EDITORIAL

Dear Colleagues,

Welcome to the third issue in the year 2010. This issue contains mostly original articles, thus I am pleased to present to you many interesting research findings.

Despite its proven efficacy and safety, electroconvulsive therapy (ECT) has a negative image and attracts widespread public criticism. In contrast, perceptions of patients who have received ECT appear to be more favourable. Subho Chakrabarti and colleagues from India present a review and provide evidence on knowledge and views concerning ECT among its recipients. Interestingly, it seems that patients undergoing ECT were usually poorly informed about it. Thus, it is suggested to improve the pretreatment procedures for patients undergoing ECT.

Christine Wiebking and colleagues investigated behavioural and neural correlates of interoception in healthy and depressed subjects using the Body Perception Questionnaire (BPQ) and a well established heartbeat perception task in fMRI. Indeed, the results provide evidence that behavioural and neural abnormalities are closely related to the patients' somato-vegetative abnormalities and their abnormal "material me".

Breno Satler Diniz and colleagues from Brazil present a study with 29 elderly subjects with major depression and 42 healthy older adults. All depressed patients were antidepressant-free for at least one month prior to clinical and laboratorial assessments. Serum Brain-derived neurotrophic factor (BDNF) levels were determined by sandwich Enzyme-linked immunosorbent assay (ELISA). The aim of the study was to investigate serum BDNF levels in older depressed patients as compared to healthy elderly controls. The colleagues could provide evidence that reduced serum BDNF level may be a state marker of late-life depression in non-medicated elderly patients. Further, reduced neurotrophic may have an important role in the physiopathology of late-life depression

Abnormalities of brain white matter and oligodendroglia are replicated findings in schizophrenia research. Nadine Farkas and German colleagues studied the numerical density of A Disintegrin and Metalloprotease (ADAM) 12 expressing oligodendrocytes in postmortem prefrontal brains of patients with haloperidol treated, chronic schizophrenia and matched controls and found evidence that reduced ADAM12 protein contributes to a deviant metabolism of some of its substrates known to be compromised in schizophrenia.

Ultrastructural abnormalities of capillaries and of pericapillary cellular environment found suggest that blood-brain barrier dysfunction might contribute to the pathogenesis of cortical lesions in schizophrenia. Natalya Uranova and Russian colleagues conducted a study with 26 schizophrenia patients and 26 healthy controls and investigated the capillaries in PFC (BA 10) and visual cortex (VC) (BA 17) by electron microscopy and morphometry.

Beata Sebestyen and colleagues from Hungary analysed the relationship between increasing antidepressant utilization and the national suicide rate in Hungary between 1998 and 2006, with particular regard to seasonal patterns and gender differences. During the 9 years of the study period there was a significant correlation between the steadily increasing antidepressant prescription and continuous decline in total national suicide rate as well as both in females and males. Thus, increasing antidepressant utilization can be associated with significantly decreased seasonality of suicides, especially among males.

Despite its clinical importance and relevance for health care policy, the pathways between depression and stress regulation remain poorly understood. Johannes Ehrenthal and German colleagues recorded cardiovascular and autonomic reactions to two different stress tasks including anger recall and mental arithmetic in a sample of 25 severely depressed and 25 non-depressed subjects. The results provide further evidence for altered cardiovascular reactivity and impaired cardiac autonomic functioning in depression. Thus, the authors suggest further research on the psychophysiological response to either more disease-oriented or more personality-oriented stressors.

Serge Brand and colleagues from Switzerland conducted a study with 107 adolescents (of which 70 had experienced intense romantic love and 47 were controls). Participants completed the Hypomania Check List, and data were compared with those of adult outpatients suffering from bipolar II disorders. The aim of the study was to compare hypomania scores of adolescents with those of adult outpatients suffering from bipolar II disorders, and to investigate possible gender-related differences. Indeed the authors could provide evidence that adolescents' developmental tasks surrounding experiences in social, psychosexual and substance-use-related engagement may lead to temporary and gender-related hypomanic-like stages.

Yours sincerely,

Siegfried Kasper, MD
Chief Editor

ISSN 1562-2975 print/ISSN 1814-1412 online © 2010 Informa UK Ltd. (Informa Healthcare, Taylor & Francis AS)
DOI: 10.3109/15622971003721514

The World Journal of Biological Psychiatry, 2010; 11: 525–537

REVIEW ARTICLE

Electroconvulsive therapy: A review of knowledge, experience and attitudes of patients concerning the treatment

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Abstract

Objectives. Despite its proven efficacy and safety, electroconvulsive therapy (ECT) has a negative image and attracts widespread public criticism. In contrast, perceptions of patients who have received ECT appear to be more favourable. This review intended to encapsulate the evidence on knowledge and views concerning ECT among its recipients. *Methods.* Extensive electronic and manual searches were conducted to identify all relevant studies on the subject. *Results.* Seventy-five reports were found suitable. The evidence from these studies suggested that patients undergoing ECT were usually poorly informed about it. This was attributable to factors such as unsatisfactory pre-treatment explanations or post-ECT memory impairment. About one-third undergoing ECT reported feeling coerced to have the treatment. Fear of ECT and distressing side effects were also present in a majority. Despite these problems, a vast majority of patients perceived ECT to be helpful and had positive views regarding the treatment. Simultaneously, a sizeable proportion was quite critical, although little was known about the extent and nature of such disapproval. *Conclusions.* Overall, the weight of the evidence supports the notion that patients undergoing ECT are well-disposed towards it. However, much needs to be done to improve the practice of ECT and to enhance patients' satisfaction with the experience of treatment.

Key words: *ECT, knowledge, perceptions, experience, patients*

Introduction

Despite 70 years of existence and substantial proof of efficacy, electroconvulsive therapy (ECT) still continues to be one of the most controversial and misunderstood treatments in medicine (Fink 2001; Dowman et al. 2005; Bauer et al. 2007). Over the years refinements such as use of anaesthetics and muscle relaxants, and of brief pulse machines which deliver carefully titrated electrical stimuli to trigger a seizure under controlled circumstances, have rendered the procedure safe and less discomforting (Goodman et al. 1999). Yet considerable stigma still surrounds ECT, which undermines the public's acceptance of this treatment. Primitive practices of the past, negative media representations, irrational fears of electricity, all contribute to this public disapproval. Unfortunately, in this debate about its role, the opinions of patients undergoing ECT have rarely been evaluated. Professionals are partly to blame, since their research has traditionally focused on aspects such as efficacy, side effects, mechanism of action, etc. However, the realisation that mere clinical efficacy of ECT did not necessarily predict patients' perceptions or satisfaction with the treatment has eventually propelled several investigations of the knowledge, attitudes and experience of the procedure among patients (Malcolm 1989; Johnstone 1999). The present review intended to encapsulate the findings that have emerged from this exercise and their possible implications for the practice of ECT.

Method

Electronic databases such as the PUBMED, Google and PSYC INFO were searched using various combinations of the terms "knowledge", "attitudes", "experience", "perceptions", "views" and "ECT", to

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(Received 12 August 2009; accepted 14 December 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903559925

identify studies that had ascertained patients' knowledge, views and experience regarding ECT. Criteria for including studies were kept fairly broad in order to capture most of the data available. Thus, any report that yielded data on patients' awareness and perceptions of ECT was included, even if the primary focus of the study was different and regardless of its design. Consequently, the nature of reports varied a great deal. They included peer-reviewed studies (observer-rated, self-rated and survey data), as well as reports by consumer-organisations. However, individual lay or professional opinions (e.g., editorials), or personal accounts of ECT were excluded, as were studies on views of public or health-professionals. All suitable publications in English were extracted. In all cases relevant cross-references listed in these publications were subsequently searched manually for pertinent studies.

Results

Details of studies included

We found 85 reports on the subject. Eight reviews and two studies conducted solely among relatives of treated patients (Walter et al. 1999; Sethi and Williams 2003) were excluded. The final list thus included 75 studies; 68 of these were peer-reviewed; seven were reports published by consumer organisations. Fifty studies were observer-rated; nine were self-reports or subjective accounts, and 16 were based on surveyed data. These studies together included a total of about 6000 patients from 17 countries (UK 29, USA 14, India 10, Australia 7, Canada 3, Sweden 2, Belgium 2; Denmark, France, Czech Republic, Turkey, Argentina, Brazil, Hong Kong, Pakistan, Iran, Japan 1 each). Only one study (Chavan et al. 2006) had exclusively assessed patients who had not received ECT. Nine had been conducted among the elderly (> 60 years), two among adolescents, the rest included young to middle-aged adults. Majority of the patients were women. Since most reports originated from Western countries, there was a predominance of Caucasian patients, though other ethnic groups were also well represented. Most patients had received ECT for depression, followed by psychotic disorders and mania.

Knowledge of ECT among patients

Details could be extracted from 20 studies. Methods employed to elicit patients' knowledge included spontaneous reports, semi-structured interviews or administration of questionnaires. Poor knowledge about ECT among patients was the dominant trend across a majority of these reports. This was best illustrated by the small proportions of patients who had complete, or near complete knowledge and understanding of the procedure (Table I).

The only exceptions to this trend appeared to be the few studies that had reported adequate knowledge regarding ECT among a high proportion of their subjects. However, even in these reports, many patients were unaware of the finer details of the procedure such as different indications, technical aspects, mechanism of action, nature of side effects, etc. Accordingly, though Kerr et al. (1982) found that 59% of their patients got all their answers correct, 15–30% of them harboured several misconceptions about the usefulness of ECT in preventing suicide and its usual side effects. Similarly, although Walter et al. (1999) estimated that 62% of their 26 respondents had adequate knowledge about ECT, only 15% knew about the indications for its use.

Table I. Knowledge of ECT among patients.

No.	Study	Proportion of patients with full knowledge*
1.	Freeman and Kendell 1980 (n=166)	15% (30% partial knowledge)
2.	Hughes et al. 1981 (n=72)	7%
3.	Kerr et al. 1982 (n=60)	59% (15–30% had several misconceptions)
4.	Benbow 1988 (n=54)	12%
5.	Malcolm 1989 (n=100)	16%
6.	Ramachandra et al. 1992 (n=50)	6% (84–96% knew about most aspects of ECT)
7.	Riordan et al. 1993 (n=37)	10%
8.	Walter et al. 1999 (n=26)	15% (62% were aware of most aspects)
9.	Taieb et al. 2001 (n=10)	10% (50% had poor knowledge)
10.	Tang et al. 2002 (n=96)	23% (52% had knowledge of most aspects of ECT)
11.	Chavan et al. 2006 (n=89)	17% (42–89% had knowledge of most aspects of ECT)
12.	Bustin et al. 2008 (n=75)	8%
13.	Rajagopal 2008 (n=50)	0% (16% near correct answers)

*Other studies which have shown poor knowledge concerning ECT among patients (but have not given the proportion of patients with full understanding) include those by Jenaway 1993; Westreich et al. 1995; Greening et al. 1999; Rajkumar et al. 2006, 2007; Arshad et al. 2007; Malekian et al. 2009.

While Tang et al. (2002) reported that 52% of their 96 patients gave correct answers to all questions about ECT, they also acknowledged that less than half of their patients were aware of aspects such as the induction of seizures, mechanisms, safety, or the use of muscle relaxants. Finally, although two studies from India (Ramachandra et al. 1992; Chavan et al. 2006) both concluded that a high proportion of patients (> 65%) had adequate knowledge, on closer scrutiny the proportion of patients with full understanding of the treatment, particularly about placement of electrodes, duration of stimulus or fits, side effects, indications, etc., was actually much lower (6–17%).

Taken as a whole, these findings suggested that a variable proportion of patients across different studies seemed to be aware of the rudiments of the procedure (e.g., that it involves electricity or induces a seizure). Somewhat expectedly, fewer patients were aware of the more intricate aspects of ECT (e.g., different indications, techniques, side effects, mechanism, etc.). Moreover, knowledge about ECT among patients did not seem to improve even after they had undergone the treatment (Malcolm 1989; Rajkumar et al. 2006; Malekian et al. 2009). However, the extent and of nature of awareness among these patients were similar to that found among those undergoing different surgical procedures (Tang et al. 2002).

ECT and informed consent

To give informed consent to any medical treatment, individuals must have the capacity to decide, have adequate information about risks and benefits and have the freedom to choose whether or not to undergo the treatment, without being coerced (Rose et al. 2005; Rajkumar et al. 2006). Data from this review indicated that patients were often dissatisfied about all these aspects of the consent-procedure while receiving ECT.

Information offered prior to ECT. Data were available from 35 reports (Table II). In most studies only oral information was offered prior to ECT. In 20 such reports a majority of the patients (about 50–100%; average 66%) felt that they had not received adequate explanations of the treatment before undergoing ECT. Systematic reviews, based on some of these studies, have also concluded that about half of the respondents (45–55%) report that they are not given an adequate explanation before starting ECT (SURE 2002; Rose et al. 2005). The need for adequate information also emerged as one of the major themes in qualitative assessments of patients' experience (Johnstone 1999; Froede and Baldwin 1999; Rose et al. 2005; Rajkumar et al. 2006, 2007; Vamos 2008). Patients appeared to be particularly dissatisfied about not being told about side effects and risks associated with ECT (Johnstone 1999; SURE 2002; Tang et al. 2002; Rose et al. 2005; Vamos 2008; Malekian et al. 2009).

However, these observations need to be tempered by certain other considerations. Firstly, in about one-third of the studies listed in Table II, a majority of the patients (53–80%; average 69%) were actually quite satisfied with the amount of information received prior to ECT. The studies by Jenaway (1993) and Rush et al. (2008) were particularly notable in this regard, because they indicated that it was possible to obtain much higher rates of satisfaction with the information offered, simply by adhering to minimum standards of care and providing written information. Secondly, ECT-induced memory impairment often acted as a major confounder by interfering with retention of information by patients. This effect was evident in several studies which showed that the amount of information patients had about ECT tended to decline over the course of treatment (Szuba et al. 1991; Jenaway 1993; Greening et al. 1999). The considerable magnitude of this decline was further suggested by the fact that in many reports up to half of the patients (23–50%) did not recall whether they ever received an explanation (Freeman and Kendell 1980; Malcolm 1989; Greening et al. 1999; Walter et al. 1999; Taieb et al. 2001). Recall of information seemed to worsen with longer courses and greater number of treatments (Greening et al. 1999). Moreover, some of these studies had also reported that relatives were much more likely than patients to believe that they had received adequate information (Walter et al. 1999; Tang et al. 2002; Rajkumar et al. 2006; Virit et al. 2007; Rajagopal 2008). This again raises the possibility that patients might have forgotten some of what was explained prior to treatment, due to post-ECT memory loss. Finally, it is not always easy to define what is meant by the term "adequate information" and how much people should be told. Too much information may be overwhelming and too little information is often inaccurate and misleading. A compromise is obviously required, both to be able to satisfy the patients receiving ECT, and also to guide further research in this area.

Notwithstanding such uncertainties, the unfortunate conclusion from these studies was that most patients were not satisfied with the amount of information offered prior to ECT, and a considerable proportion of them attributed their poor knowledge of the procedure to this fact. However, this problem does not seem occur exclusively with ECT, since similar deficits in recall of information have been reported among patients undergoing surgical procedures (Tang et al. 2002).

Table II. Information imparted to patients prior to ECT and perceived coercion.[1]

No.	Study details	Information offered prior to ECT[2]	Proportion of patients who felt forced to have ECT[3]
1.	Vergese et al. 1968 (*n*=36)	Majority did not receive adequate information; were upset by this	28% resisted ECT; others neither volunteered for, nor resisted ECT
2.	Freeman and Kendell 1980 (*n*=166)	Information adequate in only 21%49% sure they had been given no explanation at all	23%
3.	Hughes et al. 1981 (*n*=72)	49% received no explanation but only 12% dissatisfied	–
4.	Kerr et al. 1982 (*n*=60)	56% believed that "patients are never told what is going on"	22%
5.	Aperia 1986 (*n*=30)	Information about ECT before and after the treatments had been satisfactory for all	–
6.	Baxter et al. 1986 (*n*=55)	59% given adequate information prior to ECT	–
7.	Benbow 1988 (*n*=54)	–	30%
8.	Malcolm 1989 (*n*=100)	49% did not receive an adequate explanation; 33% did not remember	29%
9.	Szuba et al. 1991 (*n*=25)	68% felt they had received an adequate explanation before ECT, the proportion dropped to 56% after ECT	–
10.	Riordan et al. 1993 (*n*=37)	About two-thirds felt they had been given an adequate opportunity to discuss ECT prior to treatment	–
11.	Jenaway 1993 (*n*=57)	65–89% believed that they had received adequate information	16% pre-ECT & 7% post-ECT
12.	UKAN1995[4] (*n*=308)	Only 28% received an adequate explanation prior to ECT	25%
13.	MIND 1995 (*n* not available)	Only 14% given adequate information prior to ECT; 9% told about side effects	–
14.	Johnstone 1999 (*n*=20)	Nearly all patients felt that pre-ECT explanations had not been adequate	All felt somewhat forced to have ECT
15.	ECT Anonymous 1999 (*n*=200)	Only 2% felt that the risks had been fully explained to them	87%
16.	Walter et al. 1999 (*n*=26)	Only 42% received adequate explanations; 23% did not remember	27%
17.	Wheeldon et al. 1999 (*n*=150)	54% received adequate pre-ECT explanations; 37% did not remember	–
18.	Pedler 2000 (MIND) (*n*=418)	50% knew why they were given ECT; 27% received information about side effects	53%
19.	Taieb et al. 2001 (*n*=10)	Only 20% received adequate information prior to ECT; 50% did not recall receiving any explanation	80% did not know whether they could refuse ECT
20.	Tang et al. 2002 (*n*=96)	Only 20% informed about ECT prior to treatment (11–25% about various aspects)	35%
21.	Sestoft et al. 1998 (*n*=113)	53% received adequate pre-ECT explanations	–
22.	Benbow and Crentsil 2004 (*n*=70)	78% of recipients thought that the treatment had been fairly well explained	–
23.	Philpot et al. 2004 (*n*=44)	54% of the patients had received written information about ECT and its side effects	30%
24.	Sienaert et al. 2005 (*n*=36)	Only 44–50% received adequate pre-ECT explanations of treatment	–
25.	Rajkumar et al. 2006 (*n*=52)	Only 25% of the patients received adequate information before ECT	21% agreed because they it was futile to refuse ECT
26.	Myers, 2007 (*n*=148)	50% patients received an adequate explanation prior to ECT; 29% were told about side effects	22%
27.	Arshad et al. 2007 (*n*=25: received ECT)	Only 44% felt the procedure had been appropriately explained prior to ECT	20% were not asked for their consent
28.	Virit et al. 2007 (*n*=70)	Only 23–33% of the patients received adequate information before ECT	–
29.	Rush et al. 2007 (*n*=51)	More than 80% of the patients received adequate information before ECT	–
30.	Rush et al. 2008 (*n*=51)	80% of patients had enough information to make an informed choice.	4%
31.	Rajgopal 2008 (*n*=50)	Only 24% felt they had received sufficient information prior to ECT	6% felt compelled to have ECT (42% unsure)
32.	Malekian et al. 2009 (*n*=22)	Only 21% adequately informed prior to treatment (5–27% about various aspects of ECT)	27%

[1]Perceived coercion refers to the feeling of compulsion to have ECT despite freely consenting.
[2]The need for adequate information also appears as an important theme in patient testimonies and several qualitative studies by Johnstone 1999; Froede and Baldwin 1999; Koopowitz et al. 2003; Rajkumar et al. 2007; Vamos 2008.
[3]Perceived coercion also appears as an important theme in patient testimonies and several qualitative studies by Johnstone 1999; Froede and Baldwin 1999; Koopowitz et al. 2003; Philpot et al. 2004; Rajkumar et al. 2007.
[4]UKAN, United Kingdom Advocacy Network.

Perceived coercion. In many instances patients consent to have ECT because they believe they have no other choice. Perceived coercion occurs when the patient signs a consent form, but still feels that there was pressure to have ECT, even when not legally compelled to do so (Rose et al. 2005). Perceived coercion can be estimated from questions relating to whether the person felt pressured to have ECT, or believed that it could not be refused. Such information was available from 23 of the studies included in Table II.

Two surveys by consumer organisations in the UK reported very high rates of perceived coercion ranging from 53% (Pedler 2000) to 87% (ECT Anonymous 1999), which could be attributed to the way the relevant questions were framed in these surveys, as well as probable selection bias (SURE 2002; Rose et al. 2005). Contrastingly, two studies (Jenaway 1993; Rush et al. 2008) found very low rates (4–7%), either because of being carried out in accredited clinics with high standards of care (Rush et al. 2008), or because of the use of a more structured process of consent (Jenaway 1993). However, if these extreme variations are ignored, rates of perceived coercion consistently ranged from 20 to 35% across the vast majority of the studies. This figure was endorsed by systematic reviews, which estimated that between one-quarter to one-third of patients who consented to ECT, did so under pressure, or in the belief that they could not refuse (SURE 2002; Rose et al. 2005). These reviews also raised the additional concern that despite improvements in consent procedures, the proportion that felt coerced into treatment seemed to be actually increasing over the years.

Why do patients consent to have ECT despite their sense of being pressurised to accept the treatment? The evidence suggested that more often than not, they do so because they place their trust in doctors (Freeman and Kendell 1980; Kerr et al. 1982; Malcolm 1989; Tang et al. 2002; Rajagopal 2008; Malekian et al. 2009). Some authors see nothing wrong in this faith that patients have in their doctors (Benbow 1988). Others are less certain as they feel that this trust could actually be emanating from feelings of powerlessness and desperation, which are often reflected in subjective descriptions of the consent process (Johnstone 1999; Froede and Baldwin, 1999; Koopowitz et al. 2003; Philpot et al. 2004; Rajkumar et al. 2007). More worryingly, certain studies have also indicated that patients might agree to have ECT based on mistaken beliefs, such as their illness has lasted too long, or that ECT would erase all their unhappy memories (Tang et al. 2002; Rajagopal 2008; Malekian et al. 2009).

Experience of ECT among patients

Technical advances have attempted to make the process of administering ECT less unpleasant and risky. Nevertheless, certain aspects of the treatment have attracted a great deal of criticism from patients.

Fear of ECT. The proportion of patients fearful of ECT could be estimated from 28 reports (Table III). In about half of these studies a significant proportion of the patients ranging from 47 to 75% (average 63%) reported feeling anxious or fearful before ECT. The majority of such fears were linked to worries about permanent brain damage due to electric shock and subsequent adverse effects such as loss of memory. This fear did not seem to diminish much even on completion of treatment. In addition, fear of ECT was commonly described in several subjective accounts (Johnstone 1999; Froede and Baldwin 1999; Koopowitz et al. 2003; Philpot et al. 2004; Rajkumar et al 2007). Reviews on the subject have also concluded that fear of ECT remains a major obstacle, despite the modifications in technique that have taken place over the years (Fox 1993; Johnstone 1999). In contrast, the other half of studies from Table III concluded that the majority of patients did not find ECT unduly frightening or upsetting. These found much lower rates of fear, from 3 to 44% (average 28%), among patients undergoing ECT. The difference in rates of fear could not be wholly attributed to differences in the settings or design of the studies, type of treatment, age, gender, previous exposure, etc. Some degree of fear may be normal or expected in any procedure that carries an element of risk, such as ECT. However, evidence from this review seemed to suggest that slightly less than half of the patients (48%) undergoing ECT usually experience a level of fear or anxiety, which is much more than expected.

Another way to assess the degree of discomfort associated with ECT has been to ask patients to compare their experience of treatment with a visit to the dentist. Several reports have indicated that a majority of the patients (50–98%) find the experience of ECT better (or no worse than) a visit to the dentist to have a tooth pulled out (Freeman and Kendell 1980; Hughes et al. 1981; Szuba et al. 1991; Pettinati et al. 1994; Tang et al. 2002; Benbow and Crentsil 2004; Rajgopal 2008).

Experience of different aspects of the ECT-procedure. Nine studies had provided data on patients' