Evaluation neuroprotektiver Strategien am Beispiel ausgewählter neurodegenerativer Erkrankungen: Amyotrophe Lateralsklerose und Alkoholabhängigkeit

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1 Einleitung

1.1 Behandlungsstrategien bei neurodegenerativen Erkrankungen: Neuroprotektion

Die Entwicklung von Behandlungsstrategien bei neurodegenerativen Erkrankungen gehört zu einem der Schwerpunkte in der medizinischen Forschung. Durch sich immer weiter verbessernde Lebensbedingungen und eine hochwertige medizinische Versorgung hat sich die durchschnittliche Lebenserwartung in den westlichen Industrienationen zunehmend erhöht. Mit steigendem Lebensalter wächst jedoch gleichfalls das Risiko, eine neurodegenerative Erkrankung zu entwickeln (Ferri et al., 2005; Tanner, 1992).

Der Begriff „Neurodegeneration“ definiert sich über eine progrediente Schädigung von neuronalen Strukturen oder Funktionen, also pathologischen Prozessen, die in einer Fehlfunktion oder dem Tod von Nervenzellen resultieren (z.B. Przedborski et al., 2003). Unter der Bezeichnung „neurodegenerative Erkrankungen“ lässt sich somit ein großes Spektrum an Störungsbildern subsumieren: neurologische und neuropsychiatrische, chronisch progredient verlaufende Erkrankungen, wie z.B. Morbus Alzheimer, Morbus Parkinson, Chorea Huntington, Amyotrophe Lateralsklerose oder Schizophrenie (Perez-Neri et al., 2006; Przedborski et al., 2003), aber auch Erkrankungen, die durch ein akutes Ereignis neurodegenerative Prozesse auslösen, wie z.B. Schlaganfall oder Neurotrauma (Mattson, 2000). Im Sinne der o.g. Definition können ebenso Abhängigkeiten von psychotropen Substanzen zu neurodegenerativen Erkrankungen gezählt werden (Nixon, 2006).

Trotz unterschiedlicher Ätiologien und klinischer Phänotypen besitzen die genannten Krankheitsbilder einige gemeinsame Merkmale. Sie verursachen immense sozioökonomische Kosten, verlaufen in den meisten Fällen progredient, sind schwer beeinträchtigend und unheilbar. Für viele neurodegenerative Erkrankungen konnten eine Reihe symptomatischer und palliativer Therapieansätze entwickelt werden. Präventive oder kausale Therapien stehen jedoch nicht zur Verfügung, haben nicht den erhofften Effekt gezeigt oder sind noch weit davon entfernt, in der klinischen Praxis überprüft werden zu können. Erschwerend für die Entwicklung erfolgversprechender Konzepte ist die Tatsache, dass allen Erkrankungen unterschiedliche Ursachen zugrunde liegen und sie zudem in den meisten Fällen multifaktoriell bedingt sind. Jede einzelne Erkrankung ist dabei durch ein einzigartiges, komplexes Zusammenspiel

verschiedenster Faktoren gekennzeichnet. Genetische Prädisposition und Umwelteinflüsse sowie deren Wechselwirkung münden dabei in krankheitsspezifische, klinische Phänotypen, die die Grundlage für die klinische Klassifikation der Störungsbilder darstellen (Abbildung 1a; Ehrenreich et al., 2006). Da in den meisten Fällen noch nicht einmal alle „key player“ bekannt sind, verringert sich die Aussicht auf Heilungschancen in naher Zukunft weiter. Neben einer Intensivierung prophylaktischer Maßnahmen gewinnt die Forderung nach innovativen Behandlungsstrategien mit realisierbareren Zielen somit zunehmend an Bedeutung.

Ein Ansatzpunkt solcher Strategien ergibt sich daraus, dass neurodegenerative Erkrankungen auf gemeinsamen pathophysiologischen Mechanismen beruhen. Ursachenunabhängig tragen dabei verminderte Neurogenese, erhöhte neuronale Apoptose, Excitotoxizität, oxidativer Stress, Inflammation, mitochondriale Dysfunktion, verzögertes axonales Aussprossen, Dysregulation von Neurotransmittersystemen, veränderte Synaptogenese oder Synapsenfunktionen, gestörter Calciummetabolismus, Akkumulation zellulärer Aggregate und neurovaskuläre Dysfunktion zur Schädigung neuronaler Funktionen oder Strukturen und somit zum Fortschreiten der Erkrankung bei. Diese „gemeinsame Endstrecke“ der Neurodegeneration bildet den Angriffspunkt krankheitsunspezifischer Behandlungsversuche und somit das Ziel von Neuroprotektion (Abbildung 1b; Ehrenreich et al., 2004; Ehrenreich et al., 2006).

Abbildung 1a: Die meisten neurodegenerativen Erkrankungen sind heterogenen Ursprungs.

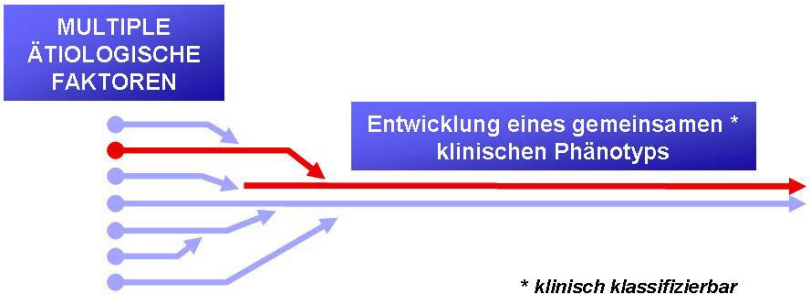
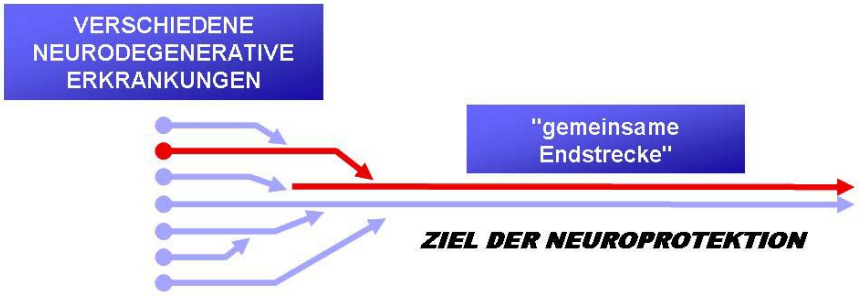


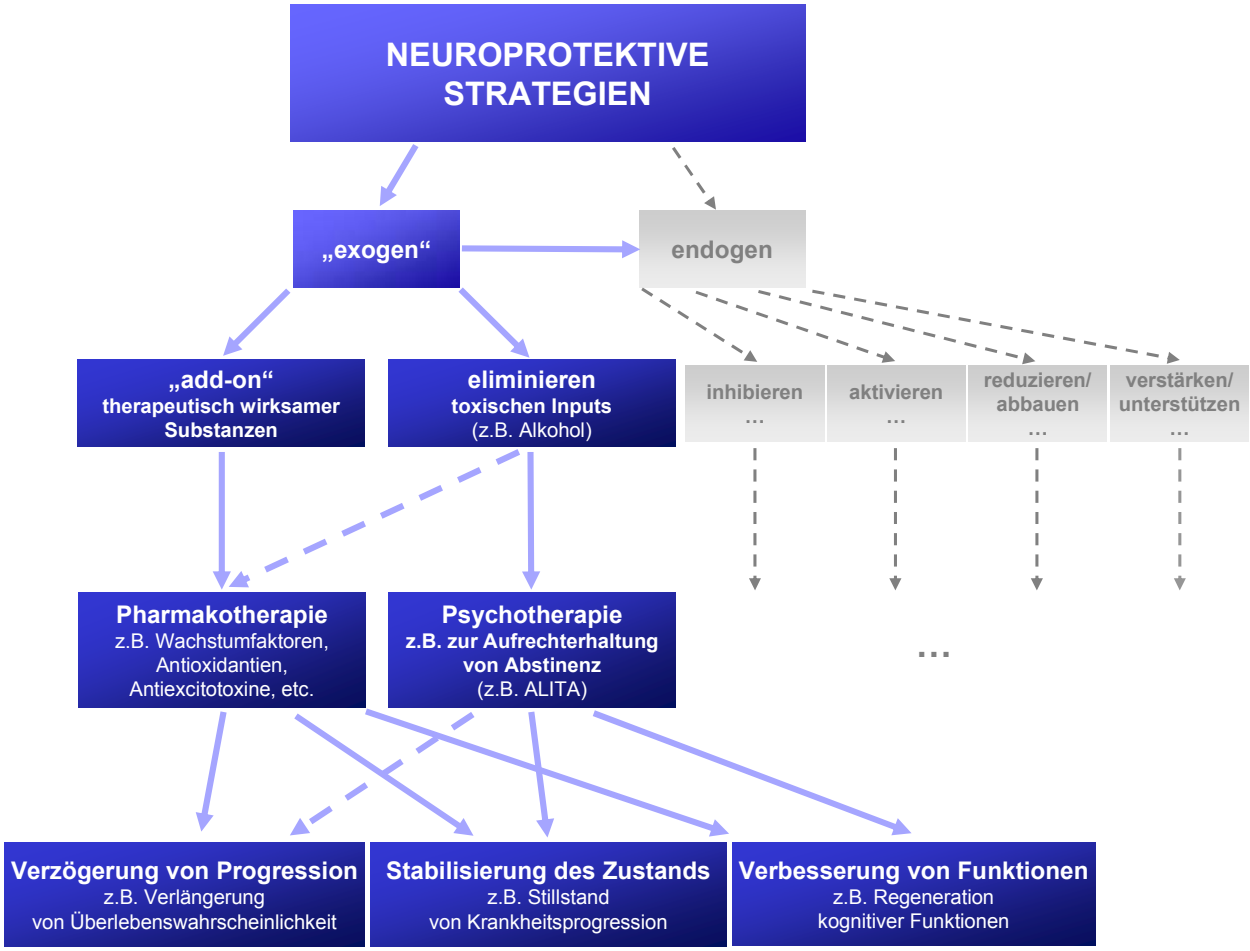
Abbildung 1b: Den meisten neurodegenerativen Erkrankungen liegen gemeinsame pathophysiologische Mechanismen zugrunde.



Abbildungen angelehnt an Ehrenreich et al (2006).

Neuroprotektion bedeutet dabei „… to maintain the highest possible integrity of cellular interactions/intracellular communication in the brain…", d.h. „… protection of neural function" (Ehrenreich & Sirén, 2001). Neuroprotektive Behandlungsstrategien sollen Neurodegeneration entgegenwirken und/oder Regeneration fördern. Aus dieser Definition ergeben sich verschiedenste Optionen für die Entwicklung neuroprotektiver Ansätze (Abbildung 2).

Abbildung 2: Neuroprotektive Ansatzpunkte und deren Realisierung



Zum einen kann – „exogen" induziert – die Verstärkung endogener Schutzmechanismen, also des zelleigenen Regenerationspotentials im Mittelpunkt stehen. Zum anderen kann es einer Manipulation „von außen" gelingen, degenerative Prozesse günstig zu beeinflussen. Eine absolute Trennung dieser Wirkweisen ist jedoch häufig nicht möglich. Auf rein endogen ablaufende neuroprotektive Überlebensprogramme, die unter pathologischen Bedingungen automatisch aktiviert werden, wird in dieser Arbeit nicht näher eingegangen. Da es, wie oben beschrieben, bei neurodegenerativen Erkrankungen zum fortschreitenden Funktionsverlust oder zum Absterben von

Nervenzellen kommt, scheinen endogene Mechanismen außerdem offensichtlich nicht ausreichend zu sein, um neurodegenerative Prozesse aufzuhalten.

Studien zur Neuroprotektion bedienen sich am häufigsten therapeutisch wirksamer Substanzen. Pharmakotherapeutisch wird – Schritt für Schritt von der Zellkultur über Tiermodelle der Erkrankung bis zur klinischen Studie – das neuroprotektive Potential einer Kandidatensubstanz geprüft (siehe auch Abschnitt 2.1.1). Ziel einer neuroprotektiven Strategie kann jedoch auch die Eliminierung eines bekannten toxischen Inputs sein, wie bei Abhängigkeiten von psychotropen Substanzen. Als Mittel zur Herstellung und Aufrechterhaltung von Abstinenz kann zwar auch auf pharmakotherapeutische Ansätze zurückgegriffen werden, diese kommen aber i.d.R. ohne psychotherapeutische Begleitung nicht aus (Ehrenreich & Krampe, 2004; Krampe et al., 2006a). Psychotherapeutische Programme stellen somit nach wie vor die am häufigsten angewandten „neuroprotektiven" Behandlungsstrategien bei Abhängigkeitserkrankungen dar. Messbar wird der Erfolg eines neuroprotektiven Konzeptes, wenn es ihm gelingt, Krankheitsprogression zu verzögern, einen Stillstand der Erkrankung zu erreichen oder im günstigsten Fall beeinträchtigte Funktionen zu verbessern. Belegen lassen sich diese Endpunkte mit einer geeigneten Auswahl krankheitsspezifischer Parameter, angefangen von Überlebens-/Abstinenzwahrscheinlichkeit über biochemische Krankheitsmarker bis hin zu strukturelle und funktionale Veränderungen erfassenden Variablen.

Für die Selektion und Durchführung neuroprotektiver Behandlungsstrategien sollten diese weitere wichtige Merkmale aufweisen: Neben der Sicherheit und Nebenwirkungsarmut der eingesetzten Mittel muss eine starke Interaktion zwischen Grundlagen- und klinischer Forschung vorliegen. Erst mit einer soliden grundlagenwissenschaftlichen Basis kann die fragliche Intervention rasch in klinische Prüfungen an einer sorgfältig ausgewählten Patientenpopulation umgesetzt werden. Den Erfolg vorausgesetzt, ist es unter Rückgriff auf weitere präklinische Forschung möglich, das Wissen über konkrete Wirkmechanismen zu erweitern und so wiederum die Behandlung zu optimieren. Um abschließend den Erfolg und die Qualität neuroprotektiver Ansätze beurteilen zu können, müssen entsprechende Studien sensitiv, valide und reproduzierbar Veränderungen in krankheitsabhängig relevanten Variablen erfassen können.

1.2 Fokus der vorliegenden Arbeit

Die beiden Publikationen, die als Bestandteile dieser Dissertation eingehen, beleuchten eingehend unterschiedliche Perspektiven neuroprotektiver Behandlungsansätze. Am Beispiel von zwei neurodegenerativen Erkrankungen sollen dabei unterschiedliche Strategien und deren Effektivität illustriert werden.

Die neurologische Erkrankung ***Amyotrophe Lateralsklerose (ALS)*** gehört zu einer der Prototypen neurodegenerativer Erkrankungen. Der selektive Untergang von Motoneuronen im motorischen Cortex (1. Motoneuron) sowie im ventralen Horn des Rückenmarks und Stammhirns (2. Motoneuron) stellt dabei das vorherrschende Merkmal dieser progredient und tödlich verlaufenden Erkrankung dar (Rowland & Shneider, 2001). Eine komplexe, multifaktorielle Kombination verschiedener genetischer und biochemischer Regulationsmechanismen ist an deren Entstehung beteiligt, wobei die genaue Ätiologie noch unbekannt ist. Unabhängig vom zugrunde liegenden molekularen/genetischen Defekt tragen jedoch u.a. folgende pathophysiologische Mechanismen zur Motoneurodegeneration bei: Apoptose, gestörter axonaler Transport, mitochondriale Dysfunktion, anormale Neurofilament-anhäufungen, Proteinaggregation, gestörte Proteasom-Funktion, Inflammation und Excitotoxizität (Boillee et al., 2006; Bruijn et al., 2004; Cleveland, 1999; Cleveland & Rothstein, 2001; Goodall & Morrison, 2006; Julien, 2001; Van Damme et al., 2005; Van Den Bosch et al., 2006). Als „final common pathway“, der diese Prozesse koordiniert, wird oxidativer Stress angenommen. Diese gestörte Balance zwischen der Bildung und Eliminierung reaktiver Sauerstoffverbindungen (*Reactive Oxygen Species*; ROS) und reaktiver Stickstoffverbindungen (*Reactive Nitrogen Species*; RNS) gilt dabei als gemeinsamer Nenner des durch Excitotoxizität und Apoptose verursachten Neuronenuntergangs (Barber et al., 2006; Emerit et al., 2004; Simpson et al., 2003). Aufgrund dessen ist oxidativer Stress einer der essentiellen Ausgangspunkte neuroprotektiver Forschungsbemühungen bei ALS.

Mit Melatonin als Antioxidanz dokumentiert **die erste Originalarbeit (Weishaupt* et al., 2006)** die sukzessive Durchführung eines neuroprotektiven, pharmakotherapeutischen „Add-on“-Ansatzes bei ALS von der Zelle bis zum Menschen.

Mit der ***Alkoholabhängigkeit*** liegt ein neuropsychiatrisches Störungsbild vor, bei dem die Ursache für Neurodegeneration offensichtlich ist. Chronischer und übermäßiger Alkoholkonsum resultiert dabei in metabolischen, morphologischen und funktionalen Schädigungen des Gehirns. Unter Einfluss der Noxe führen insbesondere

erhöhte Glutamat-Neurotransmission, Excitotoxizität, Überaktivierung von NMDA-Rezeptoren und damit einhergehende Akkumulation intrazellulären Calciums, oxidativer Stress sowie Apoptose zum kontinuierlichen Untergang von Neuronen (Fadda & Rossetti, 1998). Darüber hinaus verursacht Alkohol Veränderungen an Dendriten und Synapsen, greift schädigend in verschiedene Neurotransmitterhaushalte ein (Dopamin, Acetylcholin, Serotonin, Noradrenalin; Fadda & Rossetti, 1998) und inhibiert Neurogenese (Herrera et al., 2003; Nixon, 2006). Als Zeichen alkohol-induzierter Neurodegeneration lässt sich allgemein eine Schädigung grauer und weißer Substanz im Gehirn festhalten, die von Volumenminderung und Ventrikelerweiterung begleitet wird (Harper & Kril, 1990). Davon sind fast alle Hirnregionen betroffen, insbesondere Hypothalamus, Frontallappen, präfrontaler Cortex, Cerebellum, Amygdala und Locus coeruleus (Harper, 1998; Harper et al., 2003; Sullivan & Pfefferbaum, 2005). Der Hippocampus gilt dabei als eine der Regionen, die besonders sensitiv auf den toxischen Einfluss von Alkohol reagiert (Agartz et al., 1999; Laakso et al., 2000; Sullivan et al., 1995; White et al., 2000). Von den morphologischen Veränderungen wird wiederum angenommen, dass sie ursächlich für funktionale Beeinträchtigungen, wie kognitive Defizite sind. Viele der dokumentierten Leistungseinbußen in Exekutivfunktionen, Lernen und Gedächtnis sowie insbesondere dem visuell-räumlichen Gedächtnis indizieren dabei eine hippocampale Dysfunktion (Beatty et al., 1996; Bowden & McCarter, 1993; Kempermann et al., 2004; Matthews & Morrow, 2000; Weitemier & Ryabinin, 2003). Da bei Alkoholabhängigkeit der Auslöser für Neurodegeneration bekannt ist, muss Abstinenz als logische Konsequenz für Neuroprotektion gelten. Tatsächlich konnte bereits gezeigt werden, dass chronischem Alkoholkonsum keine irreversible Hirnatrophie folgt, sondern dass die strukturellen Veränderungen unter Abstinenz zumindest teilweise reversibel sind (Bartsch et al., 2007; Bendszus et al., 2001; Fadda & Rossetti, 1998). In diesem Zusammenhang scheint auch eine Regeneration kognitiver Funktionen möglich.

In der zweiten Publikation (Bartels et al., 2007) wird der Verlauf hippocampus-assoziierter, kognitiver Leistungen bei Alkoholabhängigen verfolgt. Als neuroprotektive Strategie zur Aufrechterhaltung der Abstinenz dient ein psychotherapeutisches Behandlungskonzept: die *A*mbulante *L*angzeit/ntensiv*T*herapie für *A*lkoholkranke.

