t*-tests were computed with a threshold of $p<.05$ and corrected for multiple comparisons (Family Wise Error, FWE; cluster size>20 voxels), implemented in a small volume correction, based on the size of the ROIs. Finally to control for interference with subacute effects, ROI-based analysis was used to compare participants with and without positive urine screenings for THC (cut-off = 50 ng/ml).

To further explore associations between BOLD response and cannabis use characteristics, whole brain correlation analyses were performed ($p<.001$, uncorrected; cluster size>20 voxels). Separate analyses were computed for the following parameters of cannabis use (1) age of onset of use, (2) time since last use, (3) average frequency of use, (4) maximum days of use, (5) estimated cumulative lifetime dose, (6) average and (7) highest daily dose ever used, as well as (8) duration of regular use. Additionally, regressions were performed for age and years of education. Because MNI coordinates do not accurately match the brain of Talairach and Tournoux (1988), we used the Matlab function mni2tal by M. Brett for nonlinear transformation of MNI to Talairach coordinates.

3. Results

3.1. Demographics, drug use and cognitive performance

Sociodemographic data and patterns of cannabis use are given in Table 1. Both groups were similar in terms of education ($t=1.93$, $p=.061$) and gender distribution ($\chi^2=1.52$, $p=.281$), yet EOU were younger ($t=3.69$, $p<.001$). The groups were identical in the average number of cigarettes smoked per day ($t=.240$, $p=.811$) and the average frequency of alcoholic drinks consumed per week ($t=.345$, $p=.732$). Regarding cannabis, the groups showed comparable patterns of use in terms of lifetime dose, duration of regular use, average and highest daily dose ever used. However, EOU used cannabis more frequently ($t=-2.28$, $p=.028$). Group differences regarding the abstinence time were analyzed by means of Mann–Whitney-*U*-test. Results from this analysis indicated that EOU had a significant shorter abstinence period (Mann–Whitney-$U=142$, $p=.040$). Regarding the qualitative drug screening on the day of the examination all participants displayed a negative drug screen for the substances amphetamines, benzodiazepines, cocaine, methadone and MDMA. Groups differed significantly regarding the distribution of positive THC screenings (19 EOUs, 4 LOUs, $\chi^2=10.14$, $p=.002$). Analysis of cognitive performance data revealed no significant differences between EOU and LOU in the Digit Span Backwards ($t=1.31$, $p=.197$), Trail Making test Trials A ($t=.05$, $p=.962$), Trials B ($t=.07$, $p=.948$) and the number of errors in the Standard Progressive Matrices ($t=-1.50$, $p=.141$) (details are given in Table 2). To estimate the impact of subacute cannabis intoxication on cognitive performance participants with and without a positive THC screening ($n=23$ and $n=20$, respectively) were compared. Findings from this analysis revealed no significant differences (all $p>.14$).

3.2. Behavioral fMRI data

All participants correctly identified most of the targets in the control task (mean = 99.15%, ±2.26 SD) and performed the working memory tasks with a high degree of accuracy. The mean number of targets identified correctly by EOU and LOU did not differ significantly for the 1-back ($t=-1.23$, $p=.228$), the 2-back ($t=1.37$, $p=.179$) and the 3-back ($t=.75$, $p=.457$) conditions (Fig. 1). Results from repeated measures ANOVA indicated that the median response latencies for the three n-back tasks differed significantly (Wilks-Lambda, $p<.001$). Response latencies increased in accordance with the hypothesized increase in memory load with shortest reaction times for the 1-back condition and longest reaction times for the 3-back condition. Regarding the direct group comparison post-hoc independent samples *t*-tests indicated significant shorter response latencies for correct detections in the LOU during the 1-back condition ($t=2.30$, $p=.029$) (details are visually presented in Fig. 1). Again,

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Table 1
Demographic features and drug use of early-onset and late-onset cannabis user.

	Early-onset users (age of onset<16 years; $n=26$)		Late-onset users (age of onset≥16 years, $n=17$)		$t/\chi^2/U$	p
	Mean (±SD)	Median (range)	Mean (±SD)	Median (range)		
Characteristics						
Present age (years)	21.0 (±2.8)	20 (18–27)	24.5 (±3.4)	25 (18–30)	3.69	.001** (≤.001)
Gender (m:f)[a]	19:7		15:2		1.52	.281
Education (years)	14.2 (±2.44)	13 (11–19)	15.7 (±2.78)	16 (10.5–20)	1.93	.061
Cannabis use patterns						
Age of first use (years)	13.9 (±1.0)	14 (12–15)	17.0 (±1.5)	16 (16–21)	8.3	.000**
Lifetime dose (gram)	695.3 (±752.9)	580 (10–3640)	486 (±408.4)	450 (10–1250)	−1.05	.301
Duration of regular use (months)	53.7 (±41.1)	39 (3–144)	46.6 (±31.7)	48 (3–120)	−.61	.547
Days of use per month (average)	17.2 (±10.7)	17.5 (.5–30)	9.8 (±9.9)	6 (.5–30)	−2.28	.028*
Days of use per month (maximum)	24.6 (±8.6)	30 (4–30)	21.7 (±10.8)	30 (3–30)	−.94	.354
Average daily dose (joints)	2.3 (±1.6)	2 (.5–6)	2.5 (±1.8)	2.5 (.3–6)	.31	.758
Highest daily dose ever used (joints)	10.3 (±7.4)	10 (1–32)	9.3 (±8.0)	8 (.5–30)	−.43	.672
Time since last use[b] (days)	97.9 (±285.4)	1 (1–1280)	64.1 (±121.6)	7 (1–365)	142	.040*

t values were calculated using unpaired t-test; 2-tailed (df 41).
*$p<.05$.
**$p<.01$.
[a] Comparison tested with χ^2 Fischer's Exact Test (df 1); Exact Significance (2-sided) are reported.
[b] Comparison tested with Mann–Whitney-U-test; Asymptotic Significance (2-sided) reported.

we tested for the impact of subacute cannabis intoxication. Participants with and without a positive THC screening ($n=23$; $n=20$) did not differ significantly in the number of correct responses or response latencies in any of the three n-back tasks (all $p>.10$).

3.3. Functional MRI results

The well established n-back paradigm reliably activated the fronto-parietal working memory networks. Participants showed working memory associated activation (more response during active than during control blocks) in several regions including bilateral prefrontal and posterior parietal areas. These regions are often reported as being activated in n-back studies of normal individuals (D'Esposito et al., 1998; Cabeza and Nyberg, 2000; Owen et al., 2005). In line with the proposed increase in memory load the 1-back paradigm showed the lowest increase in BOLD response. Compared to the 1-back task, BOLD response increased during the 2-back task in bilateral superior frontal, middle frontal and superior parietal lobes. In contrast to previous studies (e.g. Callicott et al., 1999), additional memory load during the 3-back task did not lead to a further increase in BOLD response. Specifics of the n-back task used in the present study or cannabis use in the entire sample might explain contrasting results. However, due to the lack of a non-using control group in the present study, this cannot be further investigated.

3.4. Group comparisons: regions of interest analyses

After FWE-correcting for multiple comparisons, between-group comparisons restricted to the DLPFC revealed no significant differences in any of the three n-back tasks. Likewise, between-group comparisons restricted to the SPL revealed no significant differences for the 1- and 3-back task. During the 2-back task, however, EOU showed significantly greater BOLD response in the left SPL ($t=4.84$; cluster size = 30). The maximum t-value for this cluster was located in Talairach-space at $x=-20$ $y=-60$ $z=52$. Results from this analysis are visually presented in Fig. 2A. To control for the more frequent cannabis use in the EOU, an additional ANCOVA entering the average

Table 2
Cognitive Performance of early-onset and late-onset cannabis users.

	Early-onset users ($n=26$)	Late-onset users ($n=17$)
	Mean (±SD)	Mean (±SD)
Digit Span Backwards (digits)	7.1 (±1.9)	7.8 (±1.7)
Trail Making test (seconds)		
Trials A	28.9 (±1.5)	29.1 (±2.1)
Trials B	63.8 (±3.8)	64.2 (±3.5)
Progressive Matrices (errors)	10.0 (±8.0)	6.7 (±5.9)

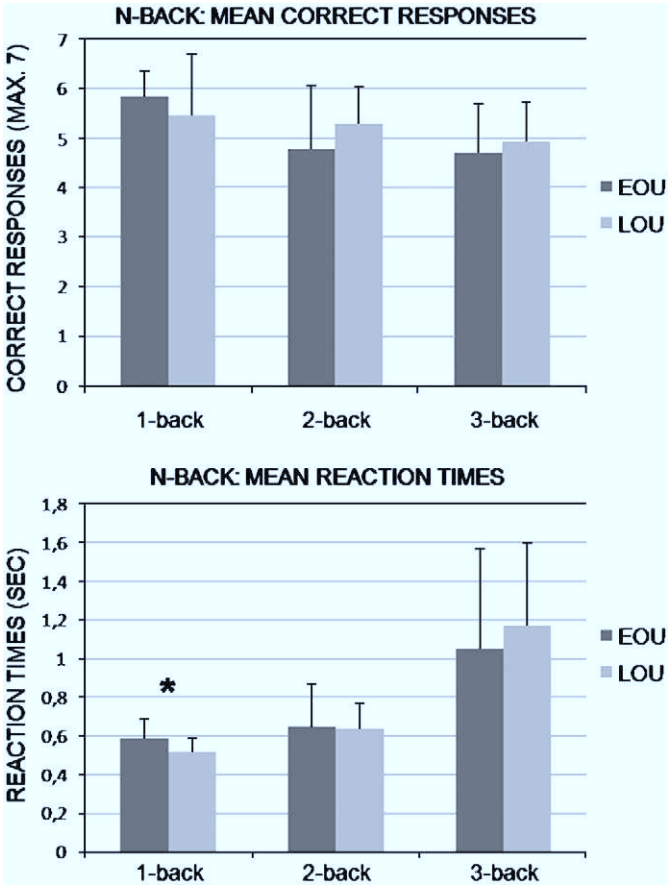

Fig. 1. Graphs showing behavioral performance during the n-back tasks. Mean number of targets correctly identified (±SD) by early-onset cannabis users (EOU) and late-onset cannabis users (LOU) and mean reaction times of correct responses on target trials (±SD) for both groups. (*) indicates significant ($p<.05$) faster responses.

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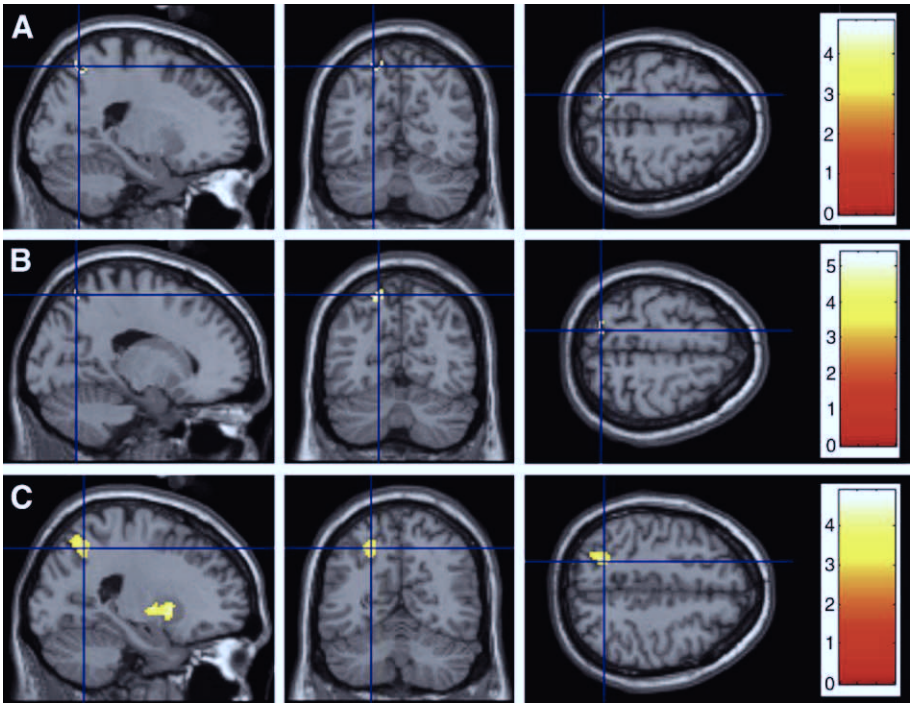

Fig. 2. Differences in BOLD response between early-onset cannabis users (EOU) and late-onset cannabis users (LOU). A) Group differences EOU − LOU in the superior parietal lobe ($p<.05$, FWE and small volume corrected; cluster size>20 voxels). B) Group differences EOU − LOU controlled for the frequency of use ($p<.05$, FWE and small volume corrected; cluster size>20 voxels). C) Simple regression between BOLD response and the age of onset ($p<.001$, uncorrected).

days of cannabis use per month as a covariate was performed. After controlling for the frequency of use EOU, continued to show greater BOLD response in the left SPL ($t=5.38$; cluster size = 37, maximum t-value located at $x=-16$ $y=-62$ $z=54$). Results from this analysis are visually presented in Fig. 2B. To control for the younger age of the EOU, an additional ANCOVA entering the current age as a covariate was performed. Again, EOU continued to show greater BOLD response in the left SPL ($t=4.86$; cluster size = 24, maximum t-value located at $x=-20$ $y=-56$ $z=49$). ANOVA and ANCOVAs exhibited a large overlap suggesting only marginal impact of the frequency of cannabis use and the current age on our imaging data. Because findings from a previous study (Chang et al., 2006) reported an association between cannabis-associated alterations in cerebellar activity and an earlier age of onset, we performed an additional analysis anatomically restricted to the cerebellum. Findings from this analysis revealed no significant ($p<.05$, FWE and small volume corrected; cluster size>20 voxels) differences between EOUs and LOUs. Finally to estimate the impact of subacute cannabis intoxication in the entire sample, users with and without positive THC screenings ($n=23$ and $n=20$, respectively) were compared. This analysis revealed no significant results.

3.5. Correlation analyses

To control for additional confounds, all cannabis use parameters, age and education were correlated with BOLD response during the tasks. However, in this exploratory whole brain analysis only the age of onset yielded significant ($p<.001$, uncorrected; cluster size>20 voxels) associations in the fronto-parietal working memory network (Table 3). An earlier start of use was associated with greater cortical activity in the inferior (right) and superior (bilaterally) frontal gyrus, the superior temporal gyrus (left), and the insula (left) for the 1-back task. During the 2-back task an earlier start of use was associated with increased activation in the precuneus spreading into the superior parietal lobe (left), the middle and inferior frontal gyrus (both left) and the right paracentral lobe (Fig. 2C). Apart from cortical activity an earlier onset was associated with increased activity in the left putamen ($x=-24$ $y=8$ $z=4$; $t=4.90$) (see Fig. 2C). No association between the onset of use and brain activity was found during the 3-back task. Additional correlational analyses between individual BOLD signal changes at the maximum of the superior parietal cluster ($x=-22$ $y=-56$ $z=47$) and the age of onset indicated that a stronger hemodynamic response was linearly associated to an earlier age of onset and that the age of onset accounts for approximately 29% of the variance ($R^2=.29$) in the BOLD signal change (Fig. 3).

4. Discussion

4.1. Summary of results

The aim of the present study was to clarify if the age of onset of cannabis use accounts for altered cortical activation patterns in cannabis users. We used fMRI to compare working memory performance and accompanied cortical activation patterns in early- and late-onset

Table 3
Results of a simple regression between age of onset and BOLD response in the n-back tasks ($p=.001$, uncorrected).

Age of onset and BOLD response	Brain region (Brodmann area)	Talairach coordinates			Cluster size (voxels)	Z score
		x	y	z		
1-back task						
1.	Inferior frontal gyrus (BA 45) R	48	25	1	125	4.77
2.	Superior frontal gyrus (BA 10) R	12	58	−10	27	4.22
3.	Superior temporal gyrus (BA 38) L	−53	15	−7	68	3.71
4.	Insula (BA 13) L	−32	13	−4	25	3.69
5.	Superior frontal gyrus (BA 8) L	−24	45	40	54	3.51
2-back task						
1.	Precuneus (BA 7) L	−22	−56	47	165	3.82
2.	Middle frontal gyrus (BA 9) L	−32	31	35	66	3.59
3.	Inferior frontal gyrus (BA 44) L	−50	16	10	33	3.49
4.	Paracentral lobule (BA 5) R	8	−42	57	44	3.39

Abbreviations: R: right hemisphere; L: left hemisphere.

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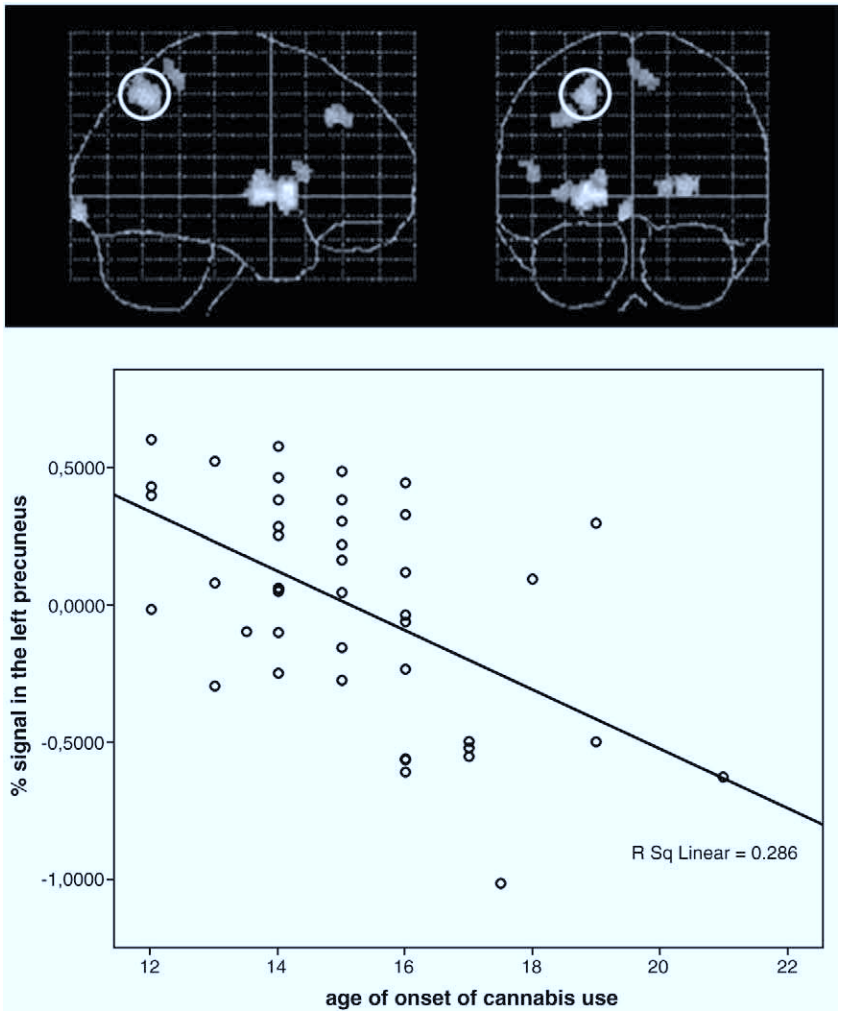

Fig. 3. Correlation of individual fMRI signal changes and the age of onset in the left precuneus.

cannabis users (EOU, LOU, respectively). Task performance and BOLD response were assessed during a verbal n-back task with increasing memory load (1-, 2- and 3-back). In terms of cognitive performance groups only differed in response latencies for the 1-back fMRI working memory task. LOU responded faster during this task condition, however during the more demanding conditions and in terms of accuracy the groups performed equally well. The overall pattern of changes in cortical activation was coherent with previous fMRI studies using n-back paradigms showing pronounced BOLD response in the frontal, parietal and occipital lobe (Cabeza and Nyberg, 2000; D'Esposito et al., 1998; Owen et al., 2005). Cortical activation increased from the first to the second levels of memory load and remained constant between the second and third levels. ROI analysis restricted to the DLPFC and the SPL indicated between-group differences in BOLD response in the SPL. In comparison to LOU, EOU displayed increased activity in the left superior parietal lobe. Differences remained stable after controlling for the frequency of regular cannabis use and current age. Further exploratory whole brain analysis indicated that among all parameters of cannabis use only the age of onset was significantly related to cortical activity within the working memory network, suggesting that an earlier start of use is associated with increased and more widespread activity during working memory processing.

4.2. Interpretation of findings

Taking into account the preliminary nature of the present study a number of possible reasons might account for the differences in BOLD response during working memory challenge in the early- and late-onset cannabis users. First, differences in cortical activity might reflect residual effects of subacute cannabis intoxication. The primary psychoactive component of cannabis Δ9-Tetrahydrocannabinol (THC) and its metabolites remain detectable in the body of frequent cannabis users several weeks after the last use (McGilveray, 2005). EOU in the present study displayed a significantly shorter abstinence period and compared to approximately one quarter (21%) of the LOU nearly three-quarter (73%) of the EOU displayed a positive qualitative THC screening on the day of the examination. An additional group analysis between participants with and without a positive THC screening revealed no interference with group differences in the task-related network, albeit between-group differences in the proportion of users with positive THC screenings might have skewed the results from this analysis. Further correlational analysis between the duration of abstinence and BOLD response revealed no significant results. However, the skewed distribution of the time since last use might have inhibited an appropriate estimation of its impact on cortical activity. The implementation of a supervised abstinence period at least several days before the examination combined with quantitative THC screenings might help to clarify this issue in future studies.

Second, given that EOU and LOU displayed comparable cannabis using habits and demographic features differences in cortical activity might be linked to the age of onset of use. Results from the ROI-based analyses indicate that hyperactivity in the left superior parietal cortex might reflect selective age of onset associated alterations. Moreover,

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findings from correlational analyses suggest that among the various parameters of use, in particular, the age of onset might have an impact upon working memory associated cortical activity and that an earlier start might be associated to increased parietal, frontal and dorsostriatal activities during working memory challenge. Several reports on altered memory and attention related neural activity in adult cannabis users have been published throughout the last years (Chang et al., 2006; Eldreth et al., 2004; Jager et al., 2006; Kanayama et al., 2004). However, to the best of the authors' knowledge to date only one study found associations between the age of onset and altered neural activity (Chang et al., 2006). In this fMRI study abstinent and current cannabis users showed decreased prefrontal, parietal and cerebellar response and increased response in several smaller frontal, parietal and occipital regions during a set of visual attention tasks. Remarkably, an earlier age of onset was related to increased cerebellar and decreased prefrontal activities. Additionally, a growing number of studies addressed memory performance and concomitant functional activity in cannabis using adolescents. Compared to age-matched controls adolescent users displayed altered neural activity during a wide range of cognitive tasks (e.g. Jacobsen et al., 2004, 2007; Padula et al., 2007; Schweinsburg et al., 2008; Tapert et al., 2007). Regarding working memory processing adolescent cannabis users displayed increased verbal memory related activity in posterior cortical regions during nicotine withdrawal (Jacobsen et al., 2007) as well as increased parietal and decreased prefrontal and occipital activities during spatial working memory challenge (Schweinsburg et al., 2008). In the latter study an earlier age of onset was related to decreased occipital activity. Findings from the present study confirm the association between early-onset cannabis use and hyperactivity within the working memory network. However, associations between an earlier onset of use and decreased cortical and cerebellar activities were not obvious in the present sample. The contrasting results might be attributable to different participant characteristics. Compared to participants studied by Chang et al. (2006) subjects in the present sample were younger, smoked cannabis less frequently and reported a shorter mean duration of use. In line with findings from a recent study, suggesting that the prolonged use of cannabis leads to different stages of neuroadaptation (Tapert et al., 2007) participants in the present study might be situated in an earlier stage of adaptive processing.

During this early stage prefrontal regions might still remain unaffected by cannabis use. In terms of cannabis using habits adolescent participants in the most recent study (Schweinsburg et al., 2008) were comparable to participants in the present study, however, had passed 28 days of monitored abstinence before the examination. In contrast, most of the participants in the present study (69%) had used cannabis within seven days prior to the examination. Based on their results Schweinsburg et al. (2008) suggested that neural recruitment changes throughout the course of abstinence. It, thus, might be suggested that an association between the onset of use and decreased activity develops as a consequence of withdrawal symptoms or a change in neurocognitive strategy with prolonged abstinence. Because of the relative short abstinence period in the present study interactions between an early onset of use and subsequent longer periods of abstinence might have remained undiscovered in the present investigation. Alternatively, the skewed distribution of the abstinence periods in the present sample might have interfered with an appropriate estimation of the impact of abstinence time on cortical activity.

4.3. Hyperactivity in early-onset users might reflect suboptimal cognitive efficiency

In contrast to LOU, EOU in the present study initiated cannabis use during early and middle adolescence. During this period several neuromaturational progressive (i.e. myelination) and regressive (i.e. synaptic pruning) changes promote increasing cognitive efficiency (Powell, 2008). The development of adult-level cognition involves increasing localization and functional specialization of cortical activity (Bunge and Wright, 2007; Konrad et al., 2005). Remarkably, adolescents have been shown to display more distributed activity throughout the parietal cortex during spatial working memory challenge, whereas adults recruit more local and functional specialized cortical networks (Scherf et al., 2006). The authors concluded that adolescents compensate for less integrated functional connectivity and less specialized computations by the recruitment of larger neural networks (Scherf et al., 2006). Given that the parietal lobes are among the brain regions that mature particularly late in ontogenetic neurodevelopment (Gogtay et al., 2004) and that the endocannabinoid system regulates fundamental neuromaturational processes (Harkany et al., 2007), EOU in the present study might have relied on more adolescent and, thus, suboptimal cortical activity patterns. However, brain dynamic changes during adolescence are affected by biological (i. e. maturation) as well as environmental (i. e. learning, experience) factors. Early-onset cannabis use might, therefore, have interfered with neuromaturational processes or, alternatively, might have led to less neural refinement due to a lack of environmental stimulation. Hyperactivity in the EOU was most pronounced in the (left) superior parietal lobe in the present study. This region has been proposed to be closely associated to focusing attention (Culham and Kanwisher, 2008; Osaka et al., 2007) and to support the central executive in working memory processes (Collette et al., 2006; Osaka et al., 2004). Differences in the amount of cortical activity in this region, thus, might, alternatively, be considered as evidence for different cognitive strategies or a difficulty in the EOU to focus attention.

4.4. Is the adolescent brain more vulnerable to the effects of cannabis use?

Findings from the present study suggest that the developing brain might be more vulnerable to the harmful effects of cannabis. Several reports on altered functional activity in adult and adolescent cannabis users provide support for this hypothesis. Studies investigating the sequelae of early-onset cannabis use reported age of onset related impairments most consistently in the domains of attention and working memory. An early study reported that beginning cannabis use before the age of 16 predicted dysfunctional visual attention (Ehrenreich et al., 1999). More recently in two electrophysiological studies, specific attentional dysfunctions in early users have been reported. Compared to controls cannabis users displayed altered steady state visual evoked potentials (Skosnik et al., 2006) and impaired information processing (Kempel et al., 2003). Increasing alterations were associated to an earlier start in both studies. Further support comes from several animal studies. In rats residual learning impairments have been reported after chronic exposure to cannabis during adolescence, yet not during adulthood (Stiglick and Kalant, 1985). More recent animal studies, directly comparing adolescent and adult rats, reported cognitive deficits specific to adolescent onset exposure. Working memory and social interaction impairments were observable in adolescent but not in adult rats treated with the cannabinoid agonist CP55,940 (O'Shea et al., 2004). Finally rats exposed to Δ9-Tetrahydrocannabinol in either adolescent or adult development stages showed residual impairments in object recognition only if treatment was applied during adolescence (Quinn et al., 2003). Taken together, these findings suggest that beginning cannabis use during early and middle adolescence might lead to enduring deficits in specific cognitive domains, most prominently attention and working memory. Our results are in line with these findings and suggest that among all parameters of cannabis use, starting to consume the drug during adolescence might, in particular, have a critical impact on neuropsychological functioning in later life. This view is supported by the fact that no other parameter of use, such as cumulative dose or frequency of use, was directly associated to cortical activity within the working memory network. If these early-

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onset related alterations persist during prolonged abstinence or just emerge in interaction with recent cannabis use remains unclear. Future studies incorporating prolonged abstinence periods are needed to address this issue. Differences in cortical activity were found only during the 2-back condition; however, not during the 1-back or the 3-back condition. The finding that no between-group differences became apparent for the less demanding 1-back condition might indicate that early-onset related cortical alterations only take effect at higher levels of working memory load. This would be in line with findings from previous studies suggesting that drug-related alterations in cortical activity only become obvious at higher levels of working memory load (e.g. Daumann et al., 2004). The finding of no between-group differences in the most demanding 3-back task might be explained in terms of a ceiling effect. Working memory has been defined as a system of limited capacity (Baddeley, 2000). Findings from neuroimaging studies suggest capacity-constrained responses in the DLPFC and parietal areas (Callicott et al., 1999). During the 3-back task both groups might have reached their capacity limits. The task might have been too difficult to detect subtle between-group differences.

4.5. Limitations

Findings from the present investigation should be regarded as preliminary and interpreted with several limitations in mind. First, because the present study did not incorporate a non-using control group we cannot exclude the possibility that EOU showed normal cortical activity, whereas LOU displayed decreased levels of activity within the context of unimpaired performance. In the context of functional compensation this finding would suggest increased working memory efficiency in the LOU. This hypothesis, however, seems unlikely, since it would be contradictory to several reports on increased activity during working memory challenge in drug using populations (e.g. Daumann et al., 2003a; Jager et al., 2006) and on neuropsychological impairments in cannabis users (e. g. Bolla et al., 2002; Messinis et al., 2006; Solowij et al., 2002). Future studies incorporating a non-using control group or a prospective design will be needed to adequately address this issue. Second, differences between groups may alternatively be associated to task-unrelated differences in regional blood flow. Compared to controls cannabis users have demonstrated increased blood volumes in frontal, temporal and cerebellar regions (O'Leary et al., 2002; Sneider et al., 2006). In chronic cannabis users alterations remain detectable after a month of abstinence (Herning et al., 2005). Between-group differences in cannabis-associated blood flow abnormalities, possibly due to differences in the age of onset or the frequency of cannabis use, could have affected the amplitude of the observed BOLD response. Third, differences in cortical activation patterns between EOU and LOU might have preceded the initiation of use. Due to the cross-sectional study design we cannot exclude this alternative explanation of the present findings. Certain subject characteristics may have an impact on the age of onset of cannabis use. For example, EOU might comprise a subgroup of users with better cognitive functioning and social skills necessary to engage in earlier cannabis use related behaviors. However, this hypothesis seems unlikely, since findings from previous studies (Ehrenreich et al., 1999; Pope et al., 2003) suggest lower cognitive functioning in users with an earlier onset compared to users with a later onset. However, to fully adjust for premorbid differences prospective data are needed. However, a comparison of EOU and LOU with comparable cannabis use patterns might help to control for confounding variables like e.g. the cannabis-associated lifestyle (e.g. altered nutrition and sleep habits). Fourth, in previous studies, early-onset cannabis use and early-onset alcohol and nicotine use were highly interrelated (e.g. Martin et al., 1996) making it difficult to disentangle the effects of the substances. Differences reported in the present study, thus, might reflect rather general effects of early-onset substance use than selective effects of early-onset cannabis use. Moreover, early-onset substance use is highly interrelated with ADHD and conduct disorder. In the present study participants with ADHD were excluded; however, conduct disorder was not specifically assessed. To control for potential confounders future studies should consider the exclusion of participants with conduct disorder. Fifth, a general problem in investigations examining cannabis users is the fact that varying potencies and variable methods of inhaling make the estimation of parameters such as cumulative lifetime dose little reliable. However, our experience is that the age of first use is well remembered by most participants. Finally we cannot exclude that (sub-)acute nicotine effects might have affected cortical activity. Acute nicotine administration has been shown to increase BOLD response in superior frontal and superior parietal cortices during working memory challenge (Kumari et al., 2003). Therefore, between-group differences in acute nicotine levels might have caused divergent BOLD response between EOU and LOU. Future studies should consider an appropriate abstinence time before scanning. However, this issue seems difficult to address, since prolonged nicotine abstinence might lead to withdrawal symptoms which in turn has an effect on cortical activity patterns.

5. Conclusion

Our findings suggest that an earlier start of cannabis use is associated with increased cortical activity in adult cannabis users. Among all cannabis use parameters the age of onset of use might have a particular critical impact on intact cognitive processing. However, based on the present results we cannot exclude that between-group differences that preceded the onset of use or differences in subacute cannabis effects might have caused between-group differences in cortical activity. Further studies are needed to confirm the impact of the age of onset of use.

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Titel:

Altered parahippocampal functioning in cannabis users is related to the frequency of use

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ORIGINAL INVESTIGATION

Altered parahippocampal functioning in cannabis users is related to the frequency of use

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Abstract
Rationale Converging lines of evidence suggest an association between cannabis use and impaired episodic memory as well as related associative learning. These deficits have been associated with the duration, frequency, and age of onset of cannabis use. However, it remains unclear whether these parameters of use differently impact memory-related hippocampal functioning.
Methods Forty-two cannabis users were examined by means of functional magnetic resonance imaging while they encoded and retrieved face–profession associations. Region of interest analysis was subsequently used to compare (para-)hippocampal functioning in users with (1) a longer and shorter duration of use, (2) a higher and lower frequency of use, and (3) an earlier and later onset. To further separate the effects of these parameters of use on performance and (para-)hippocampal activity, linear regression analysis was applied.
Results Compared to low-frequency users, high-frequency users displayed stronger blood oxygenation level-dependent response during encoding in the left parahippocampal gyrus. No differences were obvious for the groups separated according to duration of use or an earlier and later onset of use. Linear regression analysis confirmed the association between a higher frequency of use and increased activity in the left parahippocampal gyrus.
Conclusions Our findings suggest that the frequency of use might have a particular critical impact on intact parahippocampal functioning in cannabis users. Increased activity within the encoding-related network might reflect functional compensation to maintain cognitive functioning.

Keywords Cannabis · Marijuana · Memory · Cognition · Hippocampus · fMRI

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Introduction

Converging lines of evidence suggest an association between cannabis use and impaired cognition. In comparison to non-using controls, chronic cannabis users in the unintoxicated state primarily show deficient attention and memory function (Solowij and Battisti 2008; Messinis et al. 2006). More precisely, cannabis seems to selectively impair specific components of memory while leaving others relatively unaffected. In particular, deficits in working memory, episodic memory, and related associative learning have been reported (Indlekofer et al. 2008; Fried et al. 2005; Harvey et al. 2007). However, whether these impairments normalize with prolonged abstinence or reflect irreversible or at least long-lasting alterations in cognitive functioning is still at debate. It has been reported that some impairments normalize within 28 days of abstinence, and that the development of persisting deficits might be associated to an early onset of use (Pope et al. 2003). Findings from a meta-analysis investigating cannabis-associated long-term cognitive sequelae suggest marginal impairments in the domains of learning and forgetting, whereas impairments in other cognitive areas (e.g., atten-

tion) were not apparent (Grant et al. 2003). In general, deficits have been linked to specific parameters of use, in particular, to an earlier onset of use (Ehrenreich et al. 1999; Pope et al. 2003), a longer duration of use (Solowij et al. 2002; Messinis et al. 2006), and a higher frequency of use (Pope and Yurgelun-Todd 1996; Pope et al. 2001).

Given this pattern of cognitive performance with predominant memory impairments in cannabis users, it might be hypothesized that the function of the hippocampal formation, a region which is linked especially to memory processes, might be particularly vulnerable to the consequences of prolonged cannabis use. The medial temporal lobes have been consistently associated with memory encoding and retrieval (Schacter and Wagner 1999), and the hippocampal formation, in particular, has been thought to support encoding and creating new associations (Henke et al. 1997, 1999; Davachi and Wagner 2002). Interestingly, the hippocampus and the parahippocampal gyrus are among the brain regions with the highest density of cannabinoid (CB1) receptors (Herkenham et al. 1991; Tsou et al. 1998). Findings in laboratory animals suggest that the hippocampal formation mediates the effects of cannabinoids on learning and memory. For example, it has been shown that the cannabinoid agonist CP55,940 leads to deteriorated spatial working memory performance if applied directly into the hippocampal formation of rats (Lichtman et al. 1995). Findings from functional neuroimaging studies administering a wide range of tasks to investigate the neural correlates of cannabis-associated memory impairments provide further support. In comparison to non-using controls, frequent cannabis users displayed altered neural activity within the hippocampal formation up to 7 days after last use (Eldreth et al. 2004; Jager et al. 2007; Nestor et al. 2008). However, to date, no study has specifically addressed the question whether altered memory-related functioning within the hippocampal formation of cannabis users is associated to specific parameters of use. Relating altered neurophysiological functioning and associated neuropsychological capacities to specific parameters of cannabis use might help to understand the effects of cannabis on cognition, estimate the duration of these effects, and identify a subgroup of users who are at risk to develop relevant cognitive impairments. Therefore, in the present study, cannabis users with different using habits and a varying degree of cumulative lifetime exposure were recruited and examined using functional magnetic resonance imaging (fMRI). Participants were examined using an associative memory task, which has been shown to induce memory-related blood oxygenation level-dependent (BOLD) response, particularly in the hippocampal formation (Henke et al. 2003; Daumann et al. 2005). To estimate whether the age of onset, the duration, and the frequency of use account for a varying degree of altered (para-) hippocampal functioning, the entire sample was median split according to these parameters of use, resulting in three separate comparisons, namely, the comparison of (1) users with an earlier and later onset, (2) users with a longer and shorter duration of use, and (3) users with a higher and lower frequency of use. To further investigate the specific effects of these parameters on performance and (para-) hippocampal activity, linear regression analysis was applied.

Taking into account findings from two previous studies addressing associative memory and associated hippocampal functioning in cannabis users (Jager et al. 2007; Nestor et al. 2008), we expected that (1) groups would not differ in associative memory performance. However, regarding accompanying (para-)hippocampal activity, we expected (2) group differences during encoding, (3) but not during retrieval. Accumulating evidence from studies with drug-using and clinical populations suggests that neural-impaired subjects achieve normal performance through additional recruitment of larger neuronal areas during memory challenge (Callicott et al. 2000; Chang et al. 2001; Daumann et al. 2005). In line with this hypothesis on compensatory recruitment, we expected (4) that individuals with a more deleterious pattern of cannabis use (either a younger onset and/or a longer duration or a higher frequency of use) will show a stronger (para-)hippocampal activity compared to users with a less deleterious pattern of use (later onset, shorter duration, and lower frequency of use).

Materials and methods

Subjects

Forty-two cannabis users were enrolled in the present study. Subjects were included if they reported a minimum lifetime cannabis usage of 10 g. All subjects were required to be at least 18 years old and right-handed. Exclusion criteria were (1) any current or previous axis I psychiatric diagnosis (except for cannabis abuse), (2) childhood diagnosis of attention hyperactivity disorder (ADHD), (3) regular use of any other illicit substances except for cannabis (more than five occasions), (4) a history of alcohol abuse (according to Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) criteria, APA 1994), (5) regular intake of any medication, (6) intake of any legal or illegal psychotropic substances or medication except for cannabis 7 days prior to testing, (7) consumption of cannabis on the day of the examination, (8) pregnancy, or (9) other known contraindications for MRI scanning. All subjects gave written informed consent and received remuneration.

The mean age of the study sample was 22.5 (±3.5 SD) years; 21% were female (nine out of 42). Participants had a mean of 14.9 (±2.7 SD) years of education. Regarding

cannabis use parameters, participants first tried cannabis at the mean age of 15.1 (±2.0 SD) years, used cannabis on 14.2 (±11.0 SD) days per month, with an average daily dose of 2.4 (±1.6 SD) joints, for 51.3 (±37.8 SD) months, and had smoked a mean of 612.2 (±649.4 SD) g of cannabis in their lifetimes. While most participants were current cannabis users, eight (19.0%) had reported no cannabis use in the month before the fMRI investigation; the average self-reported time since last use was 86.52 (±235.66 SD) days.

Experimental procedure

All subjects underwent a structured interview according to the DSM IV. To exclude participants with childhood ADHD, all participants completed the German version of the Wender Utah Rating Scale (WURS). The WURS retrospectively assesses childhood ADHD by self-reported symptom severity between the age of 8 and 10 (Ward et al. 1993). Participants were excluded if they exceeded the recommended cut-off score of 46 (Ward et al. 1993). In addition, we took a medical history and detailed history of drug use, including the following parameters of cannabis use: (1) age of first use, (2) time since the last use in days, (3) average frequency of use measured by average days of use per month, (4) maximum days of use per month ever, (5) estimated cumulative lifetime dose, (6) average and (7) highest daily dose ever used, as well as (8) duration of regular use in months. Studies validating self-reported voluntary substance use found a high reliability of the reported drug quantity (Martin et al. 1988; Rothe et al. 1997). Randomly taken hair samples by the Institute of Legal Medicine of the University of Cologne confirmed the self-reported substance use. In addition, qualitative drug screens were performed on the day of the examination with urine samples for amphetamines, benzodiazepines, cocaine, methadone, methylenedioxymethamphetamine (MDMA), and cannabis (enzyme-multiplied immunoassay, von Minden GmbH). The study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee of the Medical Faculty of the University of Cologne.

Cognitive task

The fMRI paradigm consisted of two encoding runs separated into two fMRI time series and one retrieval fMRI time series. During the three scans, a blocked periodic design was used, incorporating eight alternating active and control conditions per run. In the encoding time series, the active or experimental condition consisted of the presentation of 16 full frontal portraits of unknown bald human faces showing neutral expressions (four in each of the experimental conditions per time series). Written professions, of which one half was academic and the other half was artistic, were displayed below the faces. The control condition consisted of the repeated presentation of a single facial contour. The encoding instruction simply required participants to learn the face–profession combinations and to watch the facial contour respectively. During the active condition of the retrieval fMRI time series, the previously presented faces were displayed again without the written professions, with the instruction to retrieve the associated professions and to indicate their category (academic or artistic) and to press the designated button accordingly. In the control condition, facial contours were displayed with the instruction to indicate by button-press whether the left or the right ear was larger. This instruction for the control condition was chosen to serve as a control for motor output and eye scanning patterns for the active condition during the first-level analysis (for further details of the stimuli, see Henke et al. 2003). All stimuli were presented for 4.6 s, resulting in a total scanning time of 2:45 min per fMRI time series and 8:15 min for the entire experiment. The three time series were separately introduced by the appropriate standardized verbal instruction presented via headphones.

Imaging parameters

MRI employing BOLD contrast was performed on a clinical 1.5 T Philips ACS NT Gyroscan (Philips, Eindhoven, The Netherlands) using a singleshot multislice T2*-weighted gradient echo EPI sequence (imaging parameters, 56 volumes; TR, 3,000 ms; TE, 50 ms; flip angle, 90°; matrix, 64×64; field of view, 192×192 mm; 30 contiguous slices parallel to the AC–PC line covering the whole brain; voxel size. 4×4×7 mm; no interslice gap). For each of the two encoding runs and the retrieval run, 56 dynamic scans were recorded. Image collection was preceded by five dummy scans to allow for equilibration of the MRI signal. For anatomic reference and to exclude subjects with apparent brain pathologies, we obtained a T1-weighted Fast Field Echo sequence (imaging parameters: TR, 25 ms; TE, 4.6 ms; TI, 400 ms; flip angle, 30°; matrix, 256×256; slice thickness, 2 mm). Images were acquired using a standard head coil.

Data analyses

Sociodemographic data, cannabis use parameters, and performance

Differences between the median-split groups for age, education, alcohol and nicotine consumption, cannabis using parameters, and memory performance during the associative memory task were analyzed by means of Student's *t* tests. Because of multiple comparisons, the statistical

threshold was adjusted (Bonferroni correction), resulting in an adjusted alpha level of $p=.017$ for each of the three group comparisons. Gender distribution was analyzed by means of Pearson Chi-square test, with Bonferroni-corrected alpha level in height of $p=.017$. Statistical tests were computed using SPSS 17 (SPSS Inc., Chicago, IL, USA).

fMRI

fMRI data were preprocessed and analyzed using SPM5 software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (version 7, The Mathworks Inc., Sherborn, MA, USA). Scans were realigned and spatially normalized into standard stereotactic MNI space. Reconstructed voxel size was $2\times2\times2$ mm^3 and smoothed with a Gaussian kernel of 8 mm. Raw time series were detrended by the application of a high-pass filter (cut-off period, 128 s). Individual and condition-specific effects in the two encoding time series and the retrieval time series were assessed in a first-level analysis by comparing the active condition of the single encoding conditions and the retrieval condition with their respective control condition for each subject.

Whole brain and region of interest (ROI) analyses

To examine neural activity patterns during associative learning and retrieval, activation maps were obtained for the single encoding and retrieval conditions by means of a one-sample *t* test including first-level contrasts of the entire sample. To explore whether the age of onset as well as the duration and frequency of use account for a varying degree of altered memory-related neural activity, the entire sample was median split according to these parameters of use. Subsequently, separate second level comparisons were computed to compare (1) users with an earlier and later onset, (2) users with a higher and lower frequency of use, and (3) users with a longer and shorter duration of regular use. Individual contrast images computed in the first-level analysis were used to perform these two-sample *t* tests for each of the two encoding and retrieval conditions. Because of a priori interest in the hippocampal formation and associated memory functioning and to increase statistical power, the analyses were rerun, restricted to the hippocampus and parahippocampus. These regions of interest (ROI) were anatomically defined by use of the WFU Pickatlas (version 2.4), which provides a method for generating ROI masks based on the Talairach Daemon database (see Maldjian et al. 2003, 2004 and Tzourio-Mazoyer et al. 2002 for a description of the WFU Pickatlas and the anatomical masks employed). The implemented atlases are in MNI space with dimensions of $91\times109\times91$ sampled at 2-mm intervals, corresponding to the SPM MNI templates. Whole brain analyses were computed with the standard height threshold of $p<.05$ and corrected for multiple comparisons (Family wise error (FWE)). ROI analyses were computed with a threshold of $p<.05$ and corrected for multiple comparisons (FWE), implemented in a small volume correction, based on the size of the ROI. Minimum cluster size was set to 20 voxels.

Regression analysis

To further explore specific effects of the parameters of cannabis use on task performance and (para-)hippocampal activity, linear regression analysis was applied. For this purpose, the regressors' age of onset, frequency of use, and duration of use were included. Performance and brain activity during associative memory challenge were entered as dependent variables. The total number of correctly retrieved profession categories defined associative memory performance. For (para-)hippocampal activity mean levels, the control was subtracted from the encoding and retrieval scans. Mean levels of activity were extracted separately for each of the four anatomically defined ROIs (hippocampus left, hippocampus right, parahippocampal gyrus left, and parahippocampal gyrus right), each condition (encoding and retrieval), and each participant. Corrected R-square values were taken as a measure of fit of the overall regression model. Standardized regression β-coefficients indicate the predictive power of the separate regressors.

Results

Qualitative drug screening

All participants displayed negative screenings on the day of the examination for amphetamines, benzodiazepines, cocaine, methadone, and MDMA. Twenty-two participants displayed a positive screening for Δ9-tetrahydrocannabinol (THC), the primary psychoactive compound of cannabis (52% of the entire sample).

Group assignment, demographics, cannabis use, and performance

Data-driven group assignment was based on three separate median splits according to the age of onset, duration, and frequency of use. Correlation analyses between these parameters of use failed to reach statistical significance ($p<.017$), indicating basically independent variation in the study sample (correlations and corresponding *p* values are given in Table 1). Based on a median split according to the age of onset of use (median=age of onset at the age of 15), two subgroups were identified: users with an earlier onset

Table 1 Correlations between the parameters of cannabis use in the present sample

	Cannabis use parameter		
	Age of onset	Frequency	Duration
Age of onset		−.293 (.059)[a]	−.031 (.790)[b]
Frequency			.192 (.091)[b]

[a] Pearson's correlation (two-sided significance) reported

[b] Spearman's rho (two-sided significance) reported because the duration of use was not normally distributed in the present sample

(n=25), who started to use cannabis in the mean age of 13.9 years (SD=1.1, range=12–15); and users with a later onset (n=17), who started to use cannabis in the mean age of 17 (SD=1.5, range=16–21). Groups were of comparable education and, regarding cannabis use, comparable in terms of the frequency of use, the cumulative lifetime dose, and the time since last use. However, users with an earlier onset were younger in mean age (t=−3.53, df=40, p=.001). Splitting the sample at the median duration of use (median=45 months of regular use) identified a subgroup with a shorter duration of use (n=21), who used cannabis in the mean for 22.6 months (SD=14.4, range=0–42), and a subgroup of users with a longer duration of use (n=21), who used cannabis for a mean of 79.9 months (SD=31.6, range=48–144). Regarding cannabis use, these subgroups showed comparable using patterns. However, short duration users were significantly younger (t=−3.72, df=40, p=.001) and less educated (t=−2.92, df=40, p=.006). Finally, a median split according to the frequency of use (median=11 days of use per months) yielded a subgroup of less frequent users (n=21), who used cannabis with a mean of 4.5 days per month (SD=3.5, range=0.—10), and a subgroup of more frequent users (n=21), who used cannabis on 23.8 days per month (SD=6.5, range=12–30). High-frequency users were significantly younger (t=3.07, df=40, p=.004), less educated (t=2.66, df=40, p=.011), and reported a higher cumulative lifetime dose (t=−2.95, df=40, p=.005). Details regarding the demographics and the cannabis use patterns of the median-split groups are given in Table 2. Regarding alcohol and nicotine use, the median-split groups differed neither for the average number of cigarettes smoked per day (p=.233–.798) nor for the average weekly consumed alcoholic beverages during the last year (p=.074–.757).

Global task effects and BOLD response in the hippocampal formation

All subjects had a normal structural MRI scan without focal brain lesions or anatomical abnormalities. Since the encoding trials were identical and the separate analysis of the

Table 2 Demographic features and cannabis use patterns of the median-split subsamples

Characteristics	Age of onset (median=onset at the age of 15)				Duration of use (median=45 months of regular use)				Frequency of use (median=11 days of use per month)			
	Early (n=25)	Late (n=17)	t/χ^2	p	Short (n=21)	Long (n=21)	t/χ^2	p	Low (n=21)	High (n=21)	t/χ^2	p
Present age (years)	21.1 (±2.8)	24.5 (±3.4)	−3.53	.001	20.8 (±3.0)	24.2 (±3.1)	−3.70	.001	24.0 (±3.5)	21 (±2.7)	3.07	.004
Gender (male:female)[a]	18:7	15:2	1.58	.208	14:7	19:2	3.56	.060	16:5	17:4	.141	.707
Education (years)	14.3 (±2.4)	15.7 (±2.8)	−1.80	.079	13.8 (±2.3)	16.0 (±2.6)	−2.92	.006	15.9 (±2.8)	13.8 (±2.1)	2.66	.011
Cannabis use patterns												
Age of first use (years)	13.9 (±1.1)	17.0 (±1.5)	−8.17	.001	14.9 (±1.5)	15.4 (±2.4)	−.849	.401	16.0 (±2.0)	14.5 (±1.8)	2.19	.035
Duration of regular use (months)	54.3 (±42.8)	46.6 (±31.7)	.66	.000	22.6 (±14.4)	80.0 (±31.6)	−7.56	.138	42.6 (±37.0)	60.0 (±37.5)	−1.51	.516
Days of use per month (average)	17.1 (±10.9)	9.8 (±9.9)	2.20	.033	12.8 (±11.5)	15.6 (±10.6)	−.82	.415	4.5 (±3.5)	23.8 (±6.5)	−12.1	.000
Lifetime dose (gram)	698 (±768)	486 (±408)	1.04	.305	393 (±394)	831 (±780)	−2.23	.027	340 (±344)	883 (±769)	−2.95	.005
Average daily dose (joints)	2.3 (±1.6)	2.5 (±1.8)	−.39	.696	2.4 (±1.7)	2.4 (±1.6)	−.06	.956	2.2 (±1.5)	2.6 (±1.8)	−.71	.483
Highest daily dose ever (joints)	10.4 (±7.6)	9.3 (±8.0)	.42	.674	9.2 (±8.1)	10.7 (±7.4)	−.61	.548	7.9 (±6.3)	12.0 (±8.5)	−1.80	.079
Time since last use (days)	102 (±291)	64 (±122)	.50	.617	144 (±315)	29 (±86)	1.62	.113	73 (±123)	100 (±313)	−.38	.709

t values were calculated using unpaired t test; two-tailed (df=40), p adjusted=.017

[a] Comparison tested with Pearson chi-square test (df= 1); asymptotic significance (two-sided) is reported, p adjusted=.017

encoding trials revealed similar activation patterns, we summarized the activation patterns of both encoding trials for further analyses. Whole brain analysis indicated that across both groups, the following regions showed significant activity during the encoding condition: primary visual cortex (bilateral), parahippocampal gyrus (right), middle frontal gyrus (bilateral), superior parietal lobule (left), and medial frontal gyrus (left). For the retrieval condition, the following regions showed significant activity: primary visual cortex (bilateral), middle frontal gyrus (right), cingulate gyrus (left), medial frontal gyrus (right), superior frontal gyrus (right), and posterior cingulate (right). Detailed results are given in Table 3. Anatomically predefined ROI analysis indicated bilateral activity in the hippocampal formation during the encoding condition; however, during retrieval, no significant activity within the ROI passed the threshold for significance (minimum voxel size, 20; $p<.05$; FWE and small volume corrected). During encoding, the activity was most pronounced in the left parahippocampal gyrus. The maximum t value was located in the MNI space at $x=-22$, $y=-10$, and $z=-16$ ($t=8.40$). Results for the summarized encoding conditions are visually presented in Fig. 1.

Between-group effects

Because groups differed with respect to age and education, we tested for effects of these demographic variables on performance and fMRI data prior to the between-group comparisons. No significant relations of age and years of education and performance ($p<.05$, two-tailed Pearson correlation) were found. SPM simple regression with age, years of education, and performance as separate regressors (minimum voxel size, 20; $p<.05$; FWE and small volume corrected) revealed no significant associations. Age and education were thus not further controlled for as potential confounding variables in the following group comparisons. Furthermore, groups differed in the proportion of participants with a positive cannabis screening on the day of the examination. However, a direct group comparison of participants with and without a positive screening result for the entire sample revealed no significant differences in performance ($t=.79$, $df=40$, $p=.432$) or in BOLD signal change (minimum voxel size, 20; $p<.05$; FWE and small volume corrected).

Performance data

The number of correctly classified profession categories did not reveal significant differences between the median-split groups according to duration (short duration users, mean=9.6±3.28; long duration users, mean=10.0±2.17; $t=-.48$, $df=40$, $p=.634$), frequency (low-frequent users, mean=10.2±2.8; high frequent users, mean=9.3±2.7; $t=.99$, $df=40$, $p=.325$), or age of onset (early onset users, mean=9.2±2.8; late onset users, mean=10.6±2.5; $t=-1.55$, $df=40$, $p=.131$).

Table 3 Clusters of significant activation during encoding and retrieval for the entire sample (minimum voxel size, 20; $p<.05$; Family wise error corrected)

Task	Region (Brodmann area)	MNI coordinates			Cluster size	Maximum t value
		x	y	z		
Encoding	Lingual gyrus (BA 18) L	−20	−102	−6	2,638	12.10
	Lingual gyrus (BA 17) R	20	−98	−10	1,373	10.69
	Parahippocampal gyrus (BA 28) L	−22	−6	−18	319	8.59
	Middle frontal gyrus (BA 9) R	50	24	30	260	7.82
	Superior parietal lobe (BA 7) L	−32	−64	44	135	7.25
	Middle frontal gyrus (BA 46) L	−50	36	26	64	7.07
	Parahippocampal gyrus (BA 35) R	22	−4	−18	37	6.77
	Medial frontal gyrus (BA 6) R	8	16	48	26	6.15
Retrieval	Lingual gyrus (BA 17) L	−20	−102	−10	751	12.99
	Inferior occipital gyrus (BA 17) R	24	−98	−8	241	9.00
	Middle frontal gyrus (BA 9) R	48	22	30	240	7.83
	Cuneus (BA 7) R	8	−68	28	179	6.93
	Cingulate gyrus (BA 32) L	−6	24	42	112	6.85
	Superior frontal gyrus (BA 10) R	26	52	2	39	6.62
	Posterior cingulate (BA 23) R	12	−36	22	35	6.51

MNI coordinates refer to maximum t value within the cluster. Cluster size is given in voxels per cluster

R right hemisphere, *L* left hemisphere

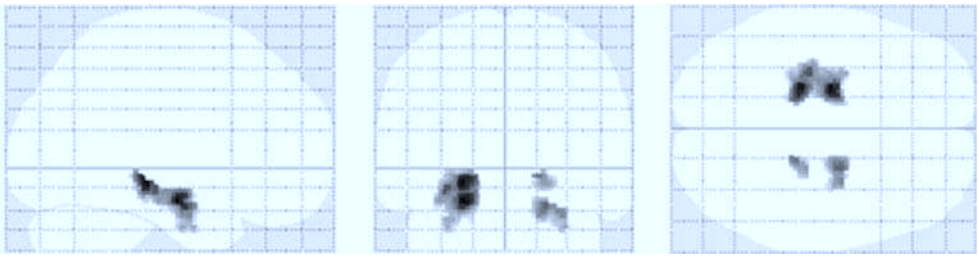

Fig. 1 Results from one-sample *t* test for the pooled encoding conditions (active>control, $p<.05$, Family wise error and small volume corrected). Maximum *t* value located at $x=-22$, $y=-10$, $z=-16$ (MNI space)

fMRI data

Group comparisons

The whole brain analysis did not reveal any significant between-group differences when correcting for multiple comparisons (minimum voxel size, 20; $p<.05$; FWE). ROI analysis indicated that the median-split groups according to the age of onset and the duration of use did not differ (minimum voxel size, 20; $p<.05$; FWE and small volume corrected). However, differences were found for both groups split according to the frequency of use. During encoding, high-frequency users showed significantly greater activity in the left parahippocampal gyrus (average *t* value for the pooled encoding runs, $t_{av}=1.76\pm1.31$, Fig. 2). This effect remained stable after controlling for task performance in terms of correct responses (analysis of covariance). Furthermore, because high-frequency users exhibited more positive THC screenings (17 vs. 5), the group comparison was replicated by applying separate SPM two-sample *t* tests for subjects with positive and negative screenings. These comparisons revealed no significant group differences (minimum voxel size, 20; $p<.05$; FWE and small volume corrected). Moreover, we did not find any significant clusters of activation differences for the retrieval run.

Regression analysis

We found no significant associations between the parameters of cannabis use, performance, and retrieval-related activity. However, the regression model significantly predicted encoding-related activity in the left parahippocampal ROI ($R^2=.125$; $F=2.95$, $df=41$, $p=.045$). Standardized β-coefficients indicated an association between higher frequency of use and stronger activity in the left parahippocampal gyrus ($\beta=.438$, $p=.007$). Detailed results are given in Table 4. To further illustrate this association, we applied Pearson's correlation analysis entering both variables ($R^2=.145$). The corresponding scatter plot is given in Fig. 3.

Discussion

Duration, frequency, and age of onset of cannabis use have been associated with cannabis-related memory impairments. To estimate whether these parameters of use impact memory-related (para-)hippocampal functioning differently in cannabis users, BOLD response during an associative memory task with separate encoding and retrieval runs was assessed in 42 cannabis users. During encoding, the associative memory task led to bilateral activation in the hippocampal formation, most pronounced in the left parahippocampal gyrus. Direct group comparison of users with a higher and lower frequency of use indicated stronger BOLD response during encoding in the left parahippocampal gyrus in the high-frequency use group. No differences were obvious for the groups split according to a longer and shorter duration or an earlier and later onset of use. Effects of the three parameters of use were further separated by means of linear regression analysis. Again, a higher frequency of use was particularly associated with stronger encoding-related BOLD response in the left parahippocampal gyrus.

The present study is the first to examine the impact of three different parameters of cannabis use on (para-)

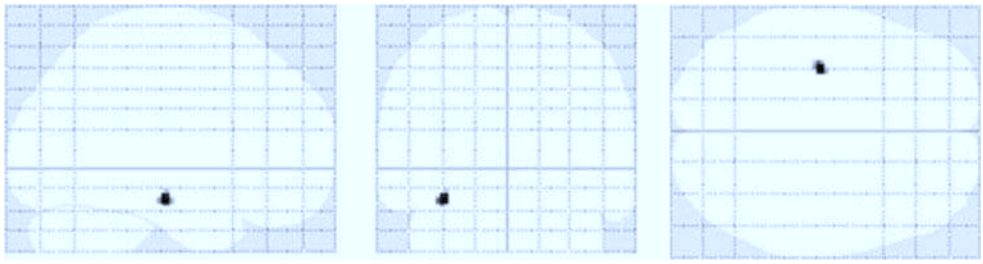

Fig. 2 Results from two-sample *t* test comparing (para-)hippocampal activity during encoding between high- and low-frequent users (high-frequent> low-frequent users, $p<.05$, Family wise error and small volume corrected). Maximum *t* value located at $x=-34$, $y=-18$, $z=-16$ (MNI space)

Table 4 Results from the linear regression analysis

Variable	Overall model fit		Standardized β-coefficients	
	R-square	Age of onset	Frequency of use	Duration of use
Performance	.005 (.380)	.218 (.220)	−.087 (.622)	.217 (.217)
Encoding-related activity				
H (left)	.016 (.317)	−.078 (.634)	−.296 (.079)	.136 (.394)
H (right)	.082 (.351)	−.198 (.232)	−.186 (.264)	.177 (.272)
P H (left)	*.139 (.034)*	.080 (.604)	*.451 (.005)*	−.199 (.185)
P H (right)	.025 (.272)	−.051 (.755)	−.259 (.121)	.228 (.155)
Retrieval-related activity				
H (left)	−.036 (.669)	−.037 (.825)	−.102 (.547)	−.161 (.326)
H (right)	.016 (.314)	−.114 (.487)	−.299 (.076)	−.049 (.756)
P H (left)	−.028 (.603)	−.156 (.354)	−.103 (.540)	−.149 (.360)
P H (right)	−.044 (.734)	−.100 (.555)	−.133 (.436)	−.105 (.524)

The three parameters of cannabis use (age of onset, frequency of use, and duration of regular use) were included as regressors. Performance and mean levels of activity within the regions of interest during encoding and retrieval were used as dependent variables. R-square values indicate the overall fit of the regression model. Standardized β-coefficients reflect the predictive power of the separate regressors. Significant values ($p<.05$) are printed in italics

H mean level of activity within the anatomically defined region of interest representing the hippocampus, *P H* mean level of activity representing the anatomically defined parahippocampal gyrus

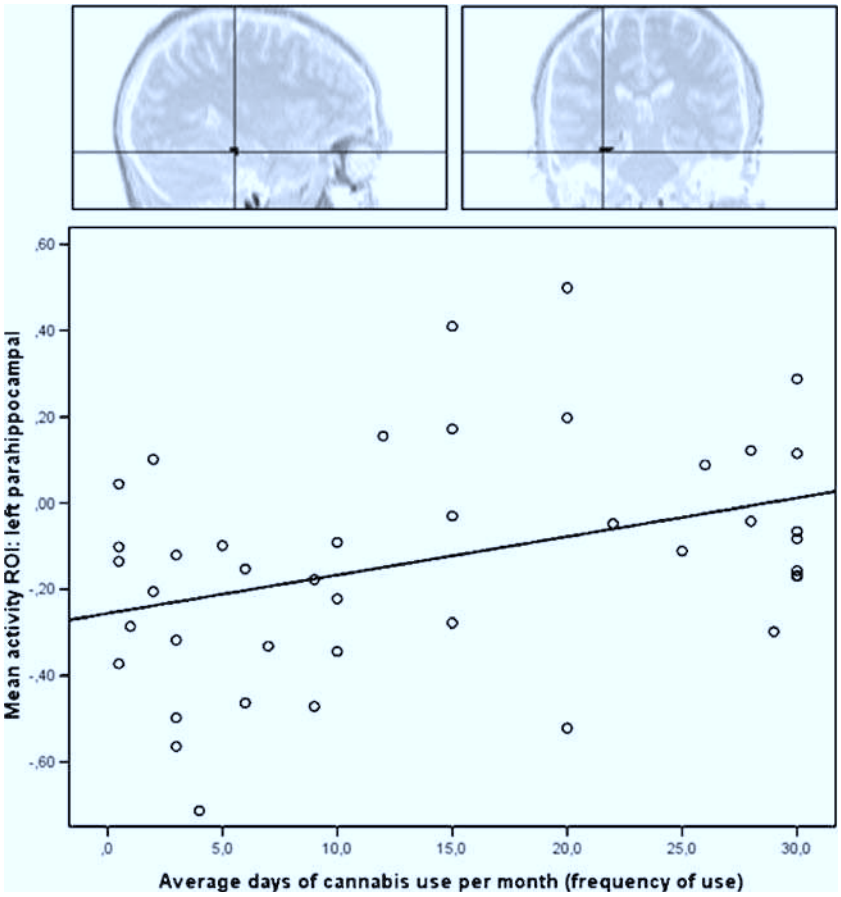

Fig. 3 Scatter plot showing the associations between individual mean levels of activity in the left parahippocampal region of interest and the average days of use per month (frequency of use)

hippocampal functioning. Therefore, it is difficult to directly link the present results to those of previous studies comparing cannabis users and non-using controls. To date, two fMRI studies have addressed hippocampal functioning in cannabis users by means of engaging primarily hippocampal-dependent task paradigms (Jager et al. 2007; Nestor et al. 2008). Both studies reported altered encoding-related parahippocampal activity in cannabis users compared to non-using controls. In the first study, decreased activity in regions involved in associative learning, particularly in the parahippocampal gyri and the dorsolateral prefrontal cortex, have been reported (Jager et al. 2007). Contrariwise, in the more recent study, increased activity in the right parahippocampal gyrus and decreased activity in superior frontal and temporal cortices were shown (Nestor et al. 2008). Findings from the present study extend these previous findings and suggest exposure-related effects of cannabis use on parahippocampal functioning. Although it must remain unclear whether the present results are directly comparable to previous findings which refer to differences between cannabis users and non-users, the association between a more deleterious pattern of use and increased activity might be suggested to support the finding that cannabis users showed parahippocampal hyperactivity relative to controls (Nestor et al. 2008). However, this finding would stand in contrast to findings of Jager et al. (2007), who reported parahippocampal hypoactivity in cannabis users. These inconsistent results might reflect different learning demands of the fMRI tasks used in the three studies. In contrast to the aforementioned investigations, in which participants had to recognize previously displayed pairs of pictures (Jager et al. 2007) or face–number combinations (Nestor et al. 2008), participants in the present study were required to learn combinations of faces and written professions. Alternatively, inconsistencies might reflect differences in abstinence periods. Participants in the study conducted by Jager et al. (2007) were required to abstain from cannabis for at least 7 days prior to testing. Compared to this, abstinence periods in the Nestor et al. (in mean, 80 h) and the present study (almost 70% of the participants had used cannabis in the week prior to the investigation) were considerably shorter. This might suggest a change in parahippocampal neural recruitment throughout the course of abstinence, possibly due to residual drug effects or withdrawal symptoms. However, studies carefully investigating the changes in parahippocampal activity during at least 28 days of abstinence will be needed to adequately address this issue. In contrast to the above-mentioned studies, the present study failed to find group differences or exposure-related effects in frontal regions. Frontal hypoactivity might have remained undetected in the present study because it represents a more general effect of cannabis use independent of specific parameters of use. Alternatively, differences between subgroups of cannabis users might simply be more subtle than differences between cannabis users and controls.

Findings from clinical populations with neurodegenerative diseases (e.g., Alzheimer's disease and HIV encephalopathy) suggest that despite subtle neuronal impairments, normal memory performance might be achieved through "working harder" within the task-related neural network and/or compensatory recruitment of additional neuronal areas (e.g., Bondi et al. 2005). Given this hypothesis of neural compensation, increased activity during cognitive challenge in drug users has been suggested to reflect compensatory neural response in order to maintain normal performance (Nestor et al. 2008; Daumann et al. 2005; Chang et al. 2006; Schweinsburg et al. 2008). High-frequency cannabis users in the present study displayed increased encoding-related activity relative to low-frequency users, particularly in the left parahippocampal gyrus. Findings from the linear regression analysis confirmed the association between the frequency of use and hyperactivity in this specific region. Although the differential role of this structure in the hippocampal formation in learning and memory is not fully understood, lesion studies suggest that it contributes predominantly to associative learning and recall of objects and faces (Weniger et al. 2004). Findings from functional imaging studies suggest that the parahippocampus is involved in the formation of face–name associations (Zeineh et al. 2003; Sperling et al. 2003; Kirwan and Stark 2004). Consequently, hyperactivity in this region might reflect difficulties in the high-frequency users to bind new associations. Moreover, findings from linear regression analysis suggest that a higher frequency of use might be accompanied by increasing cognitive demands during the formation of new associations.

The present study found exposure-related effects of cannabis use on neural activity; however, in contrast to several neuropsychological investigations reporting associative memory deficits in cannabis users (Block and Wittenborn 1985; Indlekofer et al. 2008), no associations with memory performance were found. The larger neural effort during encoding might have maintained normal task performance in the group of high-frequency users. Findings from studies investigating neurodegenerative processes and neurotoxic drug effects suggest that alterations in functional activation patterns are detectable before impairments in cognitive performance become significantly apparent (Bookheimer et al. 2000; Daumann et al. 2004; Melrose et al. 2008). fMRI, therefore, might be a more sensitive method to detect subtle memory impairments than common neuropsychological measures. Alternatively, an fMRI task simply might be a less sensitive marker for cognitive impairments. Successful retrieval of previously displayed face–name pairs has been linked to greater activity in the

hippocampal formation during encoding (Kirwan and Stark 2004). In the present study, equality of group performance might, thus, ensure that performance-associated effects will not interfere with effects of specific cannabis use parameters on (para-)hippocampal activity.

In the present study, group differences and exposure-related effects were obvious during encoding, yet not during retrieval. This effect has also been reported by recent studies comparing cannabis users and controls with similar tasks (Jager et al. 2007; Nestor et al. 2008). Together, these findings might suggest that impaired associative memory performance in cannabis users might be based on deficient encoding processes, whereas retrieval remains intact. Further support for this hypothesis comes from findings in laboratory animals. In rats, the acute exposure to THC, the primary psychoactive component of cannabis, or synthetic, or endogenous cannabinoid agonists, blocks long-term potentiation (LTP) in the hippocampus (Stella et al. 1997; Misner and Sullivan 1999). LTP represents an experimentally induced change in synaptic strengths and is thought to resemble naturally occurring long-term changes in synaptic efficacy. In a more recent study, repeated THC exposure led to impaired synaptic plasticity in the rat hippocampus for at least 7 days after the last injection (Hoffman et al. 2007). Future studies with human cannabis users investigating whether memory contents acquired before the onset of cannabis use remain intact may possibly provide further support for the hypothesis of impaired encoding but unimpaired retrieval processes in cannabis users. Alternatively, the lack of retrieval-related effects in the present study might be explained in terms of statistical power. Due to the short task duration and the small number of scans per condition, the efficiency of the fMRI design in the present study is limited. Pooling the two identical encoding sessions might have led to sufficient power to detect encoding-related effects. However, the retrieval condition was only administered once. Therefore, the lack of cannabis-associated effects during retrieval could simply be a consequence of a lack of statistical power. Future studies with more efficient fMRI designs are needed to confirm findings from the present study.

Only high- and low-frequency users differed in respect to parahippocampal activity. Additionally, only the frequency of use significantly predicted activity in the left parahippocampal gyrus during encoding. Given these findings, the frequency of use might have a particular critical impact on intact parahippocampal functioning in cannabis users. The authors of a recent review on cannabis and memory suggested that the effects of frequency and duration of use may be separable (Solowij and Battisti 2008). According to the authors, effects associated with frequency are shorter lasting and potentially reversible, whereas effects associated with duration reflect longer lasting effects, presumably based on enduring neural alterations. Hence, in respect of the duration of cannabis-associated neuronal alterations, it might be suggested that altered parahippocampal activity due to high-frequency cannabis use might reflect transitory and possibly reversible effects. This would be in line with findings from a prospective study in which current users and non-users, but not former users and non-users, differed in terms of immediate and delayed memory after adjusting for pre-drug cognitive abilities (Fried et al. 2005). However, long-term data of frequent users who are willing to abstain from cannabis for at least 28 days are needed to clarify potential reversibility.

In general, findings from the present study provide further support for the hypothesis that the hippocampal formation might be particularly vulnerable to the consequences of prolonged cannabis use. Findings from animal studies suggest that chronic administration of cannabis leads to neurotoxic alterations in the hippocampus in laboratory animals (Scallet et al. 1987; Landfield et al. 1988; Lawston et al. 2000). However, evidence for cannabis-associated altered parahippocampal integrity in human cannabis users is inconclusive. Structural imaging studies reported lower gray matter densities in the right parahippocampal gyrus and lower white matter densities in the corresponding left structure in heavy cannabis users (Matochik et al. 2005) as well as bilaterally reduced hippocampal and amygdala volumes in a sample of very heavy long-term cannabis users (Yücel et al. 2008). However, in other studies, no structural differences have been found (Block et al. 2000; Tzilos et al. 2005; Jager et al. 2007).

Findings from the present study should be interpreted with several limitations in mind. First, due to the short task duration and the small number of scans per task condition, the statistical power of the fMRI design is limited. Surprisingly, we found no activity during retrieval in the hippocampal formation. This region has consistently been linked to associative learning and memory. Pooling the data for the two identical encoding runs might have led to sufficient power to detect encoding-related activity in the hippocampal formation. Findings from the present study should, therefore, be regarded as preliminary and need to be confirmed in future studies, incorporating longer scanning sessions and a higher statistical power. Second, differences in cortical activity might reflect residual effects of sub-acute cannabis intoxication. THC and its metabolites remain detectable in the body of frequent cannabis users several weeks after the last use (McGilveray 2005). High- and low-frequency users in the present study differed with regard to positive THC urine screenings. Compared to approximately

one quarter (23%) of the low-frequency users, more than three quarters (80%) of the high-frequency users displayed a positive qualitative THC screening on the day of the examination. An additional comparison between participants with and without a positive THC screening in the subgroups of high- and low-frequency users revealed no interference with group differences in the hippocampal formation. However, because of the small number of participants with a negative screening in the high-frequency use group, results from this analysis cannot completely rule out sub-acute effects. The implementation of a supervised abstinence period of at least several days before the examination combined with quantitative THC screenings might help to clarify this issue in future studies. Third, differences in parahippocampal BOLD response might have been caused by cannabis-associated differences in regional blood flow. In comparison to controls, cannabis users demonstrated increased blood volumes in frontal, temporal, and cerebellar regions (Sneider et al. 2006; O'Leary et al. 2002). In chronic cannabis users, alterations remain detectable 1 month after the last use (Herning et al. 2005). Between-group differences in cannabis-associated blood flow abnormalities, possibly related to the frequency of use, could have affected the amplitude of the parahippocampal BOLD response. Fourth, because the present investigation did not incorporate a non-using control group, we cannot exclude the possibility that users with a higher frequency of use showed normal parahippocampal functioning, whereas low-frequent users displayed decreased levels of activity within the context of normal performance. In the context of functional compensation, this would suggest increased neural efficiency in the low-frequent users. However, the hypothesis of increased neural efficiency would stand in sharp contrast to findings from previous studies. During the last years, several functional imaging studies addressed cannabis-associated memory impairments. Increased activity or a combination of decreased activity and increased activity in other, possibly compensatory, regions have been consistently reported (e.g., Block et al. 2002; Kanayama et al. 2004; Chang et al. 2006; Nestor et al. 2008). To date, lower memory-related brain activity in the (para-)hippocampus has been reported by one study (Jager et al. 2007). The authors suggested that lower activation in cannabis users compared to controls observed in their study might be the neurophysiological expression of a non-cognitive behavioral or physiological variable related to cannabis use (e.g., vigilance or mental attitude during scanning). Moreover, the hypothesis of higher neural efficiency in low-frequency users would be contradictory to consistently reported associations between the frequency of use and the magnitude of cannabis-associated memory deficits (e.g., Pope and Yurgelun-Todd 1996; Pope et al. 2001). Taken together, the current state of research suggests that findings from the present study are unlikely to reflect a higher neural efficiency in low-frequency users. Moreover, the hypothesis of functional compensation due to high-frequency cannabis use is in line with the finding that stronger parahippocampal activity was linearly associated to a higher frequency of use in the present sample. Future studies incorporating a non-using control group or a prospective design will be needed to adequately address this issue. To date, most findings are based on comparisons between cannabis users and non-using controls, and thus cannot rule out possible differences in cognitive functioning or other unknown factors which might have affected cognition as well as the tendency to consume cannabis on a regular basis (e.g., impulsivity) before the onset of cannabis use. Furthermore, differences commonly inherent to the cannabis-associated lifestyle such as specific sleep and nutrition habits might have contributed to the neuropsychological and neurophysiological differences. To control for these factors, prospective study designs are required. However, ethical, methodological, and financial aspects often inhibit the realization of long-term investigations in this field of research. To minimize pre-existing and lifestyle-associated between-group differences in the present study, we decided to focus on the impact of specific parameters of use on memory-related (para-)hippocampal functioning within a group of cannabis users. However, due to the cross-sectional design of the present study, we cannot completely rule out that high- and low-frequency users in the present study might have differed before the initiation of use. To adjust for pre-drug differences, prospective data are needed. Fifth, a general problem in investigations examining cannabis users is the fact that varying potencies and variable methods of inhaling make the estimation of parameters such as the cumulative lifetime dose little reliable. However, in our experience, the duration of use, the age of onset of use, and the frequency of use within the last 6 months is commonly well remembered by most participants. Fifth, we cannot exclude that acute nicotine effects might have affected BOLD response. Nicotine cholinergic receptors are widely distributed throughout the brain. The alpha-7 nicotinic cholinergic subtype is particularly rich in the hippocampus (Nestler et al. 2001), and nicotine has been shown to reduce hippocampal activity during smooth-pursuit eye movement (Tanabe et al. 2006). We cannot exclude that differences in acute nicotine level between high- and low-frequency users might have caused differences in parahippocampal BOLD response. Future studies should consider an appropriate nicotine abstinence period before scanning. However, this issue seems difficult to address, since nicotine withdrawal has been shown to affect memory-related cortical activity in regular cigarette smokers (Xu et al. 2005). Moreover, findings from a recent study in adolescents suggest

interacting effects between nicotine withdrawal and cannabis-associated memory deficits (Jacobsen et al. 2007). Finally, to estimate the impact of different parameters of use, the entire sample was median split. One major disadvantage of this data-driven approach is that the assignment of each subject to one or the other group depends on the composition of the entire sample. Users classified as "low frequency" in the present sample could have been classified as "high frequency" if sample characteristics had been different. The presented findings might thus be sample-specific. However, taking a closer look at the cannabis-use characteristics of the directly compared subsamples, meaningful patterns of use are separable. With respect to the age of onset, early onset users gathered their first experiences during early to middle adolescence, whereas late onset users started to use cannabis during late adolescence and adulthood, respectively. Previous studies reported cognitive impairments in users who started before the age of 16 or 17 (Ehrenreich et al. 1999; Pope et al. 2003). Supported by findings from animal studies (for a review, see Schneider 2008), these findings suggest a higher vulnerability of the adolescent brain for cannabis use sequelae, possibly due to ongoing neuromaturational processes. With respect to the duration of use, subsamples might be regarded as users who are in the first stages of regular use as well as long-term users. Compared to users with a shorter duration, these long-term users used cannabis three times longer. A longer duration of use has been linked to increased cognitive impairments (Solowij et al. 2002; Messinis et al. 2006) and increased white matter alterations in the precentral gyrus of heavy cannabis users (Matochik et al. 2005). The median split according to the frequency of use divided the initial sample into users who use cannabis on an almost daily basis and users who use the drug primarily as a weekend recreational drug. A higher frequency of use has been linked to stronger cognitive impairments in previous studies (Pope and Yurgelun-Todd 1996; Pope et al. 2001). Additionally, findings from the median-split procedure were confirmed by linear regression analysis suggesting a linear association between the frequency of use and stronger parahippocampal activity.

In sum, findings from the present study suggest that the frequency of use might have a particular critical impact on intact parahippocampal functioning in cannabis users. Increased activity within the encoding-related network might reflect functional compensation to maintain cognitive functioning. Impaired associative memory performance in cannabis users might be based on altered encoding processes, whereas retrieval remains intact. However, given the lack of associations with the duration and the age of onset of use, this might suggest rather transient effects.

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Conflicts of interest The authors have no conflicts of interest. The experiments comply with the current laws of Germany.

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Titel:

Hippocampal dysfunction in ecstasy users: findings from a prospective fMRI study.

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Hippocampal dysfunction in ecstasy users:

findings from a prospective fMRI study.

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Abstract

Ecstasy use has been associated with subtle cognitive, particularly memory, deficits. Additionally, ecstasy-polydrug users have shown altered neural functioning in the networks engaged in associative memory and working memory processing. However, it cannot be excluded that differences might be pre-existing or related to concomitant use of amphetamine. We prospectively investigated the effects of ecstasy and amphetamine on memory-related hippocampal and working memory-related fronto-parietal functioning.

Using fMRI cognitive brain function was assessed in 43 novice ecstasy and/or amphetamine user at baseline (t1) and after 12-months (t2). Using analysis of repeated measures we compared users who reported interim regular use (n=17) with users who reported abstinence after t1 (n=12). No significant effects of ecstasy and/or amphetamine on working memory associated activity were found. However, a significant GROUP x TIME interaction effect was found in the left parahippocampal gyrus during associative learning, indicating relatively decreased activity in the interim users. Within the group of continuing users, changes in parahippocampal BOLD showed a dose-response relationship with the use of ecstasy, but not amphetamine.

Findings suggest an association between ecstasy-use and memory-related hippocampal dysfunction in ecstasy-polydrug users. Effects of ecstasy and/or amphetamine on fronto-parietal working memory networks could not be confirmed and might reflect pre-existing differences in neural functioning or effects of concomitantly used cannabis. The pattern of altered hippocampal, yet unaffected fronto-parietal functioning provide first neuroimaging evidence for a particular high vulnerability of the hippocampus to the neurotoxic effects of ecstasy.

Keywords:
ecstasy, amphetamine, fMRI, neuroimaging, cognition, brain function

Introduction

Despite numerous animal studies demonstrating the potential of 3,4-Methylenedioxymethamphetamine (MDMA, commonly sold as ecstasy) to induce selective and persistent lesions to central serotonergic nerve terminals (Fischer *et al*, 1995; Green *et al*, 2003; Hatzidimitriou *et al*, 1999), evidence for ecstasy induced long-term neurotoxic brain damage in human recreational users is still inconclusive.

In human recreational users, research has focused on indirect evidence for serotonergic neurotoxicity. In recent years numerous studies have addressed possible functional sequelae of ecstasy induced central serotonergic damage. Results have been inconsistent and confounded with methodological problems (Lyvers, 2006). Among the more consistent results are reports on subtle cognitive impairments as a potential result of prolonged ecstasy-use (Bolla *et al*, 1998; Gouzoulis-Mayfrank and Daumann, 2006b; Green *et al*, 2003; Parrott *et al*, 2000; Schilt *et al*, 2007). Recent comprehensive reviews and well-controlled studies suggest ecstasy-specific impairments in the domains of learning and memory, yet unaffected performance in other cognitive domains such as executive functions and working memory (Fox *et al*, 2002; Gouzoulis-Mayfrank and Daumann, 2009; Gouzoulis-Mayfrank *et al*, 2003; Schilt *et al*, 2008; Schilt *et al*, 2007). Given these selective pattern of mnemonic impairments, the authors consistently suggested that the hippocampal region might be particularly vulnerable to the neurotoxic effects of ecstasy.

In recent years functional magnetic resonance imaging (fMRI) has increasingly been used to investigate the neural basis of cognitive impairments in ecstasy users (Cowan, 2007). In two cross-sectional studies ecstasy polydrug users displayed altered functioning in the associative memory-related network, including parahippocampal regions (Daumann *et al*, 2005; Roberts *et al*, 2009). In addition findings from some cross-sectional (Daumann *et al*, 2003a; Daumann *et al*, 2003b; Moeller *et al*, 2004) and one longitudinal study (Daumann *et al*, 2004) suggest that ecstasy polydrug use might affect the functional integrity of the fronto-

parietal working memory network. In comparison to non-using controls ecstasy polydrug users displayed increased activity in parietal and prefrontal regions (Daumann *et al*, 2003a; Moeller *et al*, 2004). In subsequent study ecstasy users without concomitant use of other illicit drugs showed increased working memory-related parietal activity relative to non-using controls and ecstasy polydrug users (Daumann *et al*, 2003b). However, compared to both control groups ecstasy users additionally displayed relative decreased activity in multiple temporal and limbic regions. Finally, results from a longitudinal study suggest that parietal hyperactivity in heavy ecstasy polydrug users intensifies after 18-month of continued ecstasy and amphetamine use (Daumann *et al*, 2004). Interestingly, increments of neural activity in the continuing users showed a dose-response relation with the degree of interim ecstasy, but not amphetamine or cannabis, exposure. However, findings from a more recent study in which the specific effects of ecstasy and other licit and illicit drugs were separated using multiple regression analysis challenge these previous findings (Jager *et al*, 2008). This study revealed no evidence for robust effects of ecstasy on memory-related hippocampal functioning or working memory-related fronto-parietal network. Interestingly, when effects of drugs were separated memory-related parahippocampal dysfunction in polydrug ecstasy users was related to the concomitant use of amphetamine, rather than the use of ecstasy (Jager *et al*, 2008).

Summarizing, findings from fMRI studies remain inconclusive and partly contradictory regarding the specific effects of ecstasy and commonly co-used amphetamine on cognitive brain function and the specific brain regions affected. Moreover, interpretation of data is limited by methodological problems, including poorly matched control groups, cross-sectional study designs, lack of pre-use data and polydrug use.

The present study investigated the effects of moderate recreational ecstasy and amphetamine use on the neural correlates of working memory and associative memory. To control for known confounders in this field of research a prospective longitudinal design was implemented. To separate the specific effects of ecstasy and commonly co-used amphetamine dose-response relationships between specific parameters of use of these

substances and functional brain activity were explored. Based on findings from neurocognitive studies (Fox et al., 2002, Gouzoulis-Mayfrank et al., 2003, Schilt et al., 2007, Schilt et al., 2008) and previous cross-sectional fMRI studies (Daumann *et al*, 2003a; Daumann *et al*, 2005; Daumann *et al*, 2004) we hypothesized a specific effect of ecstasy on memory-related hippocampal functioning.

Methods and materials

Participants

Subjects in the present study were part of a larger prospective study on the effects of ecstasy and amphetamine on cognition and brain function. A total of 140 volunteers underwent an extensive cognitive testing at baseline (t1) and were re-examined after an interval of 12 months (t2). A subsample (n=50) was examined by means fMRI. Main inclusion criterion at baseline was a high probability of future ecstasy and/or amphetamine use, operationalized as having 'first but very limited experience with ecstasy and/or amphetamine'. Exclusion criteria at baseline were (1) having used ecstasy and/or amphetamine on more than five occasions, (2) the use of all other illicit substances except for cannabis, (3) childhood diagnosis of attention-deficit hyperactivity disorder (ADHD) and (4) any current or previous axis I psychiatric diagnosis (except for cannabis abuse). Further exclusion criteria on both study days were: (1) history of alcohol abuse and/or dependence (according to DSM IV criteria, APA 1994), (2) regular intake of any medication, (3) intake of any psychotropic substances except for cannabis seven days prior to testing, (4) use of cannabis on the day of the examination. Additional exclusion criteria for the fMRI investigation were (1) left-handedness, (2) pregnancy and (3) other known contraindications for MRI scanning.

Procedure

Following a detailed study description, written informed consent was obtained from all participants. Subsequently, all subjects underwent a structured interview according to the

Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) on both study days. To exclude participants with childhood ADHD all participants completed the German version of the Wender Utah Rating Scale (WURS) (Ward *et al*, 1993). Participants were excluded if they exceeded the recommended cut-off score of 46 (Ward *et al*, 1993). On both study days, subjects underwent a detailed structured interview assessing the use of amphetamine and ecstasy, including the following parameters of use: (1) age of first use, (2) time since the last use in days, (3) average frequency of use measured by average days of use per month, (4) maximum days of use per month ever, (5) estimated cumulative lifetime dose, as well as (6) average and (7) highest daily or one night dose ever used. Studies validating self-reported voluntary substance use found a high reliability of the reported drug quantity (Martin *et al*, 1988; Rothe *et al*, 1997). Randomly taken hair samples by the Institute of Legal Medicine of the University of Cologne confirmed the self-reported substance use. In addition, qualitative drug screens were performed on the day of the examination with urine samples for amphetamines, benzodiazepines, cocaine, methadone, MDMA and cannabis (enzyme-multiplied immunoassay, von Minden GmbH).

In order to control for confounding variables, psychopathology, concomitant substance use and health behaviour related variables were assessed on both study days. Additionally, current intellectual functioning was assessed at baseline by the Raven Standard Progressive Matrices (Raven, 2000). Self-reported psychiatric symptoms were assessed using the Symptom Checklist-90-Revised (SCL-90-R) (Derogatis, 1994). Parameters of cannabis use were assessed by a cannabis-specific version of the structured drug interview. Additionally, the following aspects were assessed for the period of the previous year: (1) use of alcohol and tobacco (frequency of alcoholic drinks per week, cigarettes per week, years of tobacco use), (2) use of medication (number of uses: hypnotic, analgesic, stimulating and sedative medications per week) and (3) sleep (average hours of sleep per night, frequency of sleep problems).

The study was in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee of the Medical Faculty of the University of Cologne.

Cognitive tasks

For both baseline and follow-up, participants performed a working memory task and an associative memory task. In previous studies both tasks were used to assess differences in cortical activity patterns between ecstasy users and non-using controls (Daumann *et al*, 2003a; Daumann *et al*, 2005). For a detailed description of the tasks we refer to previous publications from our research team (Becker *et al*, 2010a, b; Daumann *et al*, 2005).

Briefly, the working memory paradigm consisted of three verbal n-back tasks with increasing memory load, separated in three fMRI time-series. In each task a blocked periodic design was used incorporating alternating active and control conditions. The control condition was identical for each n-back task (0-back), whereas the active condition (1-, 2-, 3-back) changed per fMRI time-series. Total scanning time was 9:27 minutes. The associative memory fMRI-paradigm consisted of two encoding runs separated into two fMRI time-series and one retrieval fMRI time-series. A blocked periodic design was used with alternating active and control conditions. In the active condition of the encoding runs participants had to learn visually presented face-profession combinations. During the active condition of the retrieval run the faces were displayed without the profession. Participants had to retrieve the associated professions and indicate to which of two given categories (academic or artistic) it belonged. In the control condition facial contours were displayed, during the retrieval fMRI series participants had to indicate whether the left or right ear of the facial contour was larger. Total scanning time was 8:15 minutes.

Imaging parameters

MRI employing blood oxygenation level-dependent (BOLD) contrast was performed on a clinical 1.5 T Philips ACS NT Gyroscan (Philips, Eindhoven, The Netherlands) using a singleshot multislice T2* weighted gradient echo EPI sequence (imaging parameters: TR: 3000 ms, TE: 50 ms, flip angle: 90°, matrix: 64 × 64, field of view: 192 × 192 mm, 30 contiguous slices parallel to the AC-PC line covering the whole brain, voxel size: 4 × 4 × 7 mm, no interslice gap). 60 dynamic scans per n-back run and 56 dynamic scans for each of the two encoding runs and the retrieval run were recorded. Each time-series was preceded by five dummy scans to allow for equilibration of the MRI signal. For anatomic reference and to exclude subjects with apparent brain pathologies, a T1-weighted Fast Field Echo sequence (imaging parameters: TR: 25 ms, TE: 4.6 ms, TI: 400 ms, flip angle: 30°, matrix 256 × 256, slice thickness: 2 mm) was obtained. Images were acquired using a standard head coil.

Data analysis

To obtain information about the impact of continued ecstasy and amphetamine use on cognitive performance and associated neural activity, the sample was divided into two groups: (1) users who completely stopped ecstasy and/or amphetamine use after t1, who served as a control group (controls), and (2) users who began using ecstasy and/or amphetamine on a regular basis after t1 (at least 5 ecstasy tablets and/or 5 grams of amphetamine in the 12-month follow-up period) (users).

Between-group differences for age, education, substance use, confounding variables and performance at t1 and t2 were analyzed by means of unpaired Student t-tests and in case the normality assumption was violated by means of non-parametric Mann-Whitney-U-test. Gender distribution was analyzed by means of χ^2 Fischer´s Exact Test. Differences in performance and confounding variables between both study days were analyzed by means of repeated measures analyses of variance (ANOVA) with the between-subject factor GROUP (controls vs. users) and the within-subject factor TIME (t1 vs. t2). Analyses were computed using SPSS Statistics 18.0 software (SPSS Inc., Chicago, Ill).

Functional magnetic resonance imaging data were preprocessed and analyzed using SPM5 software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab7 (The Mathworks Inc., Sherborn, MA). To correct for motion-related variance components the scans obtained for each subject and each condition were initially realigned to the first image of each scan. All mean images were subsequently normalized with the SPM5 MNI template (resampled to 2×2×2 mm^3 voxels), and smoothed with a Gaussian kernel (triple voxel size). Raw time series were detrended by the application of a high-pass filter (cutoff period: 128 seconds). The preprocessed data was analyzed using a two stage procedure for repeated measures ANOVA (Henson and Penny, 2003). To increase the statistical power of the present study design to detect drug-related changes in brain activity, activation patterns of the task conditions were summarized on the first level. Separate analyses of the three n-back tasks revealed typical activations in the frontoparietal working memory network at both t1 and t2 (for an individual analysis of the three task conditions we refer to Becker et al. 2010). Consequently, the three tasks were summarized on the first level analysis (1-back, 2-back, 3-back > 0-back). For the associative memory paradigm separate analyses of the two identical encoding trials revealed similar activation patterns. Therefore, the two encoding trials were summarized on the first level analysis.

In a first step subject-specific changes in BOLD response were assessed using linear contrasts of the GLM parameters. Separate contrasts for the main effects of task (1-back 2-back 3-back > 0-back / encoding-1 encoding-2 > control / retrieval > control) and time (t2 – t1) were computed for each subject. Additionally for each time point (t1 and t2) separate contrasts for the effect of task (1-back 2-back 3-back > 0-back / encoding-1 encoding-2 > control / retrieval > control) were computed for each subject. In a second step the main effects of task and time as well as the interaction of GROUP x TIME were computed entering the appropriate first level contrasts in two-sample t-tests. To explore between-group

differences at baseline and follow-up individual first level contrasts of task at baseline and follow-up were entered in two separate two-sample t-tests.

To further increase statistical power and based on findings from previous studies analyses of GROUP x TIME interaction effects and between-group differences at t1 and t2 were restricted to task-specific regions of interest (ROI). ROIs were anatomically defined by use of the WFU pickatlas (Maldjian *et al*, 2004; Maldjian *et al*, 2003; Tzourio-Mazoyer *et al*, 2002). Analyses of working memory data was restricted to the dorsolateral prefrontal cortex (DLPFC, anatomically defined as Brodmann areas 9 and 46) and the superior parietal lobe (SPL, anatomically defined as Brodmann area 7). Both regions have consistently been associated to working memory processing (D'Esposito *et al*, 1998) and in previous studies the use of ecstasy and amphetamine has been related to alterations in neural functioning in these regions (Daumann *et al*, 2003a; Daumann *et al*, 2004; Jager *et al*, 2008; Moeller *et al*, 2004). Analyses associative memory data was restricted to the hippocampus and parahippocampus. These regions are known to play a key role in associative encoding and retrieval (Davachi and Wagner, 2002; Henke *et al*, 1997; Henke *et al*, 1999) and findings from previous studies with ecstasy users suggest a particular high vulnerability of the hippocampal formation to the neurotoxic effects of ecstasy (Daumann *et al*, 2005; Gouzoulis-Mayfrank *et al*, 2003; Jacobsen *et al*, 2004; Roberts *et al*, 2009).

All analyses were computed with the standard height threshold of $p < .05$ and corrected for multiple comparisons (Family wise error, FWE). For the ROI analyses the FWE correction was implemented in a small volume correction, based on the size of the ROIs. Minimum cluster size was set to 10 voxels. MNI coordinates were transformed to Talairach coordinates (Talairach and Tournoux, 1988) by the application of a nonlinear mapping method (Lacadie *et al*, 2008).

Results

Participants and group-assignment

Of the 50 users who participated in the fMRI investigation at baseline, 43 users (32 males, 11 females; age range at baseline: 18-30 years, mean: 22.30 years, SD: 3.48) could be re-examined at follow-up (86%). Four subjects moved without giving notice of their new address and three participants lost interest in the study. Based on an initial analysis of the follow-up data three subjects had to be excluded from further analyses. One subject displayed a positive screening result for benzodiazepines and one had to be excluded due to severe head movement during scanning. Finally one participant reported an interim use of 130 grams amphetamine and 144 ecstasy pills and therefore was not comparable to the rest of the interim users.

From the 40 subjects left for the analysis 12 had stopped to use ecstasy and amphetamine after baseline, they served as a control group. A total of 22 participants reported ecstasy use during the follow-up period (mean: 7.30 ecstasy pills, SD: 7.35; range: .5-30). A total of 23 participants reported amphetamine use during the follow-up period (mean: 10.12 grams, SD: 11.92; range: .3-30). Overall 17 participants fulfilled the criteria regular use of ecstasy and/or amphetamine during the follow-up period (at least 5 ecstasy tablets and/or 5 grams of amphetamine between baseline and follow-up). The remaining 11 participants reported only sporadic use of ecstasy and/or amphetamine during the follow-up period and, therefore, were excluded from the analyses.

Demographics, drug use and confounders at baseline and follow-up

Demographic features and drug use patterns at baseline for controls and users are presented in table 1. Groups were of comparable age ($t = .536$, $p = .597$; $df = 27$), education ($t = .337$, $p = .738$; $df = 27$) and gender distribution ($p = .662$, $df = 1$). At baseline, controls and interim users reported similar patterns of previous ecstasy and amphetamine use. However, compared to controls users reported a significantly shorter time since last use for

ecstasy (t = 2.81, p = .010; df = 22) and amphetamine (t = 2.16, p = .041; df = 24), as well as a significantly higher lifetime dose of amphetamine (t = -2.19, p = .037; df = 27).

Regarding potential confounding variables at baseline both groups reported similar patterns of previous cannabis use (all p > .126) and did not differ in the number of errors in the Raven Standard Progressive Matrices (t = .174, p = .863; df = 27), SCL-90-R symptom dimension scores or global indices of psychological distress (all p > .234), frequency of alcoholic drinks per week (t = .825, p = .416; df = 27), number of cigarettes smoked per week (t = -.571, p = .573; df = 27), years of tobacco use (t = .203, p = .841; df = 27), use of medication (t = .621, p = .540; df = 27), average hours of sleep per night (t = -.138, p = .891; df = 27) or the frequency of sleep problems (t = .232, p = .818; df = 27). At follow-up the groups did not differ in any of the SCL-90-R scales (all p > .071), the use of alcohol (t = -.106, p = .916; df = 27), nicotine (t = .267, p = .791; df = 27), and medication (t = -.198, p = .845; df = 27), or sleep related variables (both p > .611). To assess the development of confounding variables in the course of the study separate repeated measures ANOVA were performed for self-reported psychopathology and health behaviour related variables. These analyses yielded no significant main effects (GROUP, TIME) and no significant interaction effect (GROUP x TIME) (all p > .220). Mean time between the baseline and follow-up measurements were 55.6 (± 4.12) weeks in the user group and 57.4 (± 7.52) weeks in the control group (t = .815, p = .422; df = 27).

Patterns of interim drug use

Patterns of interim drug use are given in table 2. Interim abstinent users reported complete abstinence from ecstasy and amphetamine but continued to use cannabis. Importantly groups did not differ significantly in any of the reported parameters of interim cannabis use (all p > .192).

Performance

Reaction times and correct responses in the working memory n-back tasks at baseline (t1) and follow-up (t2) are visually presented in figure 1. Controls and users did not differ significantly in the number of correct responses or reaction times in any of the n-back tasks at baseline or follow-up (all $p > .338$). Correct retrieved profession categories during the retrieval run of the associative memory task are visually presented in figure 2. The groups did not differ significantly in the number of correct responses at baseline (t1) or follow-up (all $p > .546$). Repeated measures ANOVA performed on reaction times and accuracy yielded no significant main effects (GROUP, TIME) and no significant interaction effect (GROUP x TIME) (all $p > .218$).

Functional MRI

All subjects had a normal structural MRI scan without focal brain lesions or anatomical abnormalities.

Working memory

Whole-brain analysis of the working memory data revealed a significant BOLD effect of the pooled n-back tasks for the main effect of task (1-back 2-back 3-back > 0-back) in the working memory network including the SPL and the DLPFC (for a detailed description of the activity maps we refer to a previous study from our research team using the same fMRI paradigm (Becker *et al*, 2010b). Whole-brain and ROI analyses restricted to the SPL and DLPFC revealed no significant main effect of TIME (t1 > t2 / t2 > t1). Whole-brain and ROI analyses restricted to the SPL and DLPFC of the GROUP x TIME interaction revealed no significant results. Likewise whole-brain and ROI analyses restricted to the SPL and DLPFC revealed no significant differences between users and controls for the effect of task (1-back 2-back 3-back > 0-back) at both time points (t1 and t2).

Associative memory

Whole-brain analysis of the pooled encoding tasks revealed a significant BOLD effect for the main effect of task (encoding > control) in the associative memory network (for a detailed description we refer to a previous study from our research team using the same fMRI paradigm (Becker *et al*, 2010a)). ROI analysis restricted to the hippocampus and parahippocampus indicated bilateral activity within the hippocampal formation, most pronounced in the right parahippocampal gyrus. The maximum t-value was located in Talairach-space at x = 31 y = -9 z = -13 (t = 9.66). Results from this analysis are visually presented in figure 3. Whole-brain and ROI analysis of the hippocampal formation revealed no significant main effect of TIME (t1 > t2 / t2 > t1). Whole-brain analysis of the GROUP x TIME interaction revealed no significant results; however ROI analysis of the hippocampal formation indicated a significant GROUP x TIME effect in the left parahippocampal gyrus (maximum at x = -19 y = -40 z = -4; cluster size 14 voxels; t = 6.33, p = .001, FWE corrected for the hippocampal formation). Results are visually presented in figure 4. Analysis of the individual BOLD response differences between baseline and follow-up (t2 > t1) at the maximum of the parahippocampal cluster indicated increased activity in the controls between t1 and t2; but decreased activity in the group of users who reported interim ecstasy and/or amphetamine use. Whole-brain and ROI analyses restricted to the hippocampal formation revealed no significant differences between users and controls for the effect of task (encoding > control) at both time points (t1 and t2).

Whole-brain analysis of the retrieval task revealed a significant main effect of task (retrieval > control) in the associative memory network (detailed description see (Becker *et al*, 2010a). However, ROI analysis revealed that during retrieval no significant activity passed the threshold for significance within the hippocampal formation (minimum voxel size: 10, $p < .05$, FWE and small volume corrected). Whole-brain and hippocampal ROI analyses revealed no significant main effect of TIME (t1 > t2 / t2 > t1) and no significant GROUP x TIME interaction for the retrieval run. Analysis of between-group differences for the effect of task (retrieval > control) at both time points (t1 and t2) revealed no significant results.

To further explore the GROUP x TIME interaction found in the pooled encoding tasks, individual values for percent signal change for the active and control condition at both time points were extracted using the SPM toolbox rfxplot (Glascher, 2009). To assess percent signal changes, individual beta images from the first level analysis for the regressors of the on and off conditions during encoding were used. Computation of the percent signal change was restricted to suprathreshold voxels in a sphere with 2mm radius around coordinates of the maximum t-value from the second level interaction analysis, and defined as signal change relative to the mean signal in the suprathreshold voxels. Mean percent signal changes are given in figure 5. Findings from this analysis indicate that controls display significantly higher activity in the left parahippocampal gyrus at t2 compared to t1, whereas users display significantly lower activity in the left parahippocampal gyrus at t2 compared to t1.

Correlational analyses

To further explore associations between changes in BOLD response between t1 and t2 in the user group we performed SPM simple regressions entering individual contrast images for the effect of time (t2 > t1) and different parameters of interim amphetamine and ecstasy use (interim cumulative dose, highest reported interim 1-night dose, time since last use at t2). Analysis of the left parahippocampal ROI indicated an association between a higher cumulative interim ecstasy dosage and lower activity at t2 relative to t1 (maximum at x = -28 y = -42 z = 0; cluster size 12 voxels; t = 5.76, uncorrected). However, this result did not survive correcting for multiple comparisons.

Discussion

The present prospective study investigated effects of moderate recreational ecstasy use on cognitive brain function. At baseline and after a follow-up period of 12 months 43 young adults with first but very limited experience with ecstasy and/or amphetamine were examined

using fMRI tasks of verbal working memory and associative learning. To control for potential confounders the use of alcohol, nicotine, medication and cannabis, as well as psychopathology and health related variables were assessed at both time points. Participants reporting regular use of ecstasy and/or amphetamine between baseline and follow-up (n = 17) were compared to participants reporting complete abstinence after the baseline examination (n = 12). At baseline groups were comparable in terms of sociodemographics, current intellectual functioning and previous use of ecstasy and amphetamine; however interim users reported shorter abstinence periods at baseline for both substances. Regarding potential confounders the groups did not differ at baseline or follow-up, most importantly groups showed comparable patterns of interim cannabis use. At both time points the groups showed similar working memory and learning performance. Analysis of repeated measures revealed no main effects and no GROUP x TIME interaction effect in performance. Repeated measures analysis of the imaging data revealed no significant GROUP x TIME interaction in DLPFC or SPL during working memory challenge. However, a significant GROUP x TIME interaction effect was found in the left parahippocampal gyrus during associative learning, indicating relatively decreased activity in the interim users. Further analysis indicated a dose-response relationship between interim decreased parahippocampal activity and the use of ecstasy, but not amphetamine.

In contrast to previous studies incorporating cross-sectional (Daumann *et al*, 2003a; Moeller *et al*, 2004) and longitudinal (Daumann *et al*, 2004) designs the present study found no effects of ecstasy and/or amphetamine use on cortical activity in the fronto-parietal working memory network. Differences in study design and participant characteristics might explain the diverging results. In previous studies differences in cannabis use between ecstasy poly-drug users and controls (Daumann *et al*, 2003a; Moeller *et al*, 2004), or continuing and abstinent ecstasy poly-drug users (Daumann *et al*, 2004) were not controlled for. Findings from neuroimaging studies with cannabis users suggest an association between cannabis use and altered parietal and frontal activity during working memory challenge (Jager *et al*, 2006; Kanayama *et al*, 2004), in one study even regular cannabis users with an earlier and

later onset of cannabis use have shown differences in parietal activity (Becker *et al*, 2010b). The concomitant use of cannabis in ecstasy poly-drug users, or differences in specific parameters of cannabis use, therefore might account (at least partly) for fronto-parietal alterations found in previous studies. Alternatively, differences in the cumulative lifetime dose of ecstasy and amphetamine between study samples might have contributed to the lack of fronto-parietal alterations in the present study. Compared to participants in studies reporting fronto-parietal alterations in ecstasy poly-drug users (Daumann *et al*, 2003a; Daumann *et al*, 2004; Moeller *et al*, 2004), participants in the present study had used relatively low cumulative doses of ecstasy and amphetamine. Alterations in fronto-parietal regions might only develop as a consequence of prolonged heavy use. This would be in line with a recent prospective study reporting no effects of a single low dose of ecstasy on brain activity under cognitive challenge (Jager *et al*, 2007). However, future prospective studies with longer follow-up periods and closely matched cannabis using controls are needed to completely address this issue.

Although effects of moderate ecstasy and/or amphetamine use on fronto-parietal regions were not apparent in the present investigation, findings from the associative memory task suggest effects on hippocampal regions. During associative encoding interim abstinent users showed a relative increase in parahippocampal activity, whereas continuing users showed relatively decreased activity in this region. This finding is in line with previous reports on decreased parahippocampal activity in heavy poly-drug ecstasy users (Daumann *et al*, 2005) and adolescents with moderate ecstasy use (Jacobsen *et al*, 2004). Changes in parahippocampal activity were associated with extend of interim ecstasy use, but not with parameters of interim amphetamine or cannabis use, suggesting ecstasy-specific effects. Admittedly, associations between interim ecstasy use and changes in neural activity were rather weak in the present sample. However, due to the relatively low doses of interim ecstasy use and the short follow-up period, parameters of ecstasy use showed very limited variation in the present sample. Furthermore, confounders such as variations in the amount of MDMA within the ecstasy tablets used, or inaccuracies in self-reported drug use are likely

to have a stronger biasing effect on dose-response relationships in samples with moderate use, compared to samples with heavier use patterns.

The pattern of altered hippocampal, yet unaltered neocortical neural functioning in moderate ecstasy users in the present sample is in striking accordance with previous reports on specific cognitive impairments in ecstasy users (Fox *et al*, 2002; Gouzoulis-Mayfrank *et al*, 2003; Schilt *et al*, 2007). In these studies extensive neurocognitive test batteries, including tests on attention, working memory, planning ability and memory were used to investigate the specific profile of cognitive deficits in ecstasy poly-drug users. Ecstasy poly-drug users consistently showed specific impairments of mnemonic functions, yet unimpaired performance in other cognitive domains. In addition findings from a recent study investigating the specific effects of ecstasy relative to amphetamine, cannabis and cocaine suggest a specific negative effect of ecstasy on verbal memory (Schilt *et al*, 2008). Given that the hippocampus has consistently been associated to memory processes and neocortical regions subserve higher cortical functions such as executive control and working memory, the authors consistently concluded that the pattern of predominant memory impairments suggest a particular vulnerability of the hippocampal region to the neurotoxic effects of ecstasy. Findings from the present study provide further support for this hypothesis.

Although the precise mechanism of MDMA-induced neurotoxicity remains to be fully elucidated findings from several animal studies suggest highly specific neurotoxicity to the serotonergic system in all but one species (Capela *et al*, 2009). Long-term reductions in biochemical markers of the serotonergic system have been reported in laboratory animals (Capela *et al*, 2009), as well as in human ecstasy users (Bolla *et al*, 1998; Reneman *et al*, 2001; Thomasius *et al*, 2003). Group x Time Interaction effects in the present study therefore might be explained in terms of ecstasy-related altered serotonergic functioning in the continuing users. Serotonin has been shown to influence a broad range of physiological systems and a variety of behavioral functions (Lucki, 1998), therefore different mechanisms of altered serotonergic functioning might have caused the present findings. Serotonin

mediates vasoconstriction and MDMA has been shown to induce acute and persisting effects on cerebral blood flow in laboratory animals (Ferrington *et al*, 2006; Quate *et al*, 2004; Rosa-Neto *et al*, 2004). Findings from a recent prospective study in low dose ecstasy users suggest sustained effects of ecstasy on brain microvasculature (de Win *et al*, 2008). Given that it has been proposed, that changes in vasculature directly affect the BOLD signal underlying functional MRI (Carusone *et al*, 2002), findings from the present study might suggest ecstasy related changes in brain microvasculature, possibly mediated by lower serotonergic tone in the continuing users. However, this hypothesis would not account for the specific localization of ecstasy associated changes in the hippocampal area, since regions in the cerebral cortex display comparable dense serotonergic enervations (Jacobs and Azmitia, 1992; Leger *et al*, 2001). Apart from its vasoconstrictatory properties serotonin has been suggested to act as a trophic factor, enhancing neurogenesis in the adult mammalian dentate gyrus (Djavadian, 2004; Gould, 1999). The dendate gyrus receives input mainly from the entorhinal cortex, a region in the parahippocampal gyrus, and in turn projects to the hippocampal circuits. The influence of serotonin on neurogenesis in the hippocampal area seems to be very consistent: its depletion reduces, whereas its increased level increases the rate of neurogenesis in the dentate gyrus (Djavadian, 2004). The newly generated dentate gyrus neurons have been suggested to play an important role in hippocampus dependent memory processes (Suzuki and Clayton, 2000). Disruptions in adult hippocampal neurogenesis due to ecstasy induced decreased levels of serotonin therefore might be suggested to underlie the present findings. However, further analysis of the interaction effect in the parahippocampal region revealed, that the interaction was mainly driven by relatively increased activity in the interim abstinent users. Therefore we cannot exclude the possibility that recovery processes in the abstinent users might underlie the present findings. To recruit participants with a high probability of future ecstasy use and to avoid large oversampling, we decided to recruit participants who had first but very limited experience with ecstasy and/or amphetamine at baseline. However, recent findings from prospective studies with novice ecstasy users suggest, that even first low cumulative doses of ecstasy might lead to

measureable alterations in serotonergic functioning (de Win *et al*, 2008; de Win *et al*, 2007; Schilt *et al*, 2007). Given that adolescent ecstasy users with moderate use have shown to display reduced hippocampal activity (Jacobsen *et al*, 2004) and serotonergic alterations in ecstasy users might be reversible (Buchert *et al*, 2004), interaction effects in the present sample might reflect long-term recovery processes in the abstinent users. However, in other studies the moderate use of ecstasy was not associated with measurable alterations (Gouzoulis-Mayfrank *et al*, 2003; Halpern *et al*, 2004) and findings from a follow-up study suggest that altered cerebral activation patterns, at least in former heavy ecstasy, users do not reverse after several months of abstinence (Daumann *et al*, 2004).

Although the prospective design of this investigation might help to overcome some methodological shortcomings of previous studies, we are well aware of its methodological limitations, inherent to virtually every open-field study. First, although this study incorporated a prospective design, most known confounders were controlled for and a broad range of methods was used to recruit participants (advertisement in magazines and newspapers, notifications posted on campus, radio interviews), we cannot exclude that unknown factors or selective sampling might have contributed to the present findings. Only experimental designs with randomly selected samples could offer evidence of causality; however, direct experimental approaches in humans remain controversial. Second, most participants used amphetamine and ecstasy. During the last years this pattern of poly drug use has established in recreational ecstasy users (Gouzoulis-Mayfrank and Daumann, 2006a; Smart and Ogborne, 2000) (EMCDDA, 2009). Although alterations in the present sample were only related to the extent of interim ecstasy use, and interim doses of amphetamine and ecstasy were not associated (Pearson correlation; $p = .649$) we cannot completely rule out that complex interaction effects among the drugs might have led to the present findings. Third, drug histories were assessed by self-report. Before participants were included in the baseline examination they were interviewed about their drug histories, without knowledge of the precise inclusion and exclusion criteria and analysis of randomly taken hair samples was used to confirm self-reported drug use at baseline and follow-up. However, due to short

hairstyles and the fact that the adherence of drugs onto hair varies strongly depending on the physical characteristics of the hair and the kind of care applied to it, we cannot completely rule out that inaccuracies in the reported drug histories might have biased our results. Fourth, there was no control on purity or amount of MDMA in the ecstasy tablets used. However, recent data suggest that in the years 2006 and 2007 nearly 99% of the tablets sold as ecstasy were monopreparations, with approximately 98% containing MDMA (EMCDDA, national report 2007/2008). Fifth, although detailed information about the pattern of drug use was assessed within the drug use interview, the environment in which the drug was actually used was not assessed. Findings from previous studies suggest that the neurotoxic effects of MDMA may be enhanced under certain conditions, such as hot, overcrowded surroundings and long periods of dancing; possibly mediated by an increase in body temperature (Colado *et al*, 1998; Green *et al*, 2003; Parrott, 2004). However, there seems no easy solution for this issue. Finally, we cannot exclude that a larger sample size or other MRI paradigms or techniques might have led to the detection of fronto-parietal effects. Since most participants developed only occasional ecstasy and/or amphetamine use we cannot exclude the possibility that heavier use patterns might affect fronto-parietal functioning.

In conclusion findings from the present study suggest ecstasy-specific effects on memory-related hippocampal functioning. In contrast to findings from previous reports effects on fronto-parietal working memory networks could not be confirmed. The pattern of altered hippocampal, yet unaffected fronto-parietal functioning provide further support for the notion that the hippocampal region might be specifically vulnerable to the neurotoxic effects of ecstasy.

Conflicts of interest:

The author(s) declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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Table 1 Baseline demographic features and drug use patterns of interim abstinent users (controls, n=12) and users who began using ecstasy and/or amphetamine on a regular basis (user, n=17): Mean (± standard deviation).

	Controls (n = 12)	User (n = 17)
Demographics		
Age	23.42 (±3.97)	22.71 (±3.18)
Gender (m:f)[1]	11:1	14:3
Education (years)	15.17 (±3.03)	14.80 (±2.65)
Cannabis use patterns		
Age of first use	15.58 (±1.83)	15.59 (±2.37)
Lifetime dose (gram)	814.33 (±891.34)	762.74 (±893.32)
Duration of regular use (months)	53.90 (±32.16)	56.40 (±39.79)
Days of use per month (average)	11.95 (±11.65)	13.09 (±11.61)
Days of use per month (maximum)[2]	19.92 (±12.59)	22.10 (±11.39)
Average daily dose (joints)	2.70 (±1.29)	2.53 (±2.04)
Highest daily dose ever used (joints)	9.25 (±8.99)	10.35 (±6.67)
Time since last use (days)[2]	175 (±281.20)	111.62 (±214.49)
Number of positive THC screenings at baseline	4	9
Ecstasy use patterns		
Age of first use	19.78 (±2.53)	20.56 (±3.15)
Lifetime dose (pills)	2.66 (±1.77)	3.26 (±1.59)
Average one night dose (pills)	1.01 (±.30)	1.40 (±.93)
Highest one night dose (pills)	1.61 (±.65)	2.07 (±1.19)
*Time since last use (days)***	*828.33 (±769.06)*	*194.40 (±331.87)*
Amphetamine use patterns		
Age of first use	19.67 (±2.45)	19.88 (±3.16)
*Lifetime dose (gram)**	*2.29 (±1.63)*	*3.52 (±1.38)*
Average one night dose (gram)	0.39 (±.23)	0.53 (±.33)
Highest one night dose (gram)	.85 (±.68)	1.23 (±.80)
*Time since last use (days)[2]**	*470.67 (±759.15)*	*208.31 (±481.90)*

T values were calculated using unpaired t-test; 2-tailed (df 27).
[1]Comparison tested with χ^2 Fischer´s Exact Test (df 1); Exact Significance (2-sided) are reported.
[2]Comparison tested with Mann-Whitney-U-test; Asymptotic Significance (2-sided) reported. **Significant difference, p<.01
. *Significant difference, p<.05

Table 2: Patterns of interim drug use of interim abstinent users (controls, n=12) and users who began using ecstasy and/or amphetamine on a regular basis (user, n=17): Mean (± standard deviation).

	Controls (n = 12)	User (n = 17)
Interim cannabis use patterns		
Interim cumulative dose (gram)	71.56 (±100.98)	146.65 (±194.06)
Duration of regular use (months)[2]	5.92 (±1.79)	8.64 (±5.39)
Days of use per month (average)	11.83 (±10.85)	18.67 (±9.62)
Days of use per month (maximum)	19.29 (±10.99)	23.62 (±8.29)
Average daily dose (joints)	2.00 (±1.04)	2.26 (±2.06)
Highest interim daily dose (joints)	5.87 (±6.42)	5.58 (±4.37)
Time since last use (days)[2]	13.57 (±24.46)	25.40 (±76.44)
Number of positive THC screenings at baseline	3	10
Interim ecstasy use patterns (n = 15)		
Interim cumulative dose (pills)	X	9.50 (±7.89)
Days of use per month (average)	X	.96 (±1.48)
Days of use per month (maximum)	X	2.71 (±2.36)
Average 1-night dose (pills)	X	1.36 (±.66)
Highest interim 1-night dose (pills)	X	2.02 (±1.06)
Time since last use (days)	X	86.33 (±80.12)
Interim amphetamine use patterns (n = 16)		
Interim cumulative dose (gram)	X	10.61 (±7.52)
Days of use per month (average)	X	2.11 (±2.18)
Days of use per month (maximum)	X	4.21 (±4.88)
Average 1-night dose (gram)	X	.77 (±.65)
Highest interim 1-night dose (gram)	X	1.14 (±.67)
Time since last use (days)	X	47.44 (±73.03)

T values were calculated using unpaired t-test; 2-tailed (df 27).
[1]Comparison tested with χ^2 Fischer´s Exact Test (df 1); Exact Significance (2-sided) are reported.
[2]Comparison tested with Mann-Whitney-U-test; Asymptotic Significance (2-sided) reported..

Titles and legends to figures:

Figure 1: Behavioral performance during the n-back tasks
Mean correct responses and mean reaction times of correct responses on target trials in the n-back tasks of interim abstinent users (controls, n=12) and users who began using ecstasy and/or amphetamine on a regular basis (user, n=17) at baseline and 12-month follow-up.

Figure 2: Behavioral performance during the associative memory task
Mean correct retrieved profession categories during the retrieval run of the associative memory task of interim abstinent users (controls, n=12) and users who began using ecstasy and/or amphetamine on a regular basis (user, n=17) at baseline and 12-month follow-up.

Figure 3: Main effect of task during associative encoding
Main effect of task for the pooled encoding conditions of the associative memory task (active > control; $p<.05$, Family wise error and small volume corrected) in the ROI comprising the hippocampus and parahippocampal gyrus (bilateral). Maximum t value located at x=31,y=–9, z=–13 (Talairach-space).

Figure 4: GROUP x TIME Interaction in the hippocampal ROI
GROUP x TIME interaction in left parahippocampal activity during encoding in the ROI comprising the hippocampus and parahippocampal gyrus (bilateral). Crosshairs at maximum t-value ($t = 6.33$; $p<.05$, Family wise error and small volume corrected) located in Talairach-space at x=-19, y=-40, z=-4.

Figure 5: Percent signal change in the left parahippocampal ROI for both groups at t1 and t2
Percent signal change in the left parahippocampal gyrus for the active condition of the associative encoding task and the corresponding control condition for interim abstinent users (controls) and users who began using ecstasy and/or amphetamine on a regular basis (user) at baseline and 12-month follow-up.
t1 = baseline, t2 = 12 months follow-up, on = encoding task: active condition, off = encoding task: control condition.

Figure 1

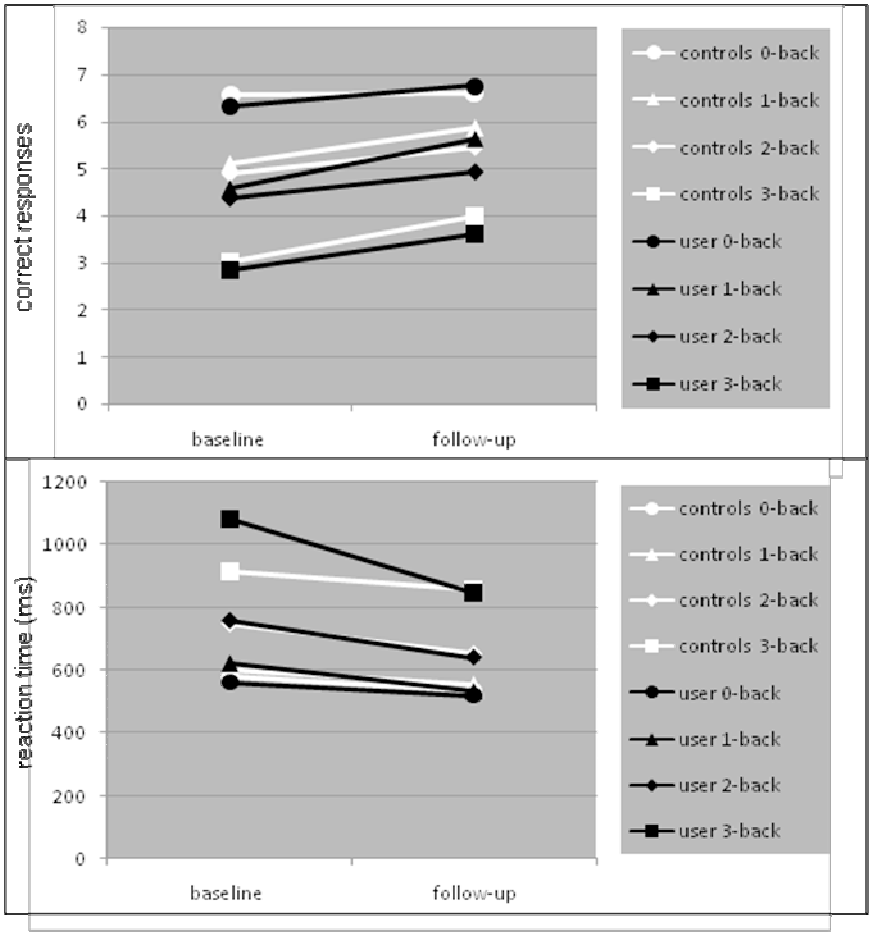

correct responses
8
7
6
5
4
3
2
1
0
baseline
follow-up
controls 0-back
controls 1-back
controls 2-back
controls 3-back
user 0-back
user 1-back
user 2-back
user 3-back
reaction time (ms)
1200
1000
800
600
400
200
0
baseline
follow-up
controls 0-back
controls 1-back
controls 2-back
controls 3-back
user 0-back
user 1-back
user 2-back
user 3-back

Figure 2

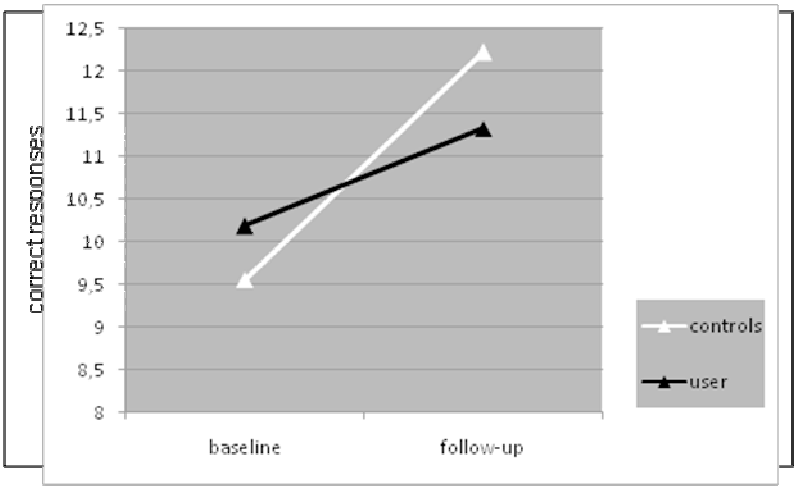

correct responses
12,5
12
11,5
11
10,5
10
9,5
9
8,5
8
baseline
follow-up
controls
user

Figure 3

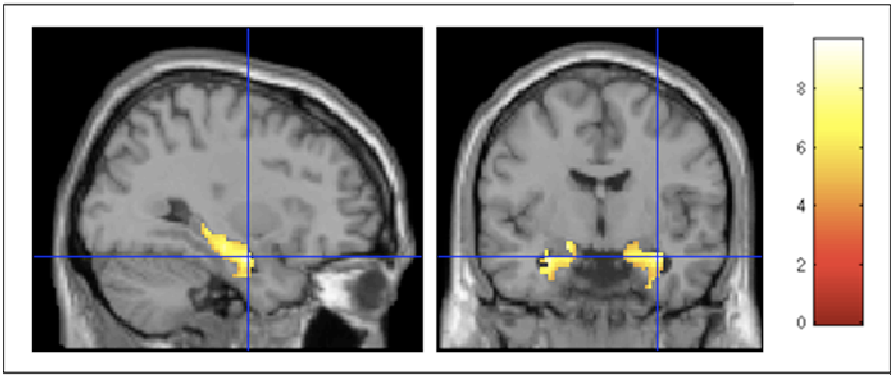


Figure 4

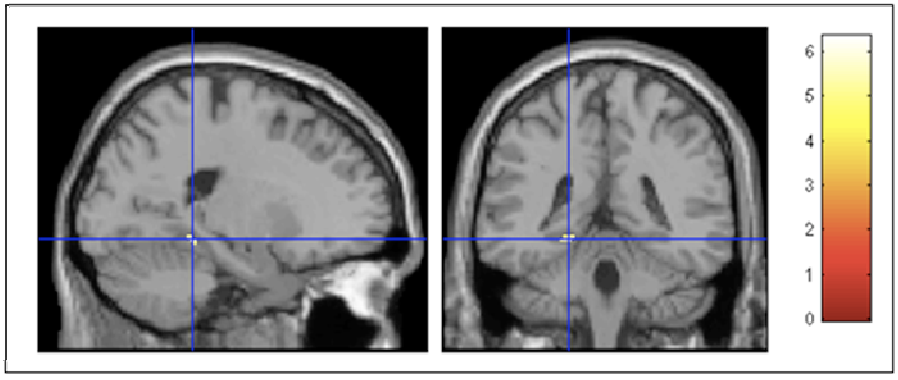
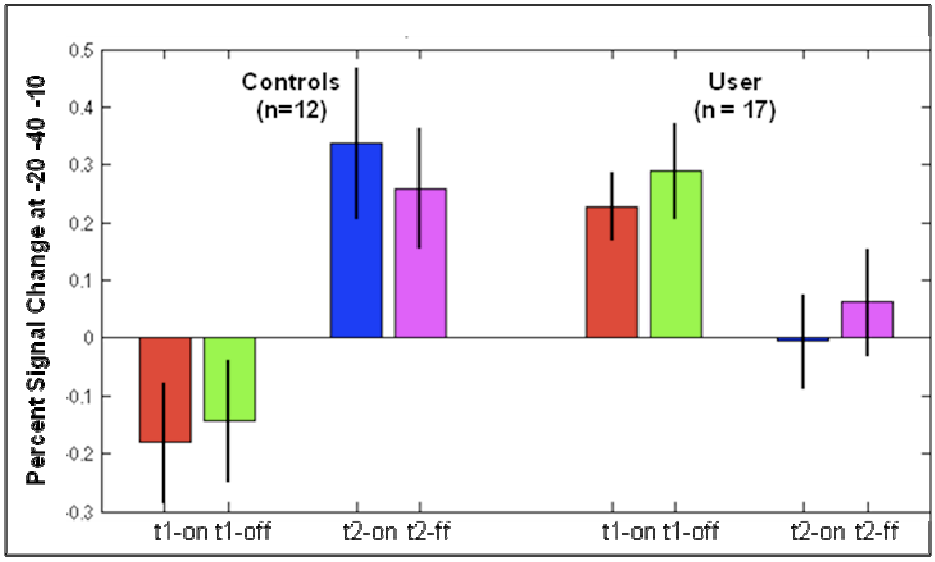


Figure 5


Percent Signal Change at -20 -40 -10
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
Controls
(n=12)
User
(n = 17)
t1-on t1-off
t2-on t2-ff
t1-on t1-off
t2-on t2-ff

Die hier vorgelegte Dissertation habe ich eigenständig und ohne unerlaubte Hilfsmittel angefertigt. Die Dissertation wurde in der vorliegenden oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

_____

(Benjamin Becker)

Köln, den 24.04.2010