2 Originalartikel

2.1 Amyotrophe Lateralsklerose: Pharmakotherapie als neuroprotektive „Add-on"-Strategie

2.1.1 Einführung in die Fragestellung

Die ‚klassische' Entwicklung neuroprotektiver, pharmakotherapeutischer Strategien gestaltet sich in folgender Weise: In der präklinischen Phase wird eine Kandidatensubstanz *in vitro* und/oder *in vivo* unter modellhaften Bedingungen der Erkrankung auf Wirkung und Wirkmechanismen getestet. Nach einer Evaluation der Verträglichkeit und Sicherheit des Wirkstoffs am Menschen (Phase I) wird in klinischen Prüfungen der Therapieerfolg placebo-kontrolliert und in der Langzeitbehandlung erhoben. Neben der Dosisfindung (Phase II) wird auch untersucht, ob die Substanz der bereits zugelassenen Standardtherapie überlegen ist (Phase III). Den Abschluss bilden große klinische Studien zur Bestätigung des Langzeitsicherheitsprofils und zur Kosten-Nutzen-Analyse im Vergleich zu anderen Medikamenten (Phase IV). Als Faustregel gilt, dass nach einer Testphase von bis zu 15 Jahren nur 1 von 10.000 gescreenten Substanzen zur Zulassung auf den Markt gelangt.

Zur Herstellung ALS-ähnlicher Bedingungen im experimentellen Setting stehen spezielle Zellkulturen und Tiermodelle zur Verfügung. Zu den am häufigsten verwandten *in vitro*-Modellen gehören Motoneurone (Silani et al., 2000) oder auch motoneuronale NSC-34 Zellen (Eggett et al., 2000), die z.B. Glutamat ausgesetzt werden oder Axiotomie-vermittelt zugrunde gehen (Elliott, 1999). Frühere *in vivo*-Modelle spontan auftretender genetischer Defekte (pmn-Mäuse; Schmalbruch et al., 1991) sind zugunsten reproduzierbarer, transgener Nagermodelle in den Hintergrund getreten (z.B. G^{93A}, G^{37R}, G^{85R}; Gurney et al., 1994; Ripps et al., 1995; Wong et al., 1995). Grundlage für die aktuell am häufigsten verwandten, SOD1 überexprimierenden Mauslinien bildete dabei die Entdeckung einer Mutation von Cu/Zn-Superoxid-Dismutase (SOD1) als Ursache für die Entwicklung einer familialen ALS-Form in 1-2% aller ALS-Fälle (Rosen, 1993). Als wichtiges Merkmal dieser Tierexperimente ist der typischerweise präsymptomatische Behandlungsbeginn zu nennen. Im Vergleich zur humanen ALS sind Modellsysteme natürlich zahlreichen weiteren Beschränkungen unterlegen, dennoch stellen sie aktuell die beste Option zur Untersuchung pathologischer Prozesse und pharmakotherapeutischer Wirkstoffe dar.

Unter Zuhilfenahme der beschriebenen Modellsysteme wurde bereits eine Vielzahl neuroprotektiver Studien bei ALS durchgeführt. Dabei zielten die eingesetzten

Substanzen im wesentlichen auf die bisher bekannten pathophysiologischen Mechanismen ab, mit Hilfe von anti-glutamatergen oder anti-inflammatorischen Wirkstoffen, neurotrophen Faktoren oder Immunmodulatoren (Goodall & Morrison, 2006; Meininger, 2005; Morrison, 2002; Traynor et al., 2006). Obwohl die meisten Konzepte sich im ALS-Mausmodell als erfolgreich erwiesen (Lebensverlängerung, Verzögerung des Krankheitsverlaufs oder Steigerung der Muskelkraft), scheiterten alle Versuche bei der Anwendung am Menschen. Darunter fallen auch bisher eingesetzte Antioxidantien (Desnuelle et al., 2001; Graf et al., 2005; Orrell et al., 2005; Pattee et al., 2003). Ergebnisse weiterer Studien stehen noch aus (z.B. Minocyclin; Gordon et al., 2004; Pontieri et al., 2005; Van Den Bosch et al., 2002; Zhu et al., 2002), andere Vorgehensweisen sind noch weit davon entfernt, in die klinische Praxis umgesetzt zu werden (Azzouz et al., 2004; Guo et al., 2003; Kaspar et al., 2003; Lepore & Maragakis, 2006; Raoul et al., 2005; Silani et al., 2004). Die einzige Ausnahme bildet Riluzol, ein Antiexcitotoxin, welches eine Lebensverlängerung von zwei bis drei Monaten bei sporadischer ALS zeigen konnte (Bensimon et al., 1994; Lacomblez et al., 1996). Nach wie vor ist Riluzol das einzige, für die Indikation ALS zugelassene Medikament. Dem marginalen Effekt stehen jedoch hohe Kosten und zahlreich dokumentierte Nebenwirkungen, wie Übelkeit, Erbrechen, Schwindel, allgemeine Schwäche und Anstieg der Lebertransaminasen gegenüber (Bensimon & Doble, 2004; Miller et al., 2007).

Aufgrund der Fülle und bisherigen Erfolglosigkeit neuroprotektiver Strategien bei ALS sind bereits einige Übersichtsarbeiten entstanden, die Kriterien für eine bessere Selektion geeigneter Kandidatensubstanzen aufstellen (Cheung et al., 2006; Meininger, 2005; Traynor et al., 2006; Turner et al., 2001).

Die folgende Veröffentlichung skizziert die Entwicklung und Durchführung eines neuroprotektiven „Add-on“-Ansatzes bei ALS von der Zelle bis zum Menschen. Ausgehend vom wissenschaftlichen/therapeutischen Rational, dass bei dieser Motoneuronerkrankung oxidativer Stress als ein gemeinsamer molekularer Nenner für Krankheitsprogression gilt, wird Melatonin, ein amphiphiles Molekül mit einem einzigartigen Spektrum direkter und indirekter antioxidativer Wirkmechanismen, als neuroprotektive Kandidatensubstanz in verschiedenen Modalitäten exploriert: 1) in motoneuronalen Zellkulturen (NSC-34), 2) im transgenen $SOD1^{G93A}$-Mausmodell und 3) im Rahmen einer offenen Sicherheitsstudie an Patienten mit sporadischer ALS.

Unter Verwendung excitotoxischer und ROS generierender Bedingungen konnte anhand einer motoneuronalen Zellkultur erfolgreich nachgewiesen werden, dass

Melatonin dosisabhängig glutamat-induzierten Zelltod im Sinne eines Radikalfängers reduziert. Im verblindeten tierexperimentellen Part erwies sich die vor Krankheitsbeginn begonnene, orale Hochdosis-Melatoninbehandlung vorteilhaft: Verabreichung von Melatonin verzögerte die Krankheitsprogression und verlängerte die Lebensdauer im Vergleich zu einer placebo-behandelten Gruppe. In der daraufhin durchgeführten Sicherheitsstudie an 31 ALS-Patienten in unterschiedlichsten Krankheitsstadien kamen im Gegensatz zu einer ersten Pilotstudie mit oraler Melatoningabe (30-60mg/d; Jacob et al., 2002) Melatonin-Suppositorien (300mg/d) als „Add-on"-Therapie zum Einsatz. Auch in der Langzeitapplikation von bis zu 24 Monaten zeigte Melatonin bei hoher Compliance eine gute Verträglichkeit, Nebenwirkungen blieben aus. Obwohl der Wirksamkeitsnachweis einer Hochdosis-Melatoninbehandlung innerhalb dieser offenen Vorstudie nicht möglich war, konnten bereits erste Hinweise zur Effektivität der Behandlung mit einer Analyse biochemischer Marker für oxidativen Stress gefunden werden: Im Serum zirkulierende, vor Behandlungsbeginn erhöhte Protein-Carbonyle normalisierten sich unter der chronischen Melatoninapplikation auf ein Niveau alters- und geschlechtsgematchter Kontrollen.

Die hier vorgeschlagene neuroprotektive Kandidatensubstanz erfüllt somit mehrere der geforderten Kriterien: 1) wissenschaftliches Rational: Relevanz oxidativen Stresses bei ALS, Melatonin als multipotenter Radikalfänger im zentralen Nervensystem, 2) ausgezeichnete Verträglichkeit Melatonins, 3) Wirksamkeit im etablierten ALS-Mausmodell ($SOD1^{93A}$) und 4) erste Hinweise auf Wirksamkeit und Wirkweise durch Reduktion oxidativen Stresses bei sporadischer ALS. Zusammengefasst liefern diese Ergebnisse die Basis für die Durchführung einer randomisierten, doppelblinden, placebo-kontrollierten, klinischen Studie mit dem Ziel der Neuroprotektion durch Erhöhung der antioxidativen Kapazität bei ALS.

2.1.2 Originalartikel

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Reduced oxidative damage in ALS by high-dose enteral melatonin treatment

Abstract: Amyotrophic lateral sclerosis (ALS) is the collective term for a fatal motoneuron disease of different etiologies, with oxidative stress as a common molecular denominator of disease progression. Melatonin is an amphiphilic molecule with a unique spectrum of antioxidative effects not conveyed by classical antioxidants. In preparation of a possible future clinical trial, we explored the potential of melatonin as neuroprotective compound and antioxidant in: (1) cultured motoneuronal cells (NSC-34), (2) a genetic mouse model of ALS ($SOD1^{G93A}$-transgenic mice), and (3) a group of 31 patients with sporadic ALS. We found that melatonin attenuates glutamate-induced cell death of cultured motoneurons. In $SOD1^{G93A}$-transgenic mice, high-dose oral melatonin delayed disease progression and extended survival. In a clinical safety study, chronic high-dose (300 mg/day) rectal melatonin was well tolerated during an observation period of up to 2 yr. Importantly, circulating serum protein carbonyls, which provide a surrogate marker for oxidative stress, were elevated in ALS patients, but were normalized to control values by melatonin treatment. This combination of preclinical effectiveness and proven safety in humans suggests that high-dose melatonin is suitable for clinical trials aimed at neuroprotection through antioxidation in ALS.

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Key words: amyotrophic lateral sclerosis, human safety study, melatonin, motoneurons, neuroprotection, reactive oxygen species, transgenic models

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, affecting predominantly motoneurons in the cerebral cortex and anterior horn of the spinal cord. Dysfunction and premature death of these neurons causes spasticity, hyperreflexia, muscular atrophy, and generalized paralysis. Respiratory failure is the main cause of death within 2–5 yr after diagnosis. Most ALS cases occur sporadic. Among the 5–10% familial forms, about 20% are associated with mutations in the gene for superoxide dismutase (SOD1) [1]. The disease is incurable and practically without treatment.

Different pathological mechanisms have been suggested to contribute to cell death in motoneuron diseases, independent of the underlying molecular/genetic defect. These include impaired axonal transport, mitochondrial dysfunction, neurofilament disorganization, protein aggregation, and impaired proteasome function [2–4]. Also excitotoxic mechanisms contribute to ALS. Indeed, the only therapeutic drug with a marginal effect on patient survival is riluzole, an antiexcitotoxin [5, 6]. Increased levels of glutamate were found in the cerebrospinal fluid of ALS patients [7]. In the spinal cord, a decreased glutamate uptake has been attributed to decreased glutamate transporter expression in the anterior horn, both in sporadic

cases of ALS and in SOD1 transgenic models [8–10]. Transgenic overexpression of the glutamate transporter EAAT2 delayed symptoms in mutant SOD1 transgenic mice [11]. Recently, a defect in AMPA-type glutamate receptor editing, leading to enhanced Ca^{2+} permeability, has been reported for a subset of ALS patients [12]. Interestingly, ocular motoneurons that are intrinsically resistant to ALS have higher calcium buffering capacities [13, 14].

It is well known that excitotoxicity by glutamate includes the generation of reactive oxygen species (ROS) [15]. For example, elevated intracellular calcium causes mitochondrial dysfunction, impairment of the respiratory chain, activation of NO synthases (NOS), and the generation of toxic radicals. An upregulation of iNOS was found in $SOD1^{G93A}$-transgenic mice [16]. It has also been proposed that aberrant enzymatic activity of mutant SOD1 leads to the production of toxic hydroxyl radicals and nitrotyrosine [4, 17]. By oxidative stress, in turn, free radical species can enhance monomer formation and aggregation of SOD1 [18]. Similarly, ROS alter the properties of neurofilaments that play a key role in axonal transport which is impaired in models of motoneuron disease [19]. Previously tested compounds with 'simple' antioxidative properties, such as vitamin C or E, have failed to prolong survival in ALS clinical trials [20–22].

Melatonin, a derivative of the essential amino acid tryptophan, is best known for its secretion by the pineal gland in the regulation of light–dark cycle [23]. It exhibits an unusually broad spectrum of antioxidative properties [24–26]. These include scavenging of hydroxyl carbonate, alkoxyl, peroxyl, and aryl cation radicals, stimulation of glutathione peroxidase, and suppression of NOS [26]. In particular, the interference of melatonin with NO metabolism is expected to have a major neuroprotective potential, because it counteracts both, cellular damage by peroxynitrite-related radicals and Ca^{2+}-dependent excitotoxicity [25, 26]. The amphiphilic chemical structure of melatonin and its rapid transfer through the blood–brain barrier [27] argue for its usefulness as a neuroprotective drug.

The present work has been designed in preparation of a possible future clinical trial of melatonin in ALS. We show that: (1) melatonin attenuates glutamate-induced death of motoneuronal cells in a dose-dependent fashion, and concomitantly reduces generation of ROS, and that (2) high-dose melatonin treatment of $SOD1^{G93A}$-transgenic mice improves outcome with respect to disease progression and survival. Importantly, long-term high-dose treatment of human ALS patients, using suppositories as a new route of melatonin application, is well tolerated and safe. A major therapeutic effect of this antioxidant is demonstrated by serum protein carbonyl levels that are initially elevated in all ALS patients and corrected to control values by melatonin treatment.

Materials and methods

NSC-34 cell culture experiments

Motoneuronal NSC-34 cells were cultured at 37 °C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. To differentiate NSC-34 cells into a motoneuronal and glutamate-responsive phenotype [28], DMEM was replaced by DMEM/Ham's F12 supplemented by 1% FCS, 1% penicillin/streptomycin and 1% modified Eagle's medium nonessential amino acids. Cells were allowed to differentiate for 8 wk under reduced serum conditions and then seeded in 96-well plates at a density of 15 000 cells/well for the following experiments.

Glutamate toxicity assay

For melatonin treatment, melatonin (Sigma, Seelze, Germany) was dissolved in dimethylsulfoxide (DMSO; Sigma) and diluted in culture-medium to a final concentration of 10 and 50 μM. The final DMSO concentration was 0.2%, and this was used as the corresponding vehicle control. Glutamate was dissolved in phosphate-buffered saline (PBS), and added to cultures at concentrations of 2 or 10 mM, 3 hr after the start of melatonin treatment. 72 hr after start of glutamate exposure, cell viability was tested with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. MTT was added to culture wells (final concentration of 0.5 mg/mL) and incubated at 37 °C for 2 hr. Viability of motoneurons was assessed by counting MTT-positive cells in six fields within a culture well with a 20× objective. Mean values of at least four independent wells from three experiments were calculated, and cell survival expressed as percent MTT-positive cells compared with untreated control conditions.

Detection of oxidative stress in NSC-34 cells

Formation of intracellular ROS following glutamate exposure for 12 hr, or treatment with 200 μM of H_2O_2 for 30 min as positive control, was detected by loading the cells with the fluorescent oxidative stress indicator dichlorodihydrofluorescein diacetate (H_2DCFDA; Molecular Probes; final concentration 20 μM) for 30 min at 37 °C. Fluorescence intensity of the oxidized dye was assessed using a LEICA TCS confocal laser scanning microscope with a 40× oil immersion objective at 496 nm excitation and 510 to 530 nm emission wavelength. Quantification of fluorescence intensity was achieved by subtraction of background fluorescence using ImageJ software. Intensity values were calculated as mean pixel intensity expressed in arbitrary fluorescence units. For each experimental condition, mean pixel intensity of at least 150 cell bodies from at least four independent wells of three experiments and seven independently taken laser scanning microscope frames was calculated.

Transgenic mice, melatonin treatment and behavioral testing

All animal experiments were approved by and conducted in accordance with the regulations of the local Animal Care and Use Committee. Mice transgenic for human $SOD1^{G93A}$ [TgN(SOD1-G93A)1 GUR] were purchased from Jackson Laboratory and bred at the University of Göttingen Medical School and Max-Planck animal facilities. A total

of 120 mice (group size 15–30, dependent on the experiments, if not indicated otherwise in the text) were used. Transgenic mice were identified using polymerase chain reaction (PCR) according to Gurney et al. [29]. Melatonin was dissolved in ethanol, then diluted in drinking water. Final ethanol concentration was 1% in all experimental groups, and vehicle controls received 1% ethanol in drinking water. To protect melatonin from light, drinking bottles were wrapped with aluminium foil at all times, and drinking water was replaced weekly. Fluorometric determination of melatonin concentrations in drinking water showed that melatonin remained completely stable under these conditions (data not shown). Depending on the experiment, treatment started at the age of 28 days or at onset of first symptoms. Locomotor function was assessed three times per week using a mouse rotarod (TCD, Germany), starting at the age of 6 wk. A constant velocity of 10 rounds/min was used. Onset of rotarod failure was defined as the first day when animals failed to perform on the rotarod for at least 5 min when three trials were allowed. In accordance with guidelines for the care and use of laboratory animals, mice were killed when they had lost more than 15% of their weight or were unable to move upright within 30 s when laid on one side. This timepoint was defined as the age of death.

Western blot analysis

Western blot experiments were performed using early symptomatic (100 days old) mice. Lumbar or cervical spinal cords were rapidly prepared and homogenized in lysis buffer containing 50 mM of Tris-HCl, 150 mM of NaCl, 1% Triton-X 100, 0.1 mM of polymethylsulfonyl fluoride (PMSF) and 2 μL/mL of pepstatin, leupeptin, and aprotinin, pH 8.3. Lysates were centrifuged at 14 000 rpm and the protein concentration of the supernatant was determined using the BCA reagent (Pierce, Rockford, IL, USA). After separating the lysates by reducing SDS-PAGE (15% gel, 20 μg protein per lane), proteins were transferred to polyvinylidene difluoride (PVDF) membrane and blocked in 5% skim milk in PBS-T (0.1% Tween 20). Proteins were detected by incubating with the following primary antibodies directed against: human SOD1 (SOD-100; Stressgen, Victoria, Canada), beta-tubulin (Sigma), phospho-ERK1/2, and phospho-AKT (Cell Signaling Technology, Beverly, MA, USA). Primary antibodies were visualized by incubation with respective HRP-conjugated secondary antibodies (Dianova, Hamburg, Germany). For densitometrical analysis, imagequant software was used and density normalized to background values. Ratios between corresponding melatonin- and vehicle-treated animals were then calculated for individual Western blot experiments after normalization to a tubulin standard. For standardization to tubulin, Western blot membranes were stripped after SOD1 staining and re-probed with a tubulin primary antibody followed by the respective HRP-conjugated secondary antibody. Lysates from at least three different animals per experimental group were used.

Immunohistochemistry

Coronal sections (20 μm) of the freshly frozen cervical spinal cord tissue were cut in a freezing cryostat (Jung CM3000; Leica Instruments, Nussloch, Germany). The tissue was postfixed for 30 min in 4% formaldehyde in PBS and rinsed with PBS. For immunoperoxidase-labeling of microglial cells with avidin-biotin, we used the following antibodies: rabbit anti-IBA-1 (Wako-Chemicals, Neuss, Germany, 1:5000), rat anti-MAC-3 (Pharmingen-BD Biosciences, Heidelberg, Germany, 1:1000), rat anti-F4/80 (Serotec, Oxford, UK, 1:1000).

Patients

Following announcement of the safety trial to the local Ethical Committee, 31 patients were included after informed consent. Inclusion required probable or definite ALS, according to El Escorial Criteria and disease duration of not more than 6 yr. Upon entry into the study, the ALS patients were admitted to a neurology ward for 5 days. Diagnosis was confirmed and treatment initiated. If patients were on riluzole (n = 25), vitamin E (n = 23), creatine (n = 4), or amitriptylin (n = 15) at admission to the study, they were allowed to continue this medication in addition to high-dose melatonin (see Table 1). Blood samples were taken before and during the initial days of treatment, and every 3–4 months during follow-up visits. ALS functional rating score (ALSFRS) was performed regularly, together with an extensive semi-structured interview of patients and relatives including questions for adverse event monitoring.

Preparation of melatonin suppositories

Melatonin suppositories were prepared through pouring of a melted cream to avoid heat destruction of the active compound. An equivalent of 300 mg pulverized high-performance liquid chromatography (HPLC)-grade melatonin (Synopharm, Barsbuettel, Germany; BUFA, Uitgeest, Netherlands) per suppository was mixed with an

Table 1. Amyotrophic lateral sclerosis baseline medication in melatonin-treated (n = 31) patients with and without continuous high-dose vitamin E (400–5000 IU/day) treatment

	Riluzole	Amitriptylin	Baclofen	Magnesium	Vitamin C	Creatine
No vitamin E premedication (n = 8)	6 (75)	5 (63)	1 (13)	4 (50)	2 (25)	1 (13)
Vitamin E premedication started 8.2 ± 2.4 months before melatonin (n = 23)	19 (83)	10 (43)	7 (30)	12 (52)	5 (22)	3 (13)

Absolute numbers of patients given, % of the respective cohort in brackets.

equivalent amount of hard fat, warmed to a cream-like consistency, mixed to homogeneity, and poured into polyethylene wrapped shapes with a 'torpedo' design (Iphas, Wuerselen, Germany). After the mixtures cooled and solidified, the suppositories were removed with warmed spatulas, and placed in special dispensing boxes (2×5). Each package was labeled as required by German laws and rules (§14 ApoBetrO, rules of AMG for clinical trials), indicating formulation of each suppository.

Melatonin radioimmunoassay

Melatonin levels in plasma and urine were determined by radioimmunoassay (RIA), as described previously [30]. In brief, plasma melatonin was measured by a direct charcoal-based RIA using tritium-labeled melatonin and a highly specific antibody (GS 704–6483) from Guildhay Antisera (Guildford, UK). The detection limit of this assay was 1 pg/300 μL. Intra- and interassay coefficients of variation were 6% and 12%, respectively. Melatonin from urine samples was extracted into chloroform under addition of acetate buffer (pH 4.0) and 0.1 mol of NaOH. The chloroform phase was evaporated to dryness and redissolved in assay buffer for RIA measurements.

Protein carbonyl determination

Protein carbonyl measurements were performed in the serum of 19 consecutively admitted ALS patients (61.7 ± 1.9 yr, 12 males, seven females) before and after ≥4 months of melatonin treatment and of ten healthy controls (61.2 ± 2.0 years, five males, five females). Protein carbonyls were determined by a variant of the 2,4-dinitrophenylhydrazine method [31]. Serum was diluted 1:10 with ice-cold 5-mM pf potassium phosphate buffer, pH 7.5, containing leupeptin (0.5 μg/mL), aprotinin (0.5 μg/mL), and pepstatin A (0.7 μg/mL); 300 μL was used for a single determination. Deviations from the original procedure concerned elimination of the chromogen: protein pellets were washed three times with 1 mL of ethanol/ethylacetate (1:1); particular attention was given to thorough resuspension (vortexing) and complete removal of the supernatant using Pasteur pipettes, and thereafter, stripes of blotting paper. Protein was measured according to Lowry et al. [32].

Chemical reagents

Laboratory chemicals were obtained from Sigma, unless otherwise stated in the text.

Statistical analysis

All numerical results are presented as mean ± S.E.M. Mean values between two groups were compared using Student t test (two-tailed unpaired t test in interindividual, dependent t test in intraindividual comparisons) in most instances, unless otherwise indicated. Statistical significance was set at $P \leq 0.05$ for all analyses.

Results

Melatonin modulates glutamate toxicity in NSC-34 motoneuronal cells

We screened candidate drugs for their motoneuron-protective potential, based on the hypothesis that excitotoxicity and the generation of ROS are major contributors to neurodegeneration in ALS. We employed NSC-34 cells, a widely used motoneuron-neuroblastoma fusion line [28], which can be differentiated by serum deprivation. After exposure to 2 or 10 mM of glutamate for 3 days, we determined a loss of 29.2% and 52.1% of cells, respectively, compared with the survival of untreated neurons. In these experiments, all viable cells were counted after staining with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Interestingly, when such cultures were pretreated for 3 hr with melatonin (10 or 50 μM), we observed a small but significant rescue effect that appeared dose dependent (Fig. 1). Some protection from cell death

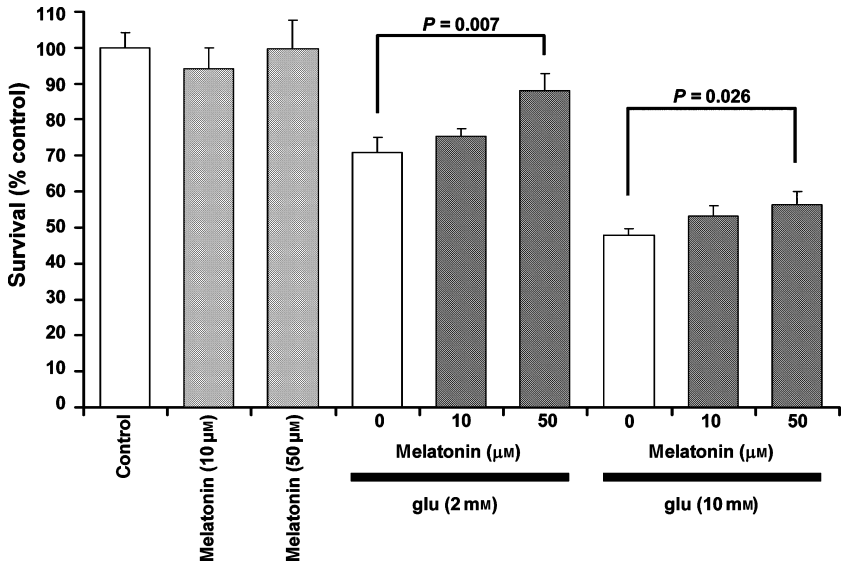

Fig. 1. Melatonin protects cultured motoneuronal cells against glutamate toxicity. Quantification of cell survival upon glutamate exposure (2 or 10 mM) for 3 days in the absence or presence of melatonin (10 or 50 μM). Number of viable cells, determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide staining, is expressed in percent of the respective untreated control cultures; n = 3–4 independent experiments; mean ± S.E.M. presented.

was even detectable for the 10 mM of glutamate challenge (8.5% more surviving cells than in unprotected cultures), but the effect was more obvious at the 'physiological' concentration of 2 mM of glutamate (17.2% more surviving cells with 50 μM of melatonin). As cell lines have acquired poorly defined antiapoptotic mechanisms, we turned to in vivo systems.

High-dose oral melatonin treatment prolongs survival of ALS mice

To determine possible neuroprotective properties of melatonin in ALS, we tested a widely used preclinical model, the SOD1^{G93A}-transgenic mouse line G1H [29]. Melatonin was given orally to female mutants at a concentration of 0.5 mg/mL in drinking water, starting at postnatal day 28. Age-matched transgenic female littermates served as respective controls. To quantify melatonin and water intake, we determined the weekly drinking volume for both experimental groups. No difference regarding body weight or fluid intake was detected (suppl. Fig. 1A,B). At about 3 months of age, SOD1^{G93A}-transgenic mice became symptomatic, starting with a fine tremor of the hindlimbs that progressed over 6–8 wk to severe paresis and premature death. Consequently, during later disease stages, drinking volumes and melatonin uptake declined (suppl. Fig. 1B,C). Between 8 and 15 wk of age, mean daily melatonin uptake was 88.3 ± 2.1 mg/kg body weight, decreasing to 56.9 ± 2.3 mg/kg between 16 and 20 wk of age, i.e., in more severely affected animals.

In this blinded study, we observed a significant benefit from melatonin treatment, with regard to disease progression and overall survival. According to national guidelines, animals had to be sacrificed at end stage (for definition, see material and methods). Mean survival time was extended by 5.9 days in melatonin-treated SOD1^{G93A}-transgenic mice as compared with untreated transgenic controls (136.9 ± 1.7 days (n = 29) versus 131.0 ± 1.2 days (n = 25) in vehicle-treated animals; Fig. 2A). The onset of symptoms, i.e., the appearance of hindlimb tremor, was not significantly changed (85.4 ± 2.3 versus 89.7 ± 2.3 days). However, disease progression, defined by the time span between the onset of tremor and premature death (41.3 ± 2.4 days in untreated mutants), was delayed by 25% in melatonin-treated mice (51.5 ± 2.7 days) (Fig. 2B). Even after failing the rotarod test, disease progression to death was prolonged (by 73.2%) in the melatonin-treated group [17.2 ± 3.6 days (n = 15) versus 9.9 ± 1.1 days (n = 15) in vehicle-treated animals; Fig. 2C]. In addition, the peak, i.e., the age at which most mutants died, was shifted by 1 wk, and a small subgroup of mice seemed to benefit overproportionally from melatonin treatment (see shoulder of red line in Fig. 2D).

Protective effects of melatonin require high dosage and early treatment

Owing to its very low solubility, the melatonin concentration in drinking water could not be further increased. We thus investigated the dose-dependency by reducing melatonin in drinking water to 10% of the tested concentration,

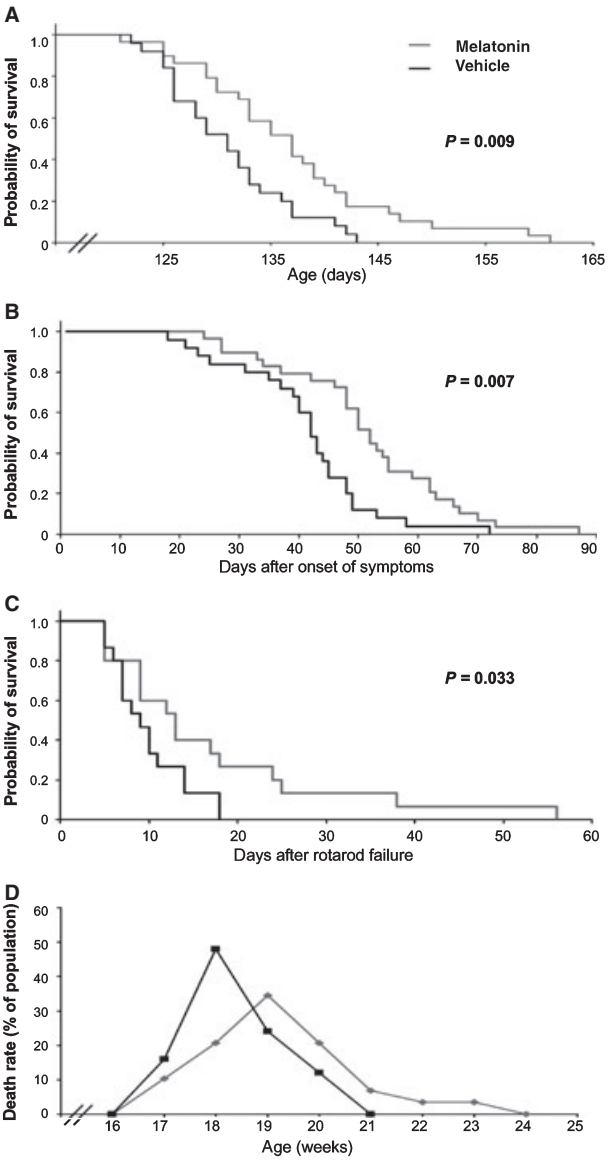

Fig. 2. Melatonin reduces disease progression and prolongs survival of SOD1^{G93A}-transgenic mice. Kaplan–Meyer curves illustrate significant benefits from melatonin treatment (0.5-mg/mL drinking water) regarding overall survival (n = 25–29 per group) (A), and disease progression from the onset of tremor (n = 25–29 per group) (B) or from rotarod failure (n = 15 per group) (C) to death in SOD1^{G93A}-transgenic mice. (D) Percentage of mouse population that reached endpoint criteria/death at the indicated age. The peak was reached later in melatonin-treated mice, and the curve is broadened to the right compared with the respective curve of the control population.

i.e., 0.05 mg/mL. This resulted in a mean melatonin uptake of 9.0 ± 0.5 mg/kg/day between 8 and 20 wk of age. At this dose, we only noticed a tendency toward increased survival times (134.6 ± 2.9 versus 132.7 ± 3.0 days) and a delay in disease progression between tremor and death (52.6 ± 6.9 versus 45.7 ± 6.0 days), without statistical significance ($P = 0.52$ and 0.15, respectively; n = 7 in each experimental group).

Similarly, when treatment was started at the onset of symptoms, which reduces the total time of treatment to

40.5 ± 2.5 days, significant differences were lost for mean survival time and disease progression (survival of 132.8 ± 2.1 days versus 134.4 ± 2.4 days in vehicle-treated control animals; $P = 0.602$; disease duration from first tremor to death 40.6 ± 2.5 versus 41.7 ± 2.8 days; $P = 0.763$).

Melatonin reduces ROS in motoneuronal cells

Although melatonin has antioxidant activities, the mechanism of neuroprotection in $SOD1^{G93A}$-transgenic mice is not known. Theoretically, melatonin might have altered the expression of the toxic SOD1-transgene. However, by Western blot analysis of spinal cord homogenates from melatonin- versus vehicle-treated mice, we were unable to detect quantitative differences at the protein level for the mutant (transgene-derived) or the endogenous SOD1 protein (suppl. Fig. 2).

As melatonin has been reported to stimulate glial-derived neurotrophic factor (GDNF) expression in the central nervous system [33], we considered the theoretical possibility that its neuroprotective effect is GDNF mediated. However, by Western blot analysis of spinal cord lysates, we found no changes in GDNF levels in mutant mice with melatonin treatment compared with untreated controls (data not shown).

Similarly, Western blot analysis of spinal cord protein lysates failed to detect differences in total amount or phosphorylation status of AKT or ERK1/2, kinases involved in the modulation of neuronal cell death [34]. Finally, melatonin treatment did not reduce microglial activation in mutant mice. Immunostaining of the cervical spinal cord, using microglial markers, was increased in all transgenic mice, when compared with wildtype mice at age P100, independent of melatonin treatment (data not shown).

Thus, the known antioxidative power of melatonin remains as the major candidate to explain the therapeutic effects observed in vivo. We focused on this potential mechanism, by studying differentiated NSC-34 motoneuronal cells in combination with dichloro-dihydro-fluoresceindiacetate (H_2DCFDA) as a fluorescent cell-permeant indicator for ROS. H_2DCFDA is nonfluorescent until the acetate groups are removed by intracellular esterases, and oxidation occurs within the cell [35]. The H_2DCFDA fluorescence in NSC-34 cells after exposure to glutamate was clearly enhanced (using H_2O_2 as positive control), but attenuated by treatment with melatonin (Fig. 3A–E). This finding is in good agreement with our hypothesis that melatonin acts as a free radical scavenger in motoneurons under distress.

Clinical safety trial

Although melatonin has been in use for almost two decades to treat jet lag at doses of 3–6 mg/day, high-dose treatments over years have not been reported. Daily melatonin uptake by SOD1-transgenic mice was 56.9 mg/kg during the symptomatic period. Because mice presumably metabolize most pharmacological compounds at least one order of magnitude faster than humans [36], we chose a dose of about 5 mg/kg for a safety study in ALS patients (corresponding to 300 mg per day). We decided to apply melatonin as suppositories at bedtime. This new form of application offered several advantages, including (1) intake

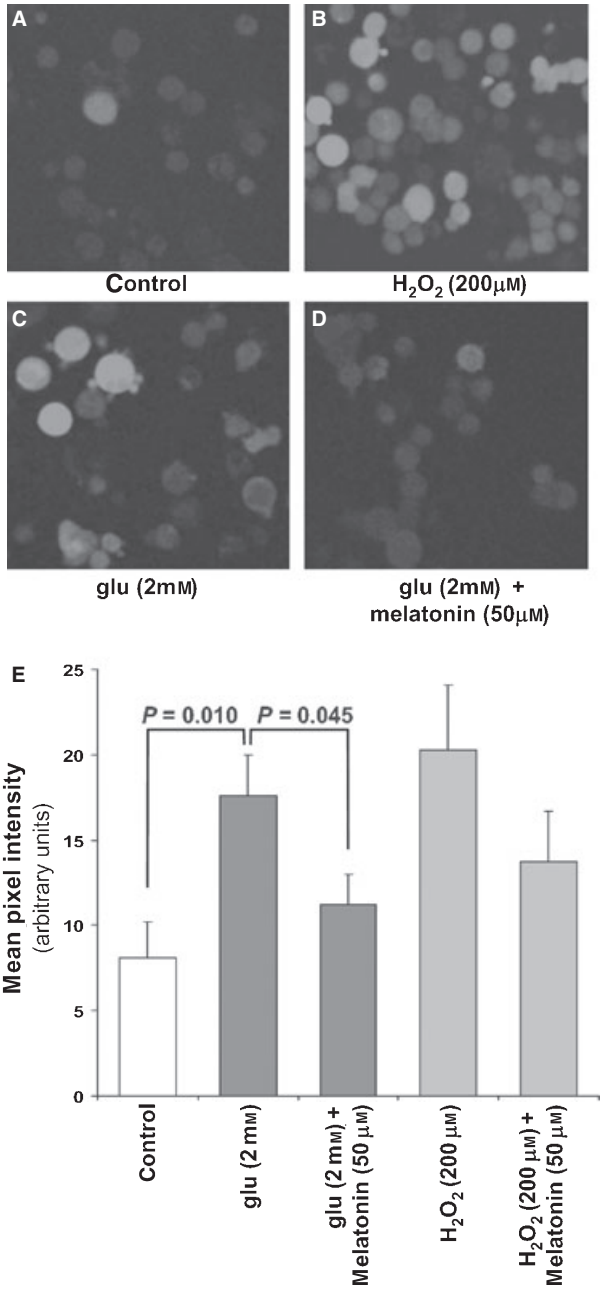

Fig. 3. Melatonin reduces the formation of reactive oxygen species in motoneuronal cells. NSC-34 cells (A, unchallenged control cultures) were exposed to 200-μM H_2O_2 for 30 min (B, positive control) or 2-mM glutamate for 12 hr (C). Cells shown in (D) were co-treated with glutamate and melatonin (50 μM). Free radical formation was assessed with the green fluorescent oxidative stress indicator H_2DCFDA. (E) Results of quantitative densitometric evaluation of confocal microscope pictures: fluorescence is given as mean pixel intensity after subtraction of background values of individual confocal pictures. Confocal laser scanning images were taken with a 40× oil immersion objective; n = 3 independent experiments; mean ± S.E.M. presented.

of high doses via an enteral route, (2) circumventing liver first-pass metabolism, and (3) avoiding swallowing that is frequently compromised in ALS patients.

A total of 31 patients (19 males, 12 females) with probable or definite ALS, according to El Escorial Criteria, but without genetic diagnosis, were enrolled over 24 months into the safety trial. Mean age at inclusion was 59.8 ± 1.9 (range 32–79) yr. The age at presumed disease onset was 58.0 ± 1.9 (28–77) yr. The latency from first symptoms to diagnosis amounted to 11.7 ± 1.2 (3–24) months. Upon entry into the study, mean duration of disease was almost 2 yr (22.1 ± 2.9 months) (range 5–76 months), the ALSFRS was 27.0 ± 1.2 (12–38; maximum = healthy 40), and forced vital capacity 62.3 ± 5.5% (13–100%), revealing that most patients were in a progressed disease state. The predominant clinical symptoms at disease onset were distributed 21:6:4 for the spinal:bulbar:mixed forms of ALS. Severe comorbidities in our patient population included pre-existing epilepsy (n = 2), prostate carcinoma (n = 1), mamma carcinoma (n = 1), cardiac diseases (n = 6), colitis ulcerosa (n = 1), bipolar disorder (n = 1), and alcoholism (n = 1).

Patients were treated for 11.5 ± 1.2 (2–24) months. Rectal application immediately achieved high plasma and urinary melatonin levels, but still maintained–at a higher niveau–a day/night pattern (Fig. 4A and suppl. Fig. 3). Follow-up analysis after 2 months showed that melatonin plasma levels stayed in the expected range, indicating that continuous melatonin administration does not lead to accumulation or increased metabolism. No adverse effects were observed nor reported. Mean routine laboratory data remained essentially unchanged. Several parameters showed typical fluctuations, e.g., associated with physical stress or intercurrent infections (creatine kinase or leucocytes), but none of them likely to be related to melatonin. In two cases, the discontinuation of riluzole revealed that temporarily increased transaminase values were the result of riluzole rather than melatonin. Some patients reported initially faster sleep onset (n = 5), improved sleep quality (n = 15), and better sleep continuation (n = 3), while others did not find their sleep changed (n = 14). No signs of hangover or increased fatigue during daytime were noted. Melatonin medication was well accepted by patients, and only eight discontinued treatment: five at the end stage, three with extremely rapidly progressing disease, who refused *any* medication. A total of 13 patients died (respiratory failure), most of them entering the safety study at an advanced disease stage (mean ALSFRS upon entry 23.6 ± 1.8; range 12–31). The ALSFRS scores from study entry to 1 yr of follow-up for all patients (n = 31) are presented in Fig. 4B.

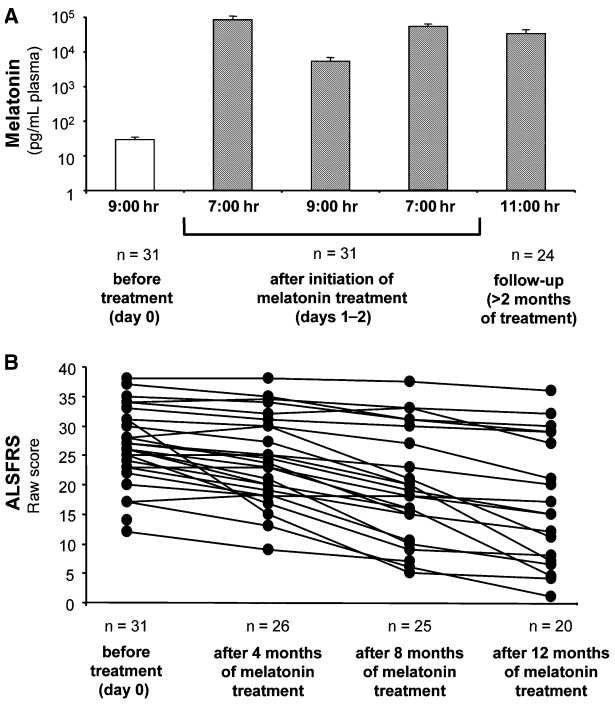

Fig. 4. Clinical safety trial in amyotrophic lateral sclerosis (ALS) patients. (A) Pharmacokinetic aspects: high-dose (300 mg) rectal melatonin treatment daily in the evening immediately potentiates plasma levels, while maintaining a day–night pattern. Plasma levels remain stable after a mean treatment time of 2.23 ± 0.23 months. (B) Clinical course: individual ALS functional rating score (ALSFRS) scores of ALS patients (n = 31) from study entry to 12 months of melatonin treatment.

Melatonin treatment reduces biochemical markers of oxidative stress in ALS

ALS autopsy material contains increased levels of protein carbonyls, representing protein modifications caused by oxidative stress [37, 38]. Recently, elevated lipid peroxides have been detected in serum from ALS patients [39]. Here, we found that an elevation of protein carbonyls can be monitored in peripheral blood samples from ALS patients, and can be used as a biochemical readout for a therapeutic effect. We measured small but significantly elevated protein carbonyl concentrations in serum of ALS patients compared with the serum of matched healthy controls. Significantly, by follow-up of the same ALS patients more than 4 months after the start of daily melatonin treatment, we found protein carbonyl levels fully reverted to control levels (Fig. 5). This is remarkable, as most of these patients (n = 12) had already been on high-dose vitamin E, another antioxidant, for 10.4 ± 2.5 months before starting

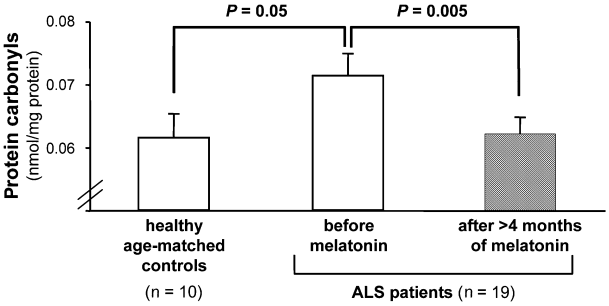

Fig. 5. Laboratory efficacy of melatonin in amyotrophic lateral sclerosis (ALS). Elevated protein carbonyls in the serum of 19 untreated ALS patients decrease to levels of matched healthy controls upon melatonin treatment (mean treatment time: 4.68 ± 0.22 months). Mean ± S.E.M. presented.

melatonin. In fact, a comparison of the clinical outcome (ALSFRS after 1 yr of melatonin treatment) of ALS patients with or without continuous high-dose vitamin E (see Table 1), did not reveal any beneficial effect of vitamin E ($P = 0.901$, $F = 0.016$).

It has been suggested that melatonin influences antioxidant enzymes by stimulating their gene expression [40]. To detect such consequences, we obtained RNA expression profiles from peripheral blood mononuclear cells (PBMC). We compared the RNA of ALS patients, just prior to melatonin treatment, with the RNA of the same patients after 1 day and after 4 months of continuous treatment. These experiments yielded no evidence for the upregulation of genes encoding antioxidant enzymes (data not shown). By quantitative reverse transcriptase polymerase chain reaction (RT-PCR), we only confirmed changes of mRNA levels for proteins associated with proteasome function, the unfolded protein response, and protein sorting (primary data and experimental details available upon request). Although these findings were negative with respect to the induction of antioxidant enzymes in PBMC, they demonstrate gene expression changes in humans triggered by melatonin. Moreover, these changes may turn out to be relevant in light of some features of ALS resembling an 'aggregopathy'. Assessing the possible contribution of melatonin-regulated genes to neuroprotection, in general, and to the formation/processing of insoluble intracellular protein aggregates, in particular, awaits the analysis of neural tissue samples.

Discussion

Radical damage is a final common pathway in the pathology of human ALS, and we have shown that melatonin treatment reduced oxidative stress in vitro and in vivo. High-dose melatonin prolonged the survival of $SOD1^{G93A}$-transgenic mice, a widely used ALS model. In an initial safety study with ALS patients, enteral high-dose application of melatonin was well tolerated and safe. Moreover, melatonin led to the normalization of serum protein carbonyls, markers for oxidative stress, which were significantly elevated prior to treatment in a diverse group of sporadic ALS patients. We do not believe that elevated peripheral protein carbonyls are derived from the central nervous system, but they appear to reflect systemic oxidative stress in ALS patients. The observations that high-dose melatonin (1) improves neuronal survival in vitro, (2) prolongs central nervous system functions in ALS mice, and (3) corrects human ALS laboratory data on oxidative damage, strongly suggest, when combined, that high-dose melatonin also provides neuroprotection in ALS patients. The neuroprotective potential of melatonin in ALS can now be addressed in a clinical proof-of-concept trial.

Till date, there is no causal or convincing symptomatic treatment for ALS. The only compound with borderline efficacy in ALS patients is riluzole, an antiexcitotoxic drug that marginally prolonged survival in clinical trials [5, 6], but lacked benefit with respect to other important outcome measures. Owing to its side effects (including asthenia, nausea, anorexia, diarrhea, headache, and increase in liver transaminases), riluzole is frequently discontinued. It also enhances catabolism in this already wasting disease [41]. Thus, when screening for new drugs in ALS, it is important to search for well-tolerated compounds that can be rapidly transferred to a clinical setting.

All mechanisms believed to explain neuronal cell death or dysfunction in ALS involve oxidative stress, either as primary insult or as part of the final common pathway of disease [4, 25, 26]. A recent 10-yr prospective study with over 900,000 individuals showed a decreased risk of developing ALS that was associated with the regular intake of vitamin E [42]. Nevertheless, none of the antioxidative compounds tested, including vitamin E, proved to be effective in the clinical setting [1, 20, 21]. This suggests that for ALS, early intervention is critical and that better compounds against oxidative stress need to be defined.

Melatonin is distinct from classical antioxidants. First, it acts on a unique broad spectrum of free radical targets by direct scavenging [24–26]. Second, its antioxidative profile extends to the activation of other antioxidative systems, such as glutathione peroxidase [24–26]. Third, melatonin is amphiphilic, and in contrast to standard antioxidants, enters both lipophilic and hydrophilic cellular environments [26].

With respect to the mechanisms underlying melatonin-mediated neuroprotection in cultured motoneuronal cells and in $SOD1^{G93A}$-transgenic mice, we could exclude alternative explanations, including GDNF induction and reduced transgene expression. Thus, the antioxidative properties are indeed the most likely reason for the observed beneficial effects of melatonin on survival in mice. Interestingly, increased protein carbonyl levels have been reported earlier in brain and spinal cord tissue samples from sporadic ALS patients [37, 38, 43, 44]. We found serum protein carbonyls, as indicators of oxidative stress, to be reduced to control levels upon chronic melatonin treatment in ALS patients. In the absence of clinical efficacy data, this finding provides indirect biochemical evidence of a therapeutic effect in human ALS.

Another potentially important mechanism of action of melatonin in ALS may be its mitigating effect on mitochondrial malfunction. Hence, melatonin-induced enhancement of ATP availability could provide additional benefits in ALS [45].

The daily dose of 300 mg melatonin for the safety study was chosen with respect to our preclinical data in mice, and taking into account up to tenfold higher metabolism rates of most drugs in rodents [36]. Jet lag in humans is usually treated with 3 to 6 mg melatonin per day, and short-term treatments with up to 2000 mg daily in cancer patients are tolerated without side effects [46]. Suppositories were effective as a new route of melatonin administration and well accepted by our patients. No unwanted effects were observed in clinical and laboratory follow-up. Some patients noted faster sleep onset and improved sleep quality, but never complained about daytime sleepiness. This novel way of melatonin application, with respect to route and dose, could prove useful in other diseases associated with the generation of ROS. We note that similar to ALS, elevated tissue protein carbonyls have been detected in Alzheimer's and Parkinson's disease [37], but the causal role of ROS in the progression of these diseases is less clear.

Similar to other experimental therapies of $SOD1^{G93A}$-transgenic mice [47], no benefit was demonstrated when melatonin was given *after* the onset of symptoms. This does not necessarily predict a lack of efficacy in ALS, even though treatment of patients typically starts many months after the onset of first symptoms. It is questionable whether the clinical stages of a motoneuron disease in mice can be quantitatively compared with that in humans. The disease course in the chosen mouse model, carrying 18 copies of a mutant SOD1 transgene [29], is extremely compressed (onset of symptoms at age 90 days), relative to human ALS (average onset at age 60 yr), independent of the shorter lifespan of mice. In addition, disease duration in mice (6–8 wk) is a small fraction of the human course (2–5 yr). Thus, *treatment-at-disease-onset* protocols may come too late in mice, but still be effective in human ALS, which develops over years. Similar differences of disease dynamics have been noted between other transgenic models and their respective human disorders, including Alzheimer's disease and multiple sclerosis [48, 49].

Only 1–2% of all ALS cases are caused by SOD1 mutations. Thus, novel therapeutic strategies should be directed against common events that contribute to all forms of ALS. Recent publications that reported delayed disease progression or improved survival in mutant $SOD1^{G93A}$-transgenic mice were, for example, based on transgenic expression of glial glutamate transporters [11] or viral delivery of neurotrophins or siRNA [50–53]. These studies have helped to understand basic disease mechanisms of ALS, but cannot be easily translated to the clinic. The search for low molecular drugs as therapeutics in ALS mouse models [54, 55] has provided compounds that failed in clinical studies (e.g., creatine [56] or vitamin E [20, 21]) or for which no safety data are available in ALS patients (e.g., minocycline [57–59] and cephalosporines [60]).

With respect to the clinical setting, disease progression and duration are more relevant parameters than overall survival time. Minocycline, which is currently under clinical investigation, postponed disease onset and death of $SOD1^{G93A}$-transgenic mice [57, 58]. However, a decreased time span (−21%) between onset of symptoms and premature death was reported [57]. Similar negative results on disease duration were observed upon vitamin E treatment [54]. In contrast, high-dose melatonin slowed disease progression by 25% or 73%, depending on the definition of 'disease onset', by the appearance of first tremor or failure on the rotarod, respectively.

Translating these findings to human ALS, we are aware that a cure by melatonin is unlikely. An increase in life expectancy, however, is a worth-while goal, specifically during the early stages of this disease. Oxidative stress is a common denominator of cell death pathways in sporadic ALS. Thus, melatonin can be expected not only to be effective as single drug treatment, but also to exert synergistic therapeutic effects, e.g., in combined treatment schemes with riluzole [5, 6], minocycline [57, 58], cephalosporines [60], or possibly COX-2 inhibitors [61]. Treatment cocktails of compounds with individually established safety records may play an important role in future ALS treatment regimes [59].

To conclude, despite these promising preclinical results with melatonin in mice, we have to stress that there are no clinical efficacy data available, and that we have only laboratory data to suggest an efficacy of melatonin in human ALS. The human safety data and unexpected findings of complete normalization of circulating protein carbonyls by melatonin treatment distinguish this study, and are encouraging to conduct a randomized double-blind trial.

Acknowledgments

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Supplementary Material

The following material is available for this article online:

Supplementary Fig. 1. Body weight, drinking water, and melatonin intake in amyotrophic lateral sclerosis (ALS) mice. No significant difference regarding body weight (A) or water intake (B) was observed between melatonin- or vehicle-treated mice. Mean body weight was 19.1 ± 0.2 and 18.8 ± 0.3 g between 6 and 19 wk of age in control and treatment groups, respectively ($P = 0.313$). Mean daily fluid intake per kg body weight was found to be 154.8 ± 9.8 and 158.9 ± 8.8 mL between 8 and 19 wk of age, respectively ($P = 0.754$). As a consequence of reduced fluid intake in later disease stages melatonin intake declined (C).

Supplementary Fig. 2. Melatonin treatment does not change SOD1 protein expression in mouse spinal cord. (A) Melatonin treatment did not change protein expression of endogenous mouse superoxide dismutase (SOD1) and transgenic human mutant SOD1 in cervical spinal cord lysates of transgenic (tg) and wildtype (wt) 100-day-old mice. (B) Densitometric quantification of protein expression levels of endogenous (wildtype) and mutant SOD1 in mutant SOD1-transgenic mice. Shown are respective ratios between melatonin-treated animals and vehicle controls, demonstrating that melatonin did not influence the expression levels of mutant or wildtype SOD1. Before calculating ratios, SOD1 protein levels were normalized to a tubulin standard ($n = 12$ per condition).

Supplementary Fig. 3. Urinary melatonin excretion of amyotrophic lateral sclerosis (ALS) patients before and after the first and second 300 mg suppository. Columns represent total amount of melatonin measured in 10-hr urine (21:00–7:00 hr) (mean ± S.E.M.).

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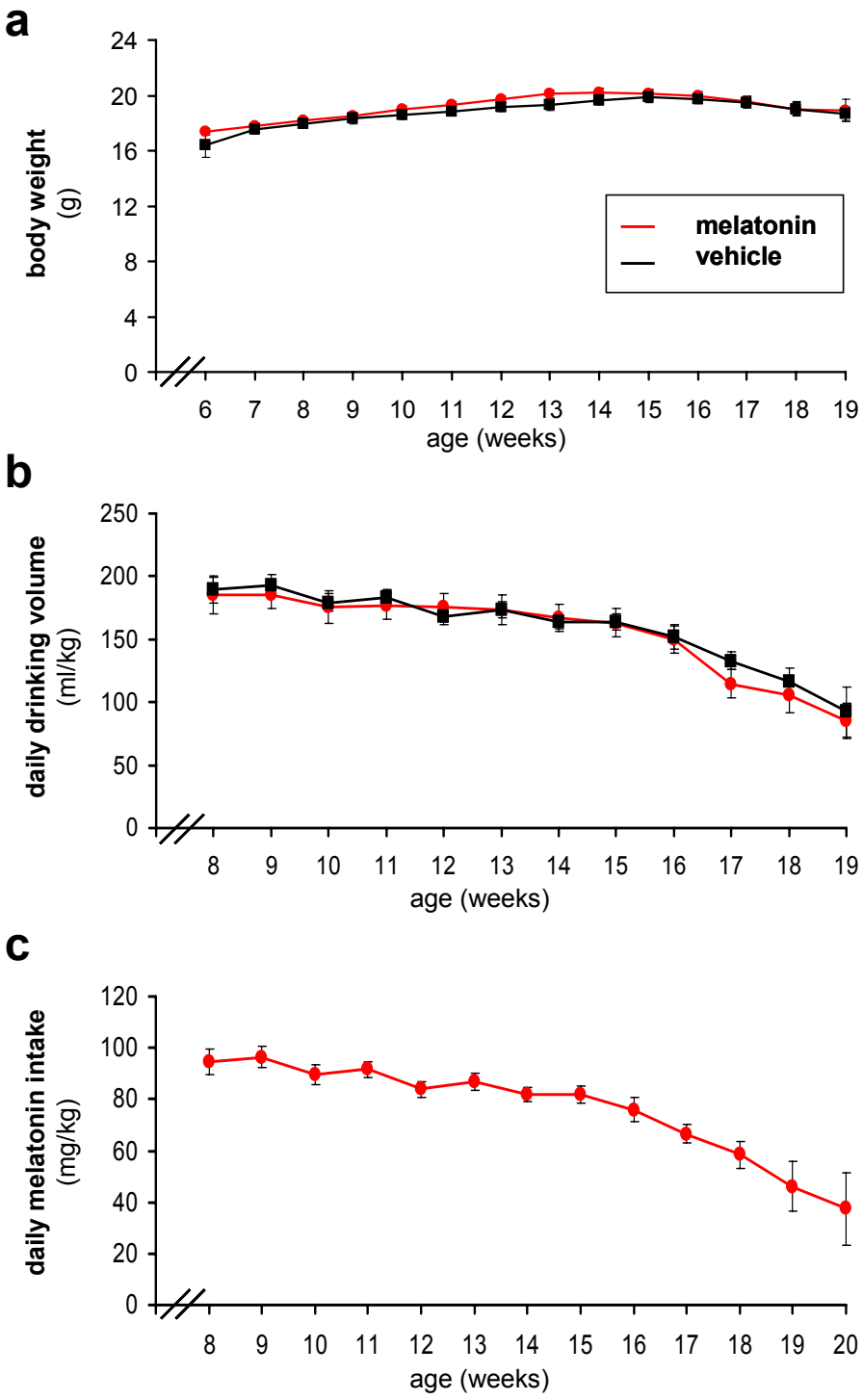
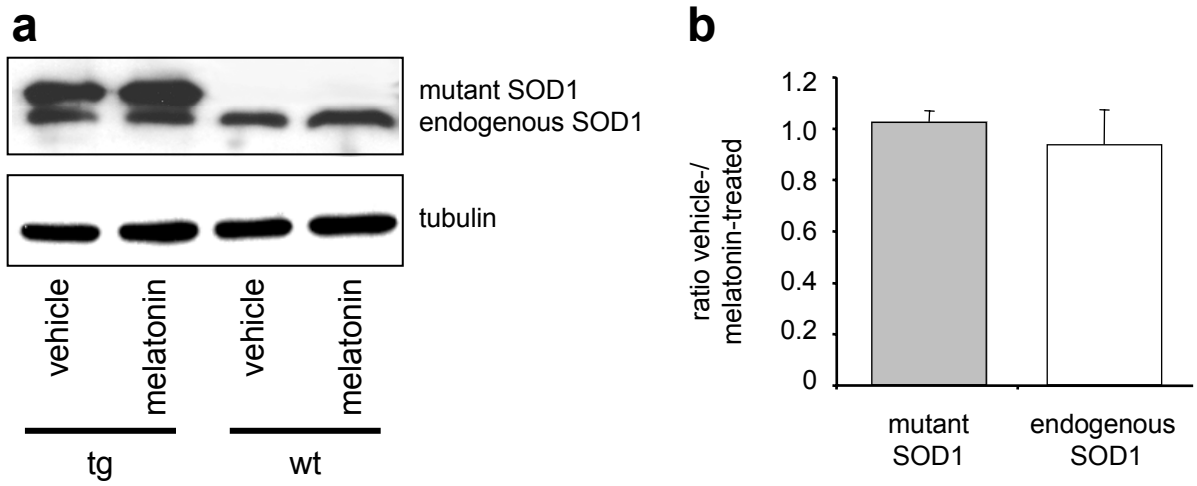
Supplementary Figure 1



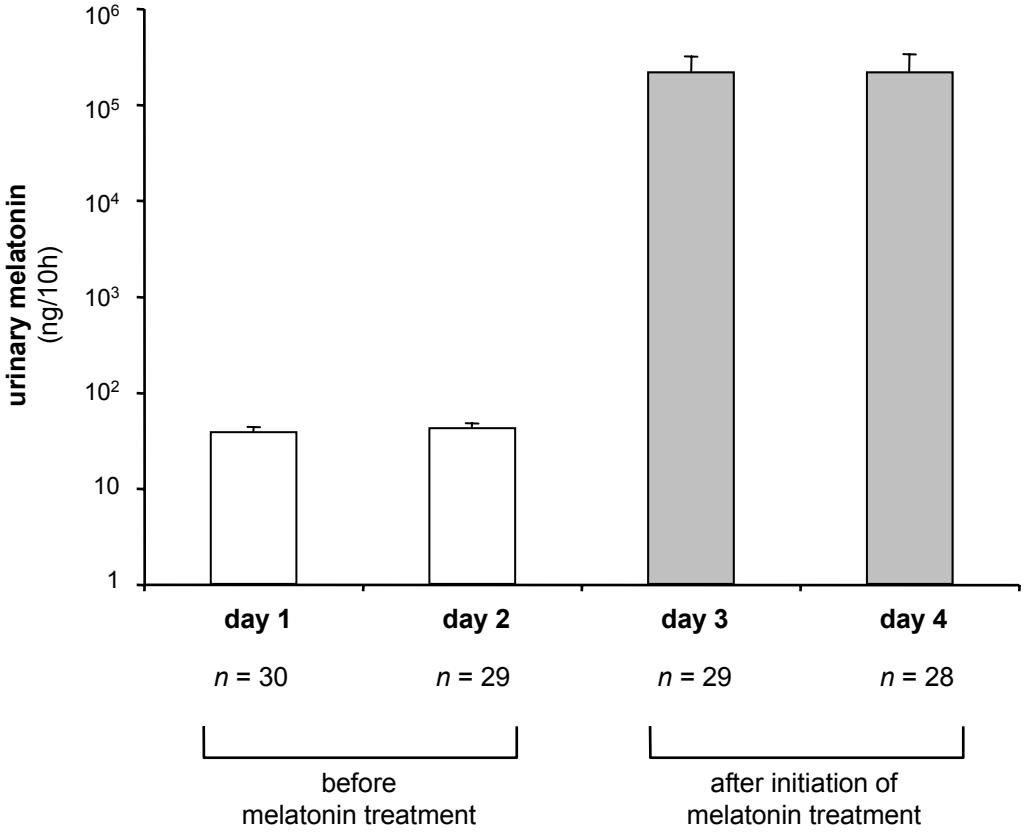
Body weight, drinking water and melatonin intake in ALS mice. No significant difference regarding body weight (**a**) or water intake (**b**) was observed between melatonin- or vehicle-treated mice. Mean body weight was 19.1±0.2g and 18.8±0.3g between 6 and 19 weeks of age in control and treatment groups, respectively (*P*=0.313). Mean daily fluid intake per kg body weight was found to be 154.8±9.8ml and 158.9±8.8ml between 8 and 19 weeks of age, respectively (*P*=0.754). As a consequence of reduced fluid intake in later disease stages melatonin intake declined (**c**).

Supplementary Figure 2



Melatonin treatment does not change SOD1 protein expression in mouse spinal cord. (**a**) Melatonin treatment did not change protein expression of endogenous mouse SOD1 and transgenic human mutant SOD1 in cervical spinal cord lysates of transgenic (tg) and wildtype (wt) 100 day-old mice. (**b**) Densitometric quantification of protein expression levels of endogenous (wildtype) and mutant SOD1 in mutant SOD1-transgenic mice. Shown are respective ratios between melatonin-treated animals and vehicle controls, demonstrating that melatonin did not influence the expression levels of mutant or wildtype SOD1. Before calculating ratios, SOD1 protein levels were normalized to a tubulin standard (*n*=12 per condition).

Supplementary Figure 3



Urinary melatonin excretion of ALS patients before and after the first and second 300mg suppository. Columns represent total amount of melatonin measured in 10h urine (9:00 p.m. – 7:00 a.m.) (mean±s.e.m.).

2.2 Alkoholabhängigkeit: Psychotherapie als neuroprotektive Strategie

2.2.1 Einführung in die Fragestellung

Folgt man dem in Abbildung 2 (S. 8) beschriebenen Modell neuroprotektiver Ansätze, sollte im Fall einer durch chronischen und massiven Alkoholkonsum induzierten Neurodegeneration eine neuroprotektive Behandlungsstrategie auf die Eliminierung dieser Noxe abzielen. Spontanremission ist bei Alkoholabhängigkeit möglich und tritt auch relativ häufig auf (Finney, 1999; Klingemann, 2001), dennoch verspricht eine Behandlung größeren Erfolg in Bezug auf das Trinkverhalten als keine Behandlung (Finney, 1999; Weisner et al., 2003), v.a. bei der Aufrechterhaltung von Abstinenz (Walton et al., 2000). Neuroprotektive Behandlungsstrategien sollten somit insbesondere auf eine Erhöhung der Abstinenzwahrscheinlichkeit fokussieren. Umsetzen lässt sich diese Strategie mittels psycho- und/oder pharmakotherapeutischer Interventionen. Bei der Auswahl eines solchen Verfahrens ist gezielt darauf zu achten, dass die angestrebte Abstinenz einerseits langfristig ist und strikt eingehalten wird. Zwischenzeitlicher Alkoholkonsum birgt die Gefahr, dass eine Erholung geschädigter Funktionen ausbleibt bzw. sich weiterer Schaden akkumuliert (Rosenbloom et al., 2003; Rourke & Grant, 1999).

Für Patienten mit Alkoholabhängigkeit stehen bereits eine Reihe verschiedener psycho- sowie pharmakotherapeutischer Behandlungsansätze zur Verfügung (Miller & Wilbourne, 2002). Als wesentliche Unterscheidungsmerkmale psychotherapeutischer Maßnahmen können das Setting (stationär vs. ambulant) und die Behandlungsdauer (wenige Tage bis Monate/Jahre) genannt werden. 12-Schritt- und kognitiv-behaviorale Therapieprogramme zählen dabei zu den am häufigsten angewandten Interventionen, die teilweise mit medikamentöser Behandlung kombiniert werden. Aufgrund einer Einflussnahme in den Glutamat-Neurotransmitterhaushalt beanspruchen sogenannte „Anti-Craving“-Substanzen (wie Acamprosat) ebenfalls, über zusätzliches neuroprotektives Potential zu verfügen (De Witte et al., 2005). Ergebnisse von Therapiestudien lieferten bisher jedoch keine überzeugende Überlegenheit einer „Anti-Craving“-Behandlung gegenüber kognitiv-behavioralen Ansätzen (Anton et al., 2006).

Meta-Analysen und Überblicksarbeiten bescheinigen dennoch, dass die verfügbaren psycho- und auch pharmakotherapeutischen Interventionen insgesamt als erfolgreich und ökonomisch zu bewerten sind (Emrick, 1974; Finney & Monahan, 1996; Holder et al., 1991; McCrady & Langenbucher, 1996; Miller et al., 2001; Miller & Wilbourne, 2002). Betrachtet man den Therapieerfolg, gemessen an der Abstinenzwahrscheinlichkeit, ergibt sich jedoch ein zweigeteiltes Bild: Relativ übereinstimmend

werden Abstinenzraten von lediglich 25-30% im Jahr nach Behandlungsende berichtet (z.B. Moos et al., 1999; Project MATCH Research Group, 1997). Die wenigen Ergebnisse zu langfristigen Therapieerfolgen (zwei bis drei Jahre nach Abschluss der Therapie) variieren dagegen in Abhängigkeit von der Erhebung des Trinkstatus: Bei objektiver Abstinenzkontrolle mittels Blut- oder Atemalkoholprüfung bleiben nur noch 6-18% der Patienten abstinent (Burtscheidt et al., 2002). Verlassen sich die Studien jedoch ausschließlich auf den Selbstbericht der Patienten, resultieren Abstinenzraten von bis zu 30% (Miller, 2001; Project MATCH Research Group, 1998). Da nur unter der Bedingung langfristiger Abstinenz eine Regeneration von beeinträchtigten Funktionen erfolgen kann, erscheinen die bisher eingesetzten Verfahren als neuroprotektive Strategien wenig vielversprechend.

Mit dem ALITA-Programm (*A*mbulante *L*angzeit-*I*ntensiv*T*herapie für *A*lkohol-kranke) liegt dagegen ein innovatives biopsychosoziales Behandlungskonzept vor (Ehrenreich et al., 1997; Krampe et al., 2004; Krampe et al., 2006b; Wagner et al., 2004). In einer 9-Jahres-Katamnese zeigte sich mit einer Abstinenzwahrscheinlichkeit von 52% ein langfristiger Therapieerfolg, der anderen Behandlungsprogrammen deutlich überlegen ist (Krampe et al., 2006a). ALITA kann somit als Neuroprotektion ermöglichende Behandlungsstrategie gelten, in der Regeneration sorgfältig evaluierbar ist. Auch über die zwei Jahre des Therapieprogramms hinaus bietet ALITA durch eine regelmäßige und objektive Abstinenzkontrolle (Blut- und Urinanalysen) als auch eine intensive Langzeitbetreuung optimale Möglichkeiten zur prospektiven und validen Längsschnitterfassung verschiedenster, geschädigter und potentiell regenerations-fähiger Parameter.

Die vorliegende Originalarbeit beschäftigt sich innerhalb des Therapiesettings von ALITA mit dem Verlauf hippocampus-assoziierter kognitiver Funktionen unter kontrollierter Langzeitabstinenz. Aufgrund zahlreicher Nachweise mit *in vitro* und *in/ex vivo*-Studien ist von dem neuroanatomischen Korrelat, der Struktur des Hippocampus (HC), anzunehmen, dass es äußerst vulnerabel auf toxische Einflüsse wie Alkohol reagiert (Agartz et al., 1999; Laakso et al., 2000; Sullivan et al., 1995; White et al., 2000). Im Gegenzug weist der HC jedoch aufgrund von Neuroplastizität ein hohes Regenerationspotential auf (Marrone et al., 2004; Schmidt-Hieber et al., 2004; Shors et al., 2001; Steiner et al., 2004). Obwohl bereits diverse Studien mittels „indirekter“ neuropsychologischer Datenerhebung belegen, dass HC-assoziierte Funktionen, wie visuell-räumliche Orientierung, Lernen und Gedächtnis bei Alkoholabhängigkeit am häufigsten von Einbußen betroffen sind, existieren kaum oder widersprüchliche Daten

über deren Verlauf bzw. deren mögliche Regeneration unter Langzeitabstinenz (Fein et al., 1990; Reed et al., 1992; Rosenbloom et al., 2004; Sullivan et al., 2000). Ziel der vorliegenden Studie war die Evaluation 1) der Häufigkeit einer alkohol-induzierten Dysfunktion in sogenannten HC-Tests, 2) des Einflusses eines solchen kognitiven Defizits auf die Abstinenzwahrscheinlichkeit und 3) der Regenerationsfähigkeit von HC-Funktionen unter strikter Alkoholabstinenz.

Dazu durchlief eine repräsentative Stichprobe schwer alkoholkranker Patienten eine HC-Funktionen, Aufmerksamkeit und Intelligenz umfassende neuropsychologische Testbatterie zwei bis drei Wochen nach einer initialen stationären Entzugsphase (T_1) sowie nach 3, 6, 12 und 24monatiger Abstinenz (T_{2-5}).

30 der 50 (60%) initial getesten Patienten wiesen eine HC-Dysfunktion auf, die in der Tat tendenziell eine geringere Langzeitabstinenzwahrscheinlichkeit vorhersagte (p=0.058). Eine Subgruppe von 32 Patienten (Alter: 44.7±6.2 Jahre, 23 männliche, 9 weibliche Patienten) blieb über den gesamten Untersuchungszeitraum abstinent und konnte somit alle Testungszeitpunkte absolvieren. Auch hier zeigte sich die Mehrheit (53%) der Patienten von einer HC-Dysfunktion betroffen. Dieses spezifische Defizit ließ sich dabei nicht durch mögliche Konfundierungsvariablen, wie suchtassoziierte Merkmale, psychiatrische Komorbidität, Einnahme von Alkoholaversiva, intellektuelles Leistungsniveau oder globale Hirnatrophie erklären. Die primär festgestellten Beeinträchtigungen HC-assoziierter Funktionen erholten sich erst nach zwei Jahren strikter Abstinenz auf ein Niveau im Normbereich, während Patienten ohne initiale Beeinträchtigung stabile, normgerechte Leistungen aufwiesen. Eine weitere Untergruppe von Patienten mit zusätzlicher hirnorganischer Schädigung blieb ebenfalls auf einem konstanten, jedoch unterdurchschnittlichen Leistungsniveau.

Der Nachweis langsamer, aber deutlicher Erholung von HC-Funktionen unter der Bedingung strikter Alkoholabstinenz untermauert erneut die Forderung nach abstinenzorientierten Langzeitbehandlungen für Alkoholkranke. Das Ausbleiben funktionaler Regeneration bei Patienten mit zusätzlichen hirnorganischen Erkrankungen lässt sich vermutlich auf die Erschöpfung des HC-Regenerationspotentials durch den „dual hit“ (hirnorganische Erkrankung + Schädigung durch chronischen Alkoholkonsum) zurückführen. Klinische Relevanz und somit therapeutische Implikationen ergeben sich durch die erhöhte Rückfallgefährdung kognitiv beeinträchtigter Patienten.

Vermittelt durch eine entsprechende, abstinenzfördernde Behandlungsstrategie kann diese Studie somit insgesamt das neuroprotektive Potential strikter Langzeitabstinenz bei Alkoholabhängigkeit belegen.

2.2.2 Originalartikel

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RECOVERY OF HIPPOCAMPUS-RELATED FUNCTIONS IN CHRONIC ALCOHOLICS DURING MONITORED LONG-TERM ABSTINENCE

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Abstract — Aims: The hippocampus (HC) is characterized by high vulnerability to noxious influence, but also by a considerable regenerative potential. Although deficits in HC-related functions are among the most commonly reported cognitive sequelae in alcoholism, little and conflicting information is available concerning regeneration upon abstinence. The present study has been designed to evaluate (i) the frequency of measurable dysfunction in so called HC tests and (ii) its predictive value for risk to relapse in a cohort of 50 severely affected chronic alcoholic patients and (iii) to monitor recovery of HC-related functions upon strict abstention from alcohol. **Methods:** Patients underwent a 2-year neuropsychological follow-up including HC-associated tests (Verbal Learning Test, VLT; Non-verbal Learning Test, NVLT; 'City Map Test' of Learning and Memory Test, LGT-3), as well as tests of intelligence and attention in the framework of OLITA (Outpatient Long-Term Intensive Therapy for Alcoholics), a programme with careful abstinence monitoring. **Results:** At study entry, 30/50 (60%) alcoholics had HC dysfunction which tended to predict a lower long-term abstinence probability ($P = 0.058$). Of the subgroup that could be followed under conditions of strictly monitored alcohol abstinence ($n = 32$; age 44.7 ± 6.2 years; 23 men, 9 women), 53% (17/32) exhibited distinct HC dysfunction at inclusion which returned to normal after 2 years. Patients with initially normal HC function (9/32) and patients with additional brain damage of different aetiologies (6/32) failed to show improvement on HC-related tests. While the former displayed stably normal HC test performance, the latter remained on a performance level below normal. **Conclusions:** Demonstrating slow but remarkable regeneration of HC functions upon strict abstention from alcohol, our data strongly support abstinence-oriented long-term treatment of alcoholics. The absence of functional recovery in patients with additional causes of brain damage might be explained by the 'dual hit' exhausting the regenerative potential of the HC.

INTRODUCTION

Chronic extensive alcohol consumption affects basically all organs, including most brain areas (Pfefferbaum *et al*., 1988; Lishman, 1990; Kril *et al*., 1997; Heather and Stockwell, 2001). Among those, the hippocampus (HC) is characterized by a particular vulnerability to any noxious input ranging from hypoxia/ischaemia or toxic compounds to inflammatory or neurodegenerative processes (Meyer *et al*., 2001; Geddes *et al*., 2003). Alcohol consumption, be it sporadically or chronically, impairs HC function and morphology as documented *in vitro* and *in/ex vivo* (Ryabinin, 1998; White *et al*., 2000), using animal experiments (Bonthius *et al*., 2001), human post mortem brains (Harding *et al*., 1997), or human imaging studies (Sullivan *et al*., 1995, 1996; Agartz *et al*., 1999; Laakso *et al*., 2000). The ethanol-induced HC damage has been explained by different mechanisms, ranging from neuronal loss (Cadete-Leite *et al*., 1988; Bengochea and Gonzalo, 1990) to glial cell diminution (Korbo, 1999), dendritic alterations in HC cells (King *et al*., 1988; Durand *et al*., 1989), decrease in HC neurogenesis (Herrera *et al*., 2003), or attenuated long-term potentiation (Tremwel and Hunter, 1994). In rodent studies, ethanol-induced reduction in HC volume was reversible upon abstinence (White *et al*., 2000). In fact, the HC has a strong regenerative potential due to considerable neuroplasticity, involving adult neurogenesis (Shors *et al*., 2001; Eriksson, 2003), gliogenesis (Steiner *et al*., 2004), synaptogenesis (Marrone *et al*., 2004), synaptic and dendritic sprouting (Spigelman *et al*., 1998; Bear, 2003; Schmidt-Hieber *et al*., 2004).

HC-related functions are intimately connected with learning and memory processes (Givens *et al*., 2000; Laroche *et al*., 2000; Riedel and Micheau, 2001; Duzel *et al*., 2003; Stark and Squire, 2003; Prickaerts *et al*., 2004), especially visuospatial memory (Epstein and Kanwisher, 1998) and visuospatial orientation (Iaria *et al*., 2003), crossmodal sensory integration (Laroche *et al*., 2000; Gottfried and Dolan, 2003), consolidation of information (Laroche *et al*., 2000), and attention (Wall and Messier, 2001). Previous neuropsychological investigations in alcoholism measured a wide range of different cognitive domains, but covered HC-related functions only partially. These studies revealed inconsistent patterns of impairment, ranging from general cognitive deficiency (Fein *et al*., 1990; Tivis *et al*., 1995; Sullivan *et al*., 2000b) to mild (Parsons, 1983), selective (Ratti *et al*., 1999; Noel *et al*., 2001), or even no cognitive deficits (Sullivan *et al*., 1995). Visuospatial learning and memory deficits, however, are among the most frequently and consistently found cognitive sequelae in chronic alcoholism, including rodent models (Bowden and McCarter, 1993; Beatty *et al*., 1996; Ryabinin, 1998; Matthews and Morrow, 2000; Weitemier and Ryabinin, 2003; Berry and Matthews, 2004).

Reports on cognitive recovery in alcoholism are diverse and conflicting, ranging from rapid, full, or partial recovery within several weeks (Kish *et al*., 1980; Leber *et al*., 1981; Mann *et al*., 1999; Tracy and Bates, 1999), several months (Drake *et al*., 1995) or several years (Fein *et al*., 1990; Reed *et al*., 1992; Sullivan *et al*., 2000a) to studies that yielded residual deficits or no cognitive improvement after a year or more of abstention from alcohol (Brandt *et al*., 1983; Yohman *et al*., 1985; Schandler *et al*., 1996). Most of these results were obtained either from cross-sectional studies, comparing

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different patient populations, or from short-term longitudinal observation. As an exception, Rourke and Grant (Rourke and Grant, 1999) demonstrated in a combined longitudinal and cross-sectional study design significant cognitive improvement, particularly of executive functions, in the same male alcoholics after 2 years of alcohol abstention. However, data concerning abstinence has solely been based on patients' self-reports and the majority of included patients failed to stay abstinent.

The aim of the present study was to prospectively evaluate in a cohort of severely affected chronic alcoholic subjects (i) the prevalence of HC dysfunction, (ii) the potential of HC recovery over 2 years of abstinence, and (iii) the impact of HC dysfunction on clinical outcome parameters. For the purpose of strict abstinence monitoring, the OLITA (Outpatient Long-term Intensive Therapy for Alcoholics) setting provided an ideal and unique opportunity of long-term follow-up of patients (Ehrenreich *et al*., 1997; Krampe *et al*., 2006a).

PATIENTS AND METHODS

Subjects

The study protocol has been approved by the Committee for Medical Ethics of the Georg-August-University, Göttingen, Germany. Subjects participated after written informed consent.

Of a total of 67 patients consecutively admitted to the OLITA programme (Ehrenreich *et al*., 1997; Krampe *et al*., 2006a) between 04/2000 and 06/2002, 50 subjects were neuropsychologically tested at 2–3 weeks after inpatient detoxification and of those, 32 subjects completed the neuropsychological study protocol (for overview and details see Figure 1 and Table 1).

This subgroup of 32 patients fulfilled DSM-IV criteria for alcohol dependence and was representative of the total OLITA sample of severely affected chronic alcoholics (Krampe *et al*., 2006a) concerning sociodemographic or addiction-related data. Mean age at entry was 44.7 ± 6.2 years with a male/female ratio of 23:9. Patients had 9.5 ± 2.0 years of school education, 3.1 ± 2.1 years of higher education, and 12.6 ± 3.5 years of total education. Duration of alcohol dependence was 19.8 ± 6.5 years, with 6.0 ± 8.8 inpatient detoxifications and 1.0 ± 1.0 inpatient long-term therapies. They had consumed beer, wine, and spirits, amounting to an average of 410 ± 207 g of pure alcohol daily before entering the OLITA programme.

Patients had no history of previous or current drug abuse other than alcohol, nicotine, and caffeine. Patients joined

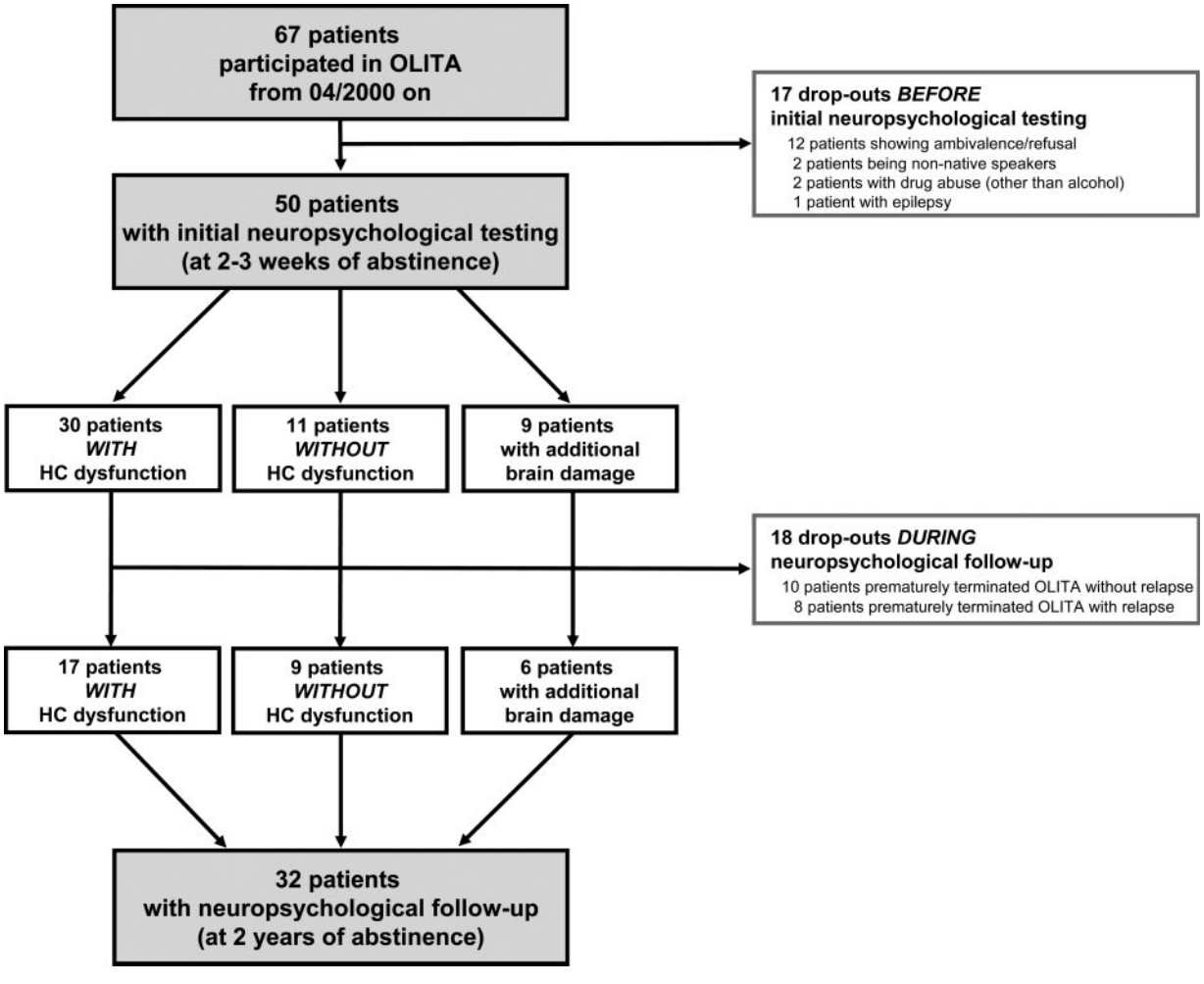

Fig. 1. Overview of subject numbers during recruitment and follow-up.

Table 1. Group characteristics at T_1 of patients with neuropsychological follow-up ($n = 32$)

Group characteristics	Patients without HC dysfunction ($n = 9$)	Patients with HC dysfunction ($n = 17$)	$P < 0.05$	Patients with additional brain damage ($n = 6$)
Present age (years)[a]	44.35 ± 6.54	44.79 ± 6.23	n.s.	44.93 ± 6.52
Male/female ratio[b]	7/2	13/4	n.s.	3/3
Handedness (right/left ratio[b])	9/0	17/0	n.s.	5/1
School education (years)[a]	10.56 ± 2.01	8.82 ± 1.70	0.029	10.00 ± 2.45
Higher education (years)[a]	4.00 ± 2.12	2.65 ± 2.09	n.s.	3.00 ± 1.90
Total education (years)[a]	14.56 ± 3.09	11.47 ± 3.30	0.029	13.00 ± 3.95
Duration of dependence (years)[a]	16.22 ± 8.80	22.29 ± 4.77	0.03	18.33 ± 4.63
Total number of inpatient detoxifications[a]	1.67 ± 1.50	7.71 ± 10.32	0.03	7.67 ± 9.22
Total number of inpatient long-term therapies[a]	0.56 ± 0.88	1.06 ± 0.90	n.s.	1.50 ± 1.38
Estimated daily alcohol dose (g)[a]	308.44 ± 165.71	508.24 ± 212.65	0.022	284.00 ± 82.75

Sociodemographic data of the two patient groups without and with hippocampal dysfunction are compared. n.s. = not significant; g = gram.
[a]independent *t*-test, two-tailed.
[b]χ^2-test, two-tailed.

Table 2. Comorbid psychiatric disorders at T_1 and T_5 of patients with neuropsychological follow-up ($n = 32$)

Group characteristics	Patients without HC dysfunction ($n = 9$)	Patients with HC dysfunction ($n = 17$)	$P < 0.05$	Patients with additional brain damage ($n = 6$)
Anxiety[a]				
T_1	2/9 (22.2%)	7/17 (41.2%)	n.s.	2/6 (33.3%)
T_5	1 (11.1%)	0 (0%)	n.s.	0 (0%)
Depression[a]				
T_1	3/9 (33.3%)	3/17 (17.6%)	n.s.	0/6 (0%)
T_5	0 (0%)	0 (0%)	n.s.	0 (0%)
Other axis I disorders[a]				
T_1	3/9 (33.3%)	1/17 (5.9%)	n.s.	1/6 (16.7%)
T_5	0 (0%)	0 (0%)	n.s.	0 (0%)
Atleast one axis I disorder[a]				
T_1	4/9 (44.4%)	9/17 (52.9%)	n.s.	2/6 (33.3%)
T_5	1 (11.1%)	0 (0%)	n.s.	0 (0%)
Personality disorders[a]				
T_1	4/9 (44.4%)	7/17 (41.2%)	n.s.	2/6 (33.3%)

Psychopathological data of the two patient groups without and with hippocampal dysfunction are compared. Absolute numbers of patients given, % of the respective group in brackets.
[a]χ^2-test, two-tailed; n.s., not significant.

the OLITA programme directly following an inpatient detoxification period of 2–3 weeks. Most subjects had typical sequelae of alcoholism during early abstinence, such as hepatomegaly/fatty liver, polyneuropathy, signs of autonomic dysregulation, and withdrawal-associated epileptic seizures. During their first week of inpatient detoxification, patients received clomethiazole, magnesium, potassium, and vitamin B1. At 2–3 weeks of abstinence, they were put on low-dose acetaldehyde dehydrogenase inhibitors (calcium carbimide or disulfiram) (Krampe *et al*., 2006a) for at least 13 months. Abstinence was strictly monitored by urine analysis at each therapeutic contact (i.e. daily for months 1–3, 3–4 times weekly for months 4–6, 2 times weekly for months 7–18, 1 time weekly for months 19–24), as well as regular blood and/or breath analyses up to the end of the programme at 2 years. For follow-up of abstinence after termination of treatment, regular weekly to quarterly urine and/or blood examinations were performed.

Psychiatric comorbidity of patients with neuropsychological follow-up is presented in Table 2. DSM-IV Axis I disorders at baseline and after 2 years (end of the treatment) were diagnosed with the MiniDIPS, a German adaptation of the Anxiety Disorders Interview Schedule (Barlow, 1988; DiNardo and Barlow, 1988; Margraf, 1994). DSM-IV Axis II disorders were diagnosed using the International Diagnostic Checklists for Personality Disorders (IDCL-P) (Bronisch and Mombour, 1998). Two prerequisites were required for personality disorder diagnosis: (i) the rater had to have observed a subject throughout a variety of situations over several months; (ii) diagnosis of personality disorder was made only for patients who had passed the third abstinent month to avoid misinterpretation of temporary withdrawal-related dysfunctions as symptoms of personality disorders [for details see (Wagner *et al*., 2004; Krampe *et al*., 2006b)].

Neuropsychological assessment

All 50 subjects underwent a standardized test battery for intelligence, attention, learning, and memory 2–3 weeks after inpatient detoxification. Of those, 32 patients completed all 5 testing time-points of the study protocol.

Each of the selected tests is extensively validated and provides normative data for direct comparison of test performance with a normal population. Neuropsychological examinations were split into two sessions to be carried out within 1 week, each lasting for about 1 h. Four different randomized test orders were defined and distributed to patients at random, to avoid systematic effects of tiredness/exhaustion on the test results. Patients were first tested 2–3 weeks after inpatient detoxification (T_1), and at 3 months (T_2), 6 months (T_3), 12 months (T_4), and 24 months (T_5) of abstinence.

Neuropsychological test battery

Intelligence. Intellectual capacity was assessed at T_1 as an important control variable for group classification and interpretation of test results. Information, Similarities, Picture Completion, and Block Design subtests of the revised German version of WAIS-R [HAWIE-R; (Tewes, 1991)] were used to determine full scale, verbal, and performance intelligence quotient (IQ). An additional estimation of premorbid intelligence was carried out (Wilson *et al*., 1978).

Attention. Global attentional functions were examined using the subtest Alertness of the TAP ('Computer-assisted battery for attentional testing'); (Zimmermann and Fimm, 1995). This test measures reaction time to a visual stimulus (Greek cross) appearing on the monitor screen, that is ('tonic alertness') or is not preceded by a cue sound. Speed of information processing, quality of reactions (omissions), and ability to enhance the level of attention in expectation of a high-priority stimulus ('phasic alertness') were measured. Crossmodal integration; This subtest of the TAP detects simultaneous control of input from different sensory information channels. The test subject has to simultaneously pay attention to an acoustic (high or low pitch of 530 or 790 Hz, respectively) and a visual stimulus (up or down arrow) and is asked to press a button whenever high pitch and upward arrow, or low pitch and downward arrow coincide (critical stimulus). Speed of information processing and quality of reactions (omissions, errors) are determined. The tertiary region of the sensory cortex, the supramodal control (Mesulam, 1981, 2000), and the superior colliculi (Calvert *et al*., 2001; Frassinetti *et al*., 2002) are believed to be neural substrates of this crossmodal integrative function, as are the temporoparietal junction (Macaluso and Driver, 2001) and, to some degree, HC connectivities (Gottfried and Dolan, 2003), providing certain overlap to the HC tests.

HC-related functions (referred to as 'HC tests'). Classical HC learning and memory functions were assessed using the recognition tasks Verbal Learning Test [VLT; German version of the Recurring Words Test; (Sturm and Wilmes, 1999)] and the Nonverbal Learning Test [NVLT; adoption of the Kimura Recurring Figures Test; (Sturm and Wilmes, 1999)]. Explicit recognition memory, as measured with the VLT and NVLT, is closely related to HC function [e.g. (Kimura, 1963; Falk *et al*., 2002; Papanicolaou *et al*., 2002; Manns *et al*., 2003), in particular in visual modality (Hammond *et al*., 2004). The number of correct positive and false-positive responses is recorded, and false-positive responses are subtracted from right responses to correct for guessing. A standardized parallel version of the VLT allows controlling for recall bias. Orientation and visuospatial memory were tested using the 'City Map Test' of the LGT-3 ['Learning and Memory Test'; (Bäumler, 1974)]. This test evaluates 2D recognition/recall of map-like spaces and areas, and requires learning and remembering visuospatial configuration. The role of the HC in managing 'spatial cognitive maps' is well documented (Aguirre *et al*., 1996; Maguire *et al*., 1998; Matthews and Morrow, 2000). Considered a human equivalent of Morris water maze (Morris, 1984), this kind of task is highly associated with HC function (Beatty *et al*., 1996, 1997; Spiers *et al*., 2001). Subjects have to learn a defined route in a virtual street map within 60 s. After an interim period of 15 min, subjects are asked to reproduce this route from memory into an identical map. Two standardized parallel versions were given to the test subjects in alternating sequence (ABABA for 5 testing time-points). The selected HC tests (VLT, NVLT, and City Map Test), turned out to be well intercorrelated ($r = 0.613$ to $r = 0.238$), whereas there was no intercorrelation with the Crossmodal Integration Test of the TAP ($r = –0.024$ to $r = 0.018$).

Group design

Alcoholic subjects were divided into three groups, based on their results in HC-related tests at baseline (T_1): (i) patients without HC dysfunction on entry; (ii) patients with HC dysfunction on entry; (iii) patients with additional brain damage (Figure 1). The inclusion criterion termed as 'HC dysfunction' was fulfilled if a patients' performance was at least 1 SD below normal (T-value $\leqslant 40$; percentile $\leqslant 16$) in at least two out of the three predefined HC tests applied (see above). None of the patients of groups 1 and 2 had any evidence (by history, physical examination, technical, or laboratory tests) of liver cirrhosis, splenomegaly, or pancreatic failure. Patients of group 3 had the following diagnoses consistent with an additional brain damage: previous stroke in the right middle cerebral artery territory (2 patients); aneurysm of the anterior cerebral artery/past subarachnoid haemorrhage (1 patient); liver cirrhosis/hepatic encephalopathy (3 patients); schizophrenia (2 patients); astrocytoma with subtotal unilateral hippocampectomy (1 patient). All of these brain syndromes could potentially show an independent influence on neuropsychological test performance, in addition to alcohol-related changes. Therefore, and also because of its heterogeneity and small number, this patient group is not included in all statistical analyses.

Imaging

For financial reasons, computed tomography (CT) or magnetic resonance imaging (MRI) of the brain have not been part of the follow-up study design. However, available routine imaging data of the patients obtained during inpatient detoxification before inclusion into the OLITA programme have been employed for analysis.

Statistical analyses

All numerical results are presented as mean ± SD. Analyses (dependent and independent t-tests, χ^2-tests, correlations, univariate and repeated measures ANOVA and ANCOVA, Kaplan–Meier survival analysis, Cox regression analysis) were carried out using SAS 8.02 (SAS, 2001) and SPSS 11.5 (SPSS, 2003). Kaplan–Meier survival analyses were performed to investigate the time-to-event measure of days from first outpatient contact to relapse over a follow-up period of up to 4 years (Kleinbaum, 1996). Cox proportional hazard models were used to examine associations of time-invariant predictors (i.e. initial neuropsychological test performance expressed as sum score after z-transformation of HC test raw scores) with time to relapse. Cases are censored if they have not experienced an event (i.e. relapse) by the end of follow-up. Statistical significance was set at 0.05 for all analyses.

RESULTS

Patient groups, cognitive performance and imaging on study entry

All patients tested at T_1 ($N = 50$) were classified according to our group design. Whereas as many as 30 patients had HC dysfunction on entry, only 11 patients were without HC dysfunction, underlining that HC dysfunction is a frequent syndrome in chronic severe alcoholism ($\chi^2 = 8.805$, df = 1, $P = 0.03$). Figure 2 demonstrates the different neuropsychological profile of these two patient groups on initial testing.

Sociodemographic and alcohol-related data for all patients of the three groups who remained abstinent and could be followed over 2 years are presented in Table 1. Comparison between groups 1 and 2 revealed significant differences in education (school education: $P = 0.029$; total education: $P = 0.029$), duration of dependence ($P = 0.03$), number of inpatient detoxifications ($P = 0.03$), and amount of daily alcohol intake ($P = 0.022$), but no difference on measurements of intelligence (WAIS-R), estimated premorbid intelligence (Wilson *et al*., 1978) and attention (Tables 3 and 4). Nevertheless, the different duration of education had to be interpreted as potential difference in intellectual capacity. Univariate ANCOVA (analysis of covariance) reassured that neuropsychological test performance at baseline, and therefore group classification, could neither be explained by intellectual capacity (covariate: intelligence – estimated full scale IQ) nor by alcohol-related variables (covariates: daily alcohol dose, duration of dependence, number of detoxifications). Only for initial performance on VLT, the IQ ($F = 10.62$, df = 1, $P = 0.003$) rather than group association ($F = 2.71$, df = 2, $P = 0.085$) predicted performance. Importantly, an unbalanced effect in the different groups of psychiatric comorbidity on neuropsychological test performance appears unlikely, since there was (i) a statistically equal distribution of anxiety, depression, other Axis I disorders as well as personality disorders at baseline and (ii) a comparable reduction of comorbid Axis I disorders from T_1 to T_5 (Table 2).

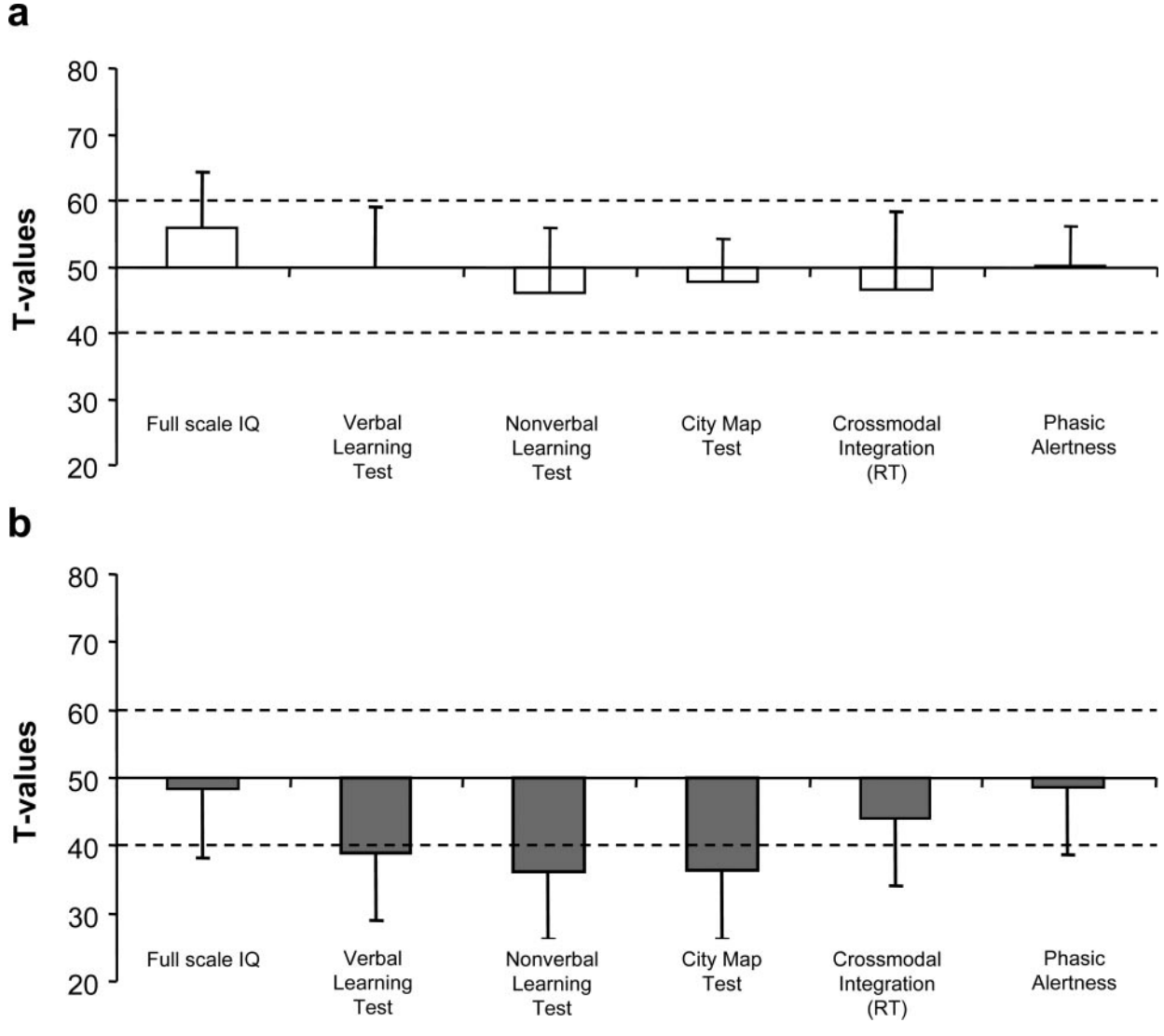

Fig. 2. Neuropsychological test profile on entry (*T*-values: mean ± SD) of (**a**) group 'without HC dysfunction on entry' ($n = 11$) and (**b**) group 'HC dysfunction on entry' ($n = 30$). Broken horizontal lines represent the normal range (test-provided normative data) of *T*-values. RT = median of reaction time.

Table 3. Stably normal performance of patients 'without HC dysfunction on entry' after 2 years of abstinence ($n = 9$ at T_1 and T_5)

Neuropsychological test results	T_1 2–3 weeks of abstinence	T_2 3 months of abstinence	T_3 6 months of abstinence	T_4 12 months of abstinence	T_5 24 months of abstinence
Intelligence					
(a) WAIS-R[a]					
Estimated full scale IQ	110.0 ± 15.1				
Estimated verbal IQ	106.7 ± 17.9				
Information	10.7 ± 3.4				
Similiarities	12.3 ± 3.0				
Estimated performance IQ	111.3 ± 15.1				
Picture completion	12.1 ± 2.3				
Block design	11.2 ± 3.0				
(b) Estimated premorbid intelligence (Wilson)[a]	99.2 ± 6.9				
Learning and memory					
(a) Verbal learning test (German version)					
Σ (correct positives–false positives)	39.0 ± 9.5	33.0 ± 15.2	41.1 ± 13.6	37.9 ± 8.4	43.6 ± 10.0
(b) Nonverbal learning test					
Σ (correct positives–false positives)	31.6 ± 7.9	31.4 ± 9.5	32.3 ± 11.5	35.1 ± 12.3	29.9 ± 9.3
(c) City map test (LGT-3)					
Delayed recall (raw score)	17.7 ± 3.6	13.9 ± 5.5	19.5 ± 4.8	15.7 ± 7.3	18.0 ± 5.7
Attention/concentration					
Computer-assisted battery for attention testing					
Crossmodal integration					
Mean of median reaction time (ms)	459.1 ± 108.5	452.2 ± 52.1	453.1 ± 60.6	453.9 ± 28.0	449.2 ± 73.9
SD of reaction time	77.9 ± 28.8	110.5 ± 52.3	88.9 ± 41.1	79.6 ± 23.4	94.1 ± 42.8
False reactions	0.2 ± 0.4	2.9 ± 6.2	1.4 ± 1.7	0.3 ± 0.5	0.0 ± 0.0
Omission errors	0.2 ± 0.7	2.3 ± 6.0	0.3 ± 0.5	0.0 ± 0.0	0.1 ± 0.3
Alertness (without cue sound)					
Mean of median reaction time (ms)	219.2 ± 30.7	239.8 ± 42.1	240.6 ± 26.1	248.4 ± 35.7	237.9 ± 24.5
SD of reaction time	41.2 ± 20.5	48.8 ± 25.1	45.3 ± 23.9	46.5 ± 18.4	36.2 ± 10.3
Alertness (with cue sound)					
Mean of median reaction time (ms)	205.3 ± 26.9	222.1 ± 34.1	222.9 ± 28.7	233.5 ± 43.2	222.1 ± 26.9
SD of reaction time	42.8 ± 22.9	37.6 ± 11.8	48.5 ± 37.7	44.4 ± 20.9	42.7 ± 20.7
Alertness (total)					
Index phase alertness	0.064 ± 0.042	0.074 ± 0.081	0.082 ± 0.112	0.068 ± 0.139	0.070 ± 0.062
Omission errors	0.2 ± 0.7	0.0 ± 0.0	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.4

Data at time points T_1 and T_5 are compared by dependent *t*-test (two-tailed). No significant differences were obtained.
[a]Measured only at T_1; SD = standard deviation; WAIS-R = Wechsler Adult Intelligence Scale-revised; LGT-3 = Learning and Memory Test (subtest 3).

Of groups 1 and 2, routine imaging data were available for 17 patients (5/9 patients of group 1, 12/17 patients of group 2). Diagnostic findings were rated in a blinded fashion as 'no cortical abnormality', or 'slight to moderate cortical abnormality'. Four out of 5 scans in group 1, and 5 out of 12 scans in group 2 were rated to have no cortical abnormality. Whereas performance in HC-related tests at baseline provided our group classification, cortical abnormality *per se* could not explain initial results of HC-related tests nor predict recovery (χ^2-tests, *t*-tests). As expected, all 6 patients with additional brain damage (group 3) were found to have 'severely abnormal CT/MRI scans'.

Neuropsychological follow-up (time and time × group effects)

Complete neuropsychological data of groups 1 and 2 are summarized in Tables 3 and 4. Abnormal scores, i.e. ⩾1 SD below the test-provided normative data, are highlighted (grey shaded fields). Group 1 started out with normal baseline values (T_1), and did not display significant alterations over time up to T_5. Group 2 exhibited distinct but circumscribed deficits upon study entry in all of the 3 HC tests. Significant improvement to values within the normal range at 2 years of abstinence was noticed comparing data of T_1 and T_5 on VLT ($P = 0.039$), NVLT ($P = 0.002$), and City Map Test ($P = 0.001$). There was also a significant reduction of omission errors on TAP subtest Crossmodal Integration ($P = 0.05$) which partly reflects HC functions. Attention test performance had remained essentially unaffected by chronic alcohol use in both groups and hence did not show signs of recovery.

The small group 3 ($n = 6$) expectedly proved heterogeneous during neuropsychological follow-up with highly varying test results in the different cognitive domains. Nevertheless, a comparison of performance between all three groups over time on the City Map Test yielded significant results when repeated measures ANCOVA adjusted by WAIS-R estimated full scale IQ was applied ($F = 5.405$, df = 2, $P = 0.01$). Figure 3 illustrates the different recovery of the three patient groups in the City Map Test.

Impact of HC dysfunction on clinical outcome

A question of high practical relevance was whether the absence or presence of HC damage and/or recovery had any effect on clinical outcome data. The impact of initial HC dysfunction on long-term abstinence probability is presented as Kaplan–Meier survival curves in Figure 4. Of all patients included (= baseline testing performed in $N = 50$), group 2 (with HC dysfunction; $n = 30$) has the tendency of a higher risk to relapse as compared to groups 1 and 3 ($n = 11$ and $n = 9$, respectively) (log rank = 3.54, df = 2, $P = 0.17$). This tendency is further supported by Cox regression analysis

Table 4. Significant improvement of HC test performance in patients 'with HC dysfunction on entry' after 2 years of abstinence (n = 17 at T_1 and T_5)

Neuropsychological test results	T_1 2–3 weeks of abstinence	T_2 3 months of abstinence	T_3 6 months of abstinence	T_4 12 months of abstinence	T_5 24 months of abstinence
Intelligence					
(a) WAIS-R[a]					
Estimated full scale IQ	98.1 ± 17.4				
Estimated verbal IQ	96.3 ± 19.3				
Information	8.9 ± 3.2				
Similiarities	11.0 ± 3.1				
Estimated performance IQ	100.9 ± 17.8				
Picture completion	11.4 ± 3.8				
Block design	8.6 ± 2.9				
(b) Estimated premorbid intelligence (Wilson)[a]	94.6 ± 6.6				
Learning and memory					
(a) Verbal learning test (German version)					
Σ (correct positive–false positive)	25.7 ± 11.6	16.3 ± 13.0	25.2 ± 14.3	27.9 ± 12.1	**32.9 ± 15.5***
(b) Nonverbal learning test					
Σ (correct positive–false positive)	18.3 ± 6.1	17.2 ± 7.3	15.8 ± 11.4	18.9 ± 11.3	**26.2 ± 10.1****
(c) City map test (LGT-3)					
Delayed recall (raw score)	9.1 ± 3.1	10.2 ± 4.3	11.2 ± 5.0	12.1 ± 5.4	**13.4 ± 3.4****
Attention/concentration					
(a) Computer-assisted battery for attention testing					
Crossmodal integration					
Mean of median reaction time (ms)	442.2 ± 68.3	424.2 ± 69.1	417.2 ± 79.5	448.6 ± 72.6	435.2 ± 74.6
SD of reaction time	86.4 ± 50.4	80.2 ± 38.8	85.9 ± 58.7	120.2 ± 65.8	92.4 ± 63.3
False reactions	0.9 ± 1.3	1.2 ± 1.4	0.9 ± 0.8	1.1 ± 1.6	0.9 ± 1.2
Omission errors	0.4 ± 0.7	0.6 ± 1.5	0.3 ± 0.6	0.4 ± 1.3	**0.1 ± 0.2***
Alertness (without cue sound)					
Mean of median reaction time (ms)	230.6 ± 36.9	251.3 ± 35.7	243.3 ± 29.9	238.8 ± 29.9	241.0 ± 39.0
SD of reaction time	43.7 ± 19.2	47.7 ± 27.0	40.7 ± 19.1	37.9 ± 12.8	40.5 ± 19.3
Alertness (with cue sound)					
Mean of median reaction time (ms)	226.9 ± 47.9	233.1 ± 31.6	233.7 ± 34.4	234.0 ± 34.6	241.3 ± 50.8
SD of reaction time	40.0 ± 16.4	40.1 ± 16.5	36.4 ± 13.0	41.6 ± 23.4	40.6 ± 21.6
Alertness (total)					
Index phase alertness	0.024 ± 0.088	0.074 ± 0.125	0.044 ± 0.094	0.024 ± 0.081	0.005 ± 0.097
Omission errors	0.5 ± 1.1	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.4	0.1 ± 0.2

Data at time points T_1 and T_5 are compared by dependent t-test (two-tailed). *$P < 0.05$; **$P < 0.01$; grey shaded fields denote values below normal range of T-values ($T \leqslant 40$).
[a]Measured only at T_1; SD = standard deviation; WAIS-R = Wechsler Adult Intelligence Scale-revised; LGT-3 = Learning and Memory Test (subtest 3).

estimating the predictive value of initial HC performance on time to relapse (B = –0.524, SE = 0.276, Wald = 3.603, df = 1, P = 0.058). In fact, the higher the degree of recovery (delta T_5–T_1 of the 3 HC tests) over 2 years of strict abstinence (which in turn depends on the degree of initial damage), the higher the cumulative probability of relapse during the 4-year follow-up period (B = 1.472, SE = 0.7, Wald = 4.422, df = 1, P = 0.035).

DISCUSSION

The present investigation demonstrates that more than two thirds of a representative population of severely affected chronic alcoholics display prominent HC dysfunction after 2–3 weeks of monitored alcohol abstinence. Most importantly, we demonstrate that, in the absence of additional brain damage, there is a slow but considerable recovery of HC performance in alcoholic patients who remain strictly alcohol abstinent. Patients with normal HC performance after detoxification as well as patients with additional brain damage do not show any improvement in performance over an observation period of 2 years of monitored abstention from alcohol.

Previous work on recovery of cognitive function in alcoholics yielded diverse and conflicting results. In contrast to our findings, several studies report on rapid improvement of functions (Kish *et al*., 1980; Leber *et al*., 1981; Drake *et al*., 1995; Mann *et al*., 1999; Tracy and Bates, 1999; Munro *et al*., 2000; Wegner *et al*., 2001) or do not find any signs of recovery but, instead, remarkable residual deficits (Brandt *et al*., 1983; Yohman *et al*., 1985; Parsons *et al*., 1990; Schandler *et al*., 1996). A recent study with long-term follow-up of female patients also reported slow recovery over as long as 4 years (Rosenbloom *et al*., 2004). Explanations of all these obvious discrepancies may be found in (i) a lack of objective abstinence control, (ii) a comparison of predominantly cross-sectionally analysed patient populations, resulting in restricted intra-individual follow-up data, (iii) the inconsistent exclusion of alcoholic patients with comorbid disorders/risk factors, (iv) the use of test batteries that do not allow to sensitively assess HC function, or (v) the time point of testing (e.g. during extended withdrawal). In fact, for investigating improvement of cognitive performance in alcoholics during periods of monitored alcohol abstinence, at least two different 'phases of recovery' have to be considered: A first phase that may be predominantly due to resolution of withdrawal symptoms together with recovery from acute intoxication, and a much more prolonged second phase of functional/morphological regeneration in which non-reported relapses may well interrupt cognitive recovery.

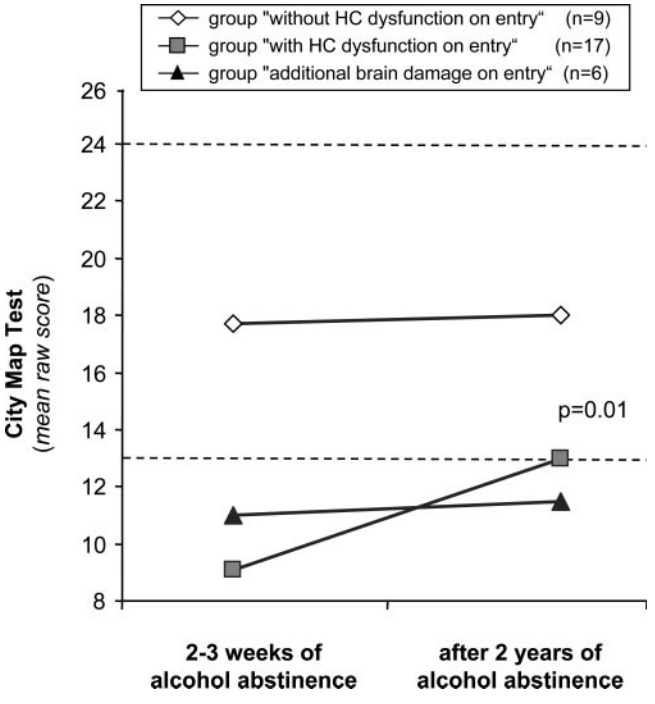

Fig. 3. Recovery of HC function in chronic alcoholics upon 2 years of strictly monitored alcohol abstinence as illustrated by the City Map Test. Presented is the course of mean raw scores from T_1 (2–3 weeks of abstinence) to T_5 (2 years of abstinence). Normal range (*T*-value = 40–60) begins at a raw score of 13 and extends to raw score 24 (dotted line; test-provided normative data). Significant group × time interactions (ANCOVA, WAIS-R estimated full scale IQ as covariate; $F = 5.405$, df = 2, $P = 0.01$).

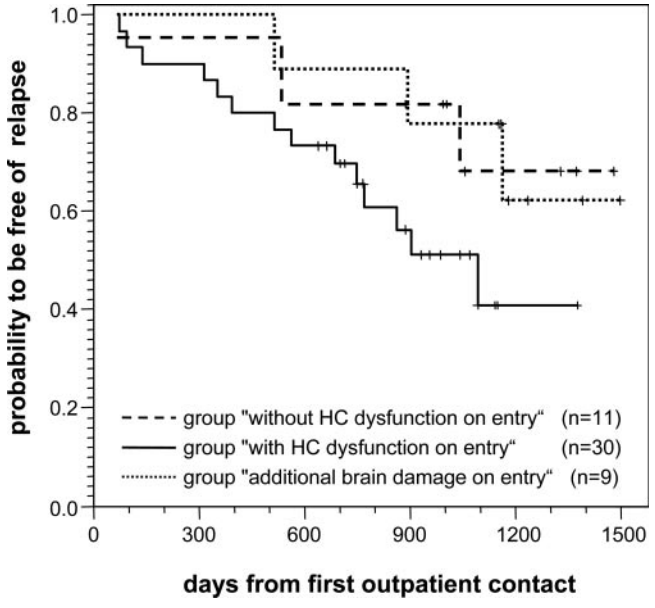

Fig. 4. Chronic alcoholics with initial HC dysfunction tend to have a higher risk to relapse. Probability of being free from relapse during a follow-up for up to 4 years (mean observation time: 1093 days, ranging from 64 to 1497 days) calculated separately for all three subgroups of patients. Patients 'with HC dysfunction on entry' were more likely to experience relapse (abstinence probability 0.41) compared to patients 'with additional brain damage on entry' (abstinence probability 0.62) and patients 'without HC dysfunction on entry' (abstinence probability 0.68) (log rank = 3.54, df = 2, $P = 0.17$). Kaplan–Meier estimates of time to relapse for defined subgroups; cases were censored if they had not experienced a relapse by the end of follow-up.

Importantly, patients with initial HC dysfunction in the absence of additional brain damage did not simply show global cognitive impairment. In most of these patients, there was a predominant HC impairment, whereas intellectual abilities and basal parameters of attention remained within the normal range. Also, preliminary analysis of a comprehensive set of executive function data did not reveal a simple association with HC performance (data not shown). The City Map Test proved to be the most sensitive test for evaluation of HC function in abstinent alcoholics and its recovery over time. It is a free recall test with purely visuospatial material, measuring orientation, processing of visuospatial information, learning, and memory. The fact that the group of patients without HC deficits upon entry showed a higher level of education and a lower amount of daily alcohol intake, raised the question of whether these two variables might explain the differences in HC function found between the two groups. Analysis of covariance, however, integrating these variables as covariates did not reveal an impact of intellectual abilities or alcohol dose on neuropsychological test results that would have changed the overall picture. Furthermore, a systematic effect of psychiatric comorbidity on HC test performance seems unlikely, with both groups showing a similar distribution and remission of comorbid psychiatric disorders. The same holds true for a potential influence of long-term deterrent medication on cognition. Groups did not differ with respect to dose and duration of intake of either disulfiram or calcium carbimide (data not shown). A major impact of practice through repeated testings can be excluded since neither the group without initial deficits in HC performance nor the group with additional brain damage showed any evidence of a 'learning-curve'.

An interesting topic of the present investigation as well as of previous work of other authors (Franceschi *et al*., 1984; Fein *et al*., 1990) is the question of why approximately one-third of severely affected chronic alcoholics do not display any measurable deficits in HC performance. The most attractive interpretation of this result is the presence of predisposing versus protective factors in the nervous system of these individuals which might be either of genetic or/and of environmental nature. Investigations to come should include genetic evaluation of various neuroprotective systems, e.g. of antioxidative pathways, of calcium binding proteins, or of components of the erythropoietin system (Neiman, 1998; Dirnagl *et al*., 2003; Juul *et al*., 2004). Furthermore, future studies should try to make use of recent developments in imaging technology for follow-up of morphological recovery. Of note is the fact that in the present study, evaluation of routine brain imaging results was neither helpful in predicting HC test performance nor estimating potential of recovery. This finding is in agreement with previous literature (Pfefferbaum *et al*., 1988; Sullivan *et al*., 1995; Ratti *et al*., 1999), and questions the importance of routine imaging technology for neuropsychological follow-up studies.

Perhaps the most important take-home message of the present study is the finding, during strictly monitored long-term abstinence, of a slow but remarkable recovery process, which may well continue over more than the 2 years

follow-up reported here (Rosenbloom *et al*., 2004) (Reed *et al*., 1992). Assuming that the degree of cognitive impairment acts as a predictor of probability to relapse (based on a clear tendency seen in our patient population), a strictly abstinence-oriented comprehensive long-term therapy programme will, by increasing the probability of cognitive recovery, improve global therapy outcome.

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3 Resumée und Ausblick

Generell ist die Möglichkeit einer Heilung neurodegenerativer Erkrankungen in den nächsten Jahren als äußerst unwahrscheinlich anzunehmen. Präventive Maßnahmen können nur limitiert eingesetzt werden oder verfügen über nur begrenzte Wirksamkeit, palliative Interventionen können nur Symptomlinderung bewirken. Aus diesem Grund sind neuroprotektive Ansätze vermehrt und zum Teil vielversprechend in den Forschungsvordergrund getreten.

Mit den beiden vorliegenden Originalarbeiten werden zwei unterschiedliche Vorgehensweisen dargestellt, die neuroprotektive Behandlungsstrategien bei zwei verschiedenen neurodegenerativen Erkrankungen veranschaulichen sollen. Als neuroprotektiv soll dabei ein von außen zugeführter Therapieansatz gelten, der in der Lage ist, eine möglichst hohe Integrität zellulärer Interaktionen im Gehirn zu erhalten bzw. wiederherzustellen oder der indirekt endogene Schutzprogramme aktiviert/verstärkt. In klinischen Prüfungen müsste sich diese neuroprotektive Wirkung darin widerspiegeln, dass entweder Krankheitsprogredienz abgemildert, eine Zustandsstabilisierung erreicht oder im günstigsten Fall eine Verbesserung des Funktionsniveaus erzielt wird. Als Read-out des Therapieerfolgs ist eine Vielzahl krankheitsspezifischer Parameter denkbar, deren klinische Relevanz natürlich im Vordergrund stehen sollte.

Am Beispiel der neurologischen Motoneuronerkrankung ***ALS*** wird dabei der klassische Weg eingeschlagen, das neuroprotektive Potential einer Substanz als „Add-on"-Therapie sukzessiv – präklinisch in der Zellkultur und im Tiermodell bis hin zu einer klinischen Sicherheitsstudie – zu explorieren. In dem dargestellten Projekt gelang es, Melatonin als neuroprotektive Kandidatensubstanz zu identifizieren, die antioxidativ *in vitro* glutamat-induzierten Zelltod verminderte sowie langsamere Krankheitsprogression und längeres Überleben im ALS-Mausmodell zeigte. Melatonin erwies sich darüber hinaus in einer Hochdosis-Sicherheitsstudie bei sporadischer ALS als sicher sowie oxidativen Stress reduzierend. Zur Bestimmung der Wirksamkeit steht eine bereits geplante, klinische Studie aus. Dabei soll die Hypothese geprüft werden, dass Hochdosis-Melatoninbehandlung bei ALS zu einer erhöhten Überlebenswahrscheinlichkeit und einer geringeren Geschwindigkeit des Krankheitsfortschritts im Vergleich zu einer Placebobehandlung führt (u.a. gemessen mit der ALS Functional Rating Scale).

In den letzten Jahren ist es zu einer hohen Dichte von i.d.R. erfolglosen Behandlungsstudien bei ALS gekommen. Zur Optimierung neuroprotektiver Strategien bei ALS sollten daher verstärkt folgende Überlegungen in die Forschungsbemühungen

einfließen: Bei ALS hat sich ein frühzeitiger Behandlungsbeginn in verschiedensten Studien (Ascherio et al., 2005; Brooks, 1999; Mackin, 1999; Weishaupt* et al., 2006) als überlegen gezeigt. Diese scheinbar simple Forderung für die Umsetzung am Patienten erweist sich jedoch bisher als kaum durchführbar, da im Durchschnitt bis zu einem Jahr von Symptombeginn bis zur Diagnosestellung vergeht (Zoccolella et al., 2006). Weiterhin stellt sich die Forderung nach einer stärkeren Selektion der Kandidaten-substanzen, da zahlreiche Ansätze erfolgreich im Tiermodell getestet wurden, in klinischen Studien der Wirksamkeitsnachweis jedoch nicht erbracht werden konnte (z.B. Cheung et al., 2006; Rothstein, 2003). Auch Melatonin kann in einer anstehenden klinischen Prüfung potentiell „nur“ neuroprotektiv, also nicht heilend, sondern maximal Progredienz verzögernd, wirken. Angesichts der Prognose bei ALS wäre es deshalb ratsam, sich nicht nur auf eine Monotherapie zu verlassen, sondern vermehrt Kombinationstherapien („drug cocktails“) zu testen, die an verschiedenen Pathomechanismen der Erkrankung ansetzen (Carri et al., 2006; Kriz et al., 2003; Ludolph, 2000). Zusammengenommen ergibt sich hier noch ein großes Aufgabenfeld für die präklinische und klinische Forschung. Eine bessere und reziproke Interaktion zwischen den einzelnen Forschungsgebieten ist jedoch Grundvoraussetzung, um eine schnellere Übersetzung in den klinischen Gebrauch sowie um eine Behandlungs-optimierung zu ermöglichen. Das Vorhandensein verlässlicher und valider biochemischer/biologischer Marker kann zusätzlich die ALS-Diagnostik erleichtern und das Urteil über die Wirksamkeit einzelner Substanzen sichern (Bowser et al., 2006; Ludolph, 2000). Mit zunehmender Forschung gelangen auch immer mehr strukturelle und/oder funktionale Abweichungen bei ALS in den Blickpunkt, die über die bereits bekannte Motoneurondegeneration hinausgehen. Solche Merkmale könnten ebenfalls als diagnostische und Verlaufsparameter bei klinischen Prüfungen genutzt werden (z.B. kognitive Leistungen, kontra-laterale Mitbewegungen; Bartels, in preparation; Krampfl et al., 2004; Massman et al., 1996; Neary et al., 2000; Wittstock et al., 2006). Diese Entwicklung bedeutet zudem eine Bereicherung zu den bisher etablierten Skalen für die Verlaufsdokumentation, da viele dieser Messinstrumente unzulänglich und wenig sensitiv für Veränderungsmessungen erscheinen (siehe auch modified Rankin Scale bei Schlaganfall, Glasgow Coma Scale bei Schädel-Hirn-Trauma, Karnofsky Performance Status Scale bei Krebserkrankungen; Rankin, 1957; Schag et al., 1984; Teasdale & Jennett, 1974; van Swieten et al., 1988). Allgemein sollten zur Beurteilung des Therapieerfolgs neuroprotektiver Behandlungen verschiedene Items kombiniert werden, um Veränderungen und Unterschiede sensitiv erfassen zu können.

Wird absolute Abstinenz bei dem psychiatrischen Störungsbild ***Alkohol-abhängigkeit*** als neuroprotektiv definiert, steht mit ALITA ein biopsychosoziales Therapieprogramm zur Verfügung, das sich durch eine besonders hohe Langzeit-abstinenzwahrscheinlichkeit ausgezeichnet hat und somit als Neuroprotektion ermöglichend angesehen werden kann. Unter objektiver Abstinenzkontrolle konnte in der vorgestellten neuropsychologischen Studie herausgearbeitet werden, dass die Mehrheit der untersuchten, schwer alkoholabhängigen Patienten von einer spezifischen Dysfunktion HC-assoziierter Funktionen betroffen ist, die sich erst über einen Zeitraum von zwei Jahren hinweg normalisiert. Patienten ohne initiales Defizit zeigten hingegen keine Leistungsverbesserung. Bei Patienten mit zusätzlicher hirnorganischer Schädigung verhinderte der „dual hit" vermutlich eine Erholung der beeinträchtigten HC-Funktionen. Klinische Relevanz erhalten diese Ergebnisse unter Berücksichtigung der unterschiedlichen Abstinenzwahrscheinlichkeiten in den Gruppen. Therapeutisch impliziert das erhöhte Rückfallrisiko kognitiv beeinträchtigter Patienten eine intensivere, nicht überfordernde und womöglich noch langfristigere Betreuung.

Alkoholabhängigkeit weist zwar eine deutlich erhöhte Mortalitätsrate auf (Bullock et al., 1992; Miller, 1999), ist aber im Vergleich zu ALS nicht ultimativ mit einer Lebensbedrohung verbunden. Neben erheblichen psychosozialen Folgen entstehen jedoch gravierende körperliche Folgeschäden fast aller Organsysteme, insbesondere des Gehirns. Zusammen mit der hohen Prävalenz verursachen Alkoholabhängigkeit und -abusus massive sozioökonomische Kosten (Uhl & Grow, 2004). Zur Kosten-reduktion und Verlängerung der Lebenserwartung Alkoholkranker müssen von den Kostenträgern insbesondere *nachweisbar* abstinenzfördernde Behandlungsprogramme unterstützt werden und aus der Vielfalt an Verfahren stärker selektiert werden. Um die Effektivität eines Therapieprogramms verlässlich einschätzen zu können, ist es insbesondere notwendig, Daten zur Alkoholkarenz unter objektiver Abstinenzkontrolle zu erheben. Studien, die auf Selbstauskunft der Patienten basieren, scheinen dagegen zu einer überschätzten Abstinenzrate zu führen. ALITA bietet sich gemäß dieser Beschreibung als ein vielversprechendes, neuroprotektives „Kandidatenprogramm" an. Mit diesem biopsychosozialen Ansatz und einem interdisziplinären Team konnten hohe Abstinenzraten im Langzeitverlauf erreicht werden (Krampe et al., 2006a). Für die Verbesserung vorhandener Behandlungskonzepte muss jedoch generell geklärt sein, welche Therapieelemente zu einer höheren Abstinenzwahrscheinlichkeit beitragen und welche nicht. Für die genaue Betrachtung des Therapieprozesses liegen erst wenige Hinweise vor, welche Ressourcen von Patientenseite, welche Therapeuteneigen-

schaften und welche Interventionen langfristigen Therapieerfolg bewirken können (z.B. Ilgen et al., 2006; Krampe et al., 2006a; Stawicki, 2007). Letztendlich müssen diese und folgende Erkenntnisse in die konkrete Behandlungsplanung integriert werden. Beispielhaft für die zweite Originalarbeit ausgeführt, bedeutet dies, dass eine alkoholinduzierte HC-Dysfunktion ein erhöhtes Rückfallrisiko mit sich bringt. Durch einen Rückfall wird wiederum eine Remission kognitiver Defizite verhindert, und neuer Schaden kann hinzukommen. Ohne besondere Berücksichtigung dieses „prognostischen" Faktors würde dies einen „Teufelskreis" für die betroffenen Patienten bedeuten. Um letztendlich abstinenzvermittelte Neuroprotektion zu bewerten, sollten – analog zu dem Beispiel – Verlaufsparameter mit möglichst hoher klinischer Relevanz ausgewählt werden. Aktuell verfügbare, bildgebende Verfahren scheinen zwar durchaus geeignet zur Abbildung von Regeneration, aufgrund der Dissoziation morphologischer und funktionaler Daten bleibt die Bedeutung für das Funktionsniveau des Patienten jedoch häufig noch fraglich (Pfefferbaum et al., 1988; Ratti et al., 1999; Sullivan et al., 1995).

Mit der Optimierung von Behandlungsstrategien neurodegenerativer Erkrankungen und einer damit einhergehenden längeren Lebenserwartung rücken weitere Outcome-Parameter zunehmend in den Vordergrund, die über das simple Überleben der Patienten oder eine Symptomentlastung hinaus gehen. Aspekte wie Lebensqualität, kognitive Leistungsfähigkeit oder soziale Unterstützung gewinnen in diesem neu gewonnenen Zeitrahmen für die Betroffenen an Relevanz. Dabei handelt es sich zum einen um deren Erhalt bei tödlich verlaufenden Erkrankungen wie ALS oder deren Wiederherstellung bei Störungsbildern wie Alkoholabhängigkeit, die mit körperlichen, psychischen und sozialen Folgeschäden verbunden sind. Für diverse neurodegenerative Erkrankungen, wie ALS und Alkoholabhängigkeit, liegen bereits einige Daten zur Lebensqualität vor, jedoch selten im Längsschnitt (Andersen et al., 2005; Borasio et al., 2001; Bremer et al., 2004; Donovan et al., 2005; Foster et al., 1999; Longabaugh et al., 1994; Nygren & Askmark, 2006; Simmons, 2005). Behandlungsstudien, die im Längsschnitt über Monate und Jahre zeigen können, durch welche Faktoren Lebensqualität beeinflusst wird bzw. sie dadurch auch günstig beeinflussbar wird, sind erst im Entstehen (z.B. Krampe, submitted). Angesichts der geringen Heilungsaussichten neurodegenerativer Erkrankungen sollten gerade neuroprotektive Behandlungsstrategien nicht nur auf das bloße Überleben oder die Verbesserung singulärer Parameter abzielen, sondern müssen auch verstärkt berücksichtigen, *wie* dieses verlängerte Überleben aussieht und in diesem Sinne Lebensqualität als zusätzlichen Zielparameter einplanen.

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5 Übersicht laufender Forschungsprojekte und Publikationsverzeichnis

Innerhalb der Division Klinische Neurowissenschaften ist die Autorin derzeit bei weiteren Projekten in klinische, psychopathologische und v.a. neuropsychologische Fragestellungen maßgeblich involviert. Diese finden in der vorliegenden Dissertation keine ausführliche Erwähnung, stehen jedoch eng mit den hier vorgestellten Untersuchungen in Verbindung. Im Forschungsschwerpunkt stehen die Entwicklung und Durchführung weiterer neuroprotektiver Behandlungsstrategien bei Multipler Sklerose und Morbus Parkinson. Als neuroprotektive Kandidatensubstanz kommt dabei der hämatopoietische Wachstumsfaktor Erythropoietin zum Einsatz. Eine weitere neuropsychologische Therapiebegleitstudie bei malignem Gliom hat gerade begonnen und untersucht Radiatio-induzierte, degenerative Veränderungen (kognitive Leistungseinbußen) und Lebensqualität im Verlauf der aggressiven Routinebehandlung.

Die bereits zum Abschluss gebrachten Fragestellungen, an denen die Autorin beteiligt war, sind der folgenden Publikationsliste zu entnehmen.

Originalartikel

*: *Die Autoren trugen zu gleichen Anteilen zu der Arbeit bei.*

**: Bei einem Teil der Publikationen wurde noch der Geburtsname (*Galwas*) geführt.

Krampe, H, Wagner, T, Stawicki, S, Reinhold, J, **Galwas, C****, Aust, C, Soyka, M, Kröner-Herwig, B, Küfner, H, Ehrenreich, H (**2003**). Chronisch mehrfach beeinträchtigte Abhängigkeitskranke" - Überprüfung des Konstrukts CMA im Rahmen der Ambulanten Langzeit-Intensivtherapie für Alkoholkranke (ALITA) [Chronic multimorbid addicts (CMA): evaluation of the construct CMA in the context of the outpatient long-term intensive therapy for alcoholics (OLITA)]. *Suchtmedizin in Forschung und Praxis* 5: 221-236.

Wagner, T, Krampe, H, Stawicki, S, Reinhold, J, Jahn, H, Mahlke, K, Barth, U, Sieg, S, Maul, O, **Galwas, C****, Aust, C, Kröner-Herwig, B, Brunner, E, Poser, W, Henn, F, Rüther, E, Ehrenreich, H (**2004**). Substantial decrease of psychiatric comorbidity in chronic alcoholics upon integrated outpatient treatment - results of a prospective study. *Journal of Psychiatric Research* 38 (6): 619-635.

Krampe, H, Stawicki, S, Wagner, T, **Bartels, C**, Aust, C, Rüther, E, Poser, W, Ehrenreich, H (**2006**). Follow-up of 180 alcoholic patients for up to seven years after outpatient treatment: Impact of alcohol deterrents on outcome. *Alcoholism Clinical & Experimental Research* 30 (1): 86-95.

Krampe, H, Wagner, T, Stawicki, S, **Bartels, C**, Aust, C, Kröner-Herwig, B, Küfner, H, Ehrenreich, H (**2006**). Personality disorder and chronicity of addiction as independent outcome predictors in alcoholism treatment. *Psychiatric Services* 57 (5): 708-712.

Weishaupt, JH*, **Bartels, C***, Pölking, E, Dietrich, J, Rohde, G, Poeggeler, B, Mertens, N, Sperling, S, Bohn, M, Hüther, G, Schneider, A, Bach, A, Sirén, AL, Hardeland, R, Bähr, M, Nave, KA, Ehrenreich, H (**2006**). Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. *Journal of Pineal Research* 41 (4): 313-323. ***Teil der Dissertation.***

Bartels ,C, Kunert, H-J, Stawicki, S, Kröner-Herwig, B, Ehrenreich, H, Krampe, H (**2007**). Recovery of hippocampus-related functions in chronic alcoholics during monitored longterm abstinence. *Alcohol and Alcoholism*. Published advanced access 2006. ***Teil der Dissertation.***

Stawicki, S, Krampe, H, Niehaus, S, Ribbe, K, Wagner, T, **Bartels, C**, Kröner-Herwig, B, Ehrenreich, H (**2007**). Multimodales Monitoring psychotherapeutischer Prozesse in der Behandlung alkoholkranker Patienten [Multimodal Monitoring of psychotherapeutic processes in the treatment of alcohol dependent patients]. *Suchtmedizin in Forschung und Praxis*. In press.

Krampe, H, **Bartels, C**, Victorson, D, Enders, CK, Beaumont, J, Cella, D, Ehrenreich, H (**submitted**). Prospective long-term investigation of quality of life in patients with Amyotrophic Lateral Sclerosis.

Bartels, C, Mertens, N, Hofer, S, Merbold, D, Küntzel, M, Dietrich, J, Frahm, J, Ehrenreich, H (**in preparation**). Clinical parameters of callosal dysfunction in amyotrophic lateral sclerosis correlate with findings in diffusion tensor imaging of the central motor system.

Übersichtsarbeiten, Bücher, Buchkapitel

Krampe, H, Wagner, T, Reinhold, J, Stawicki, S, Mahlke, K, **Galwas, C****, Barth, U, Aust, C, Haase, A, Jahn, H, Kröner-Herwig, B, Ehrenreich, H (**2003**). Therapieprozesse bei ALITA (Ambulante Langzeit-Intensiv-therapie für Alkoholkranke): Multiple Beziehungsgestaltung in der integrativen Therapie chronisch psychisch kranker Menschen [Therapeutic processes in OLITA: multiple development of therapeutic alliance during integrated treatment of chronically mental ill individuals]. *Gesprächspsychotherapie und Personzentrierte Beratung* 34: 75-84.

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6 Curriculum vitae

Persönliche Angaben

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Beruflicher Werdegang

Seit 12/2003	Verhaltenstherapeutin (in Ausbildung), Abteilung für Psychologie and Psychotherapie, Georg-August-Universität, Göttingen
10/2002-10/2003	Therapeutische Mitarbeiterin bei *ALITA* (*A*mbulante *L*angzeit-*I*ntensiv-*T*herapie für *A*lkoholkranke), Klinik für Psychiatrie und Psychotherapie, Georg-August-Universität, Göttingen
2000-2004	Psychologische Online-Ernährungsberaterin (“Slimnet”)
1999-2002	Studentische Hilfskraft, Abteilung für Psychopathologie und Neuropsychologie, Klinik für Psychiatrie und Psychotherapie, Georg-August-Universität, Göttingen

Ausbildung

1982-86	Grundschule, Vellmar
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1995-2003	Diplomstudiengang Psychologie, Georg-August-Universität, Göttingen (Gesamtnote 1,54)
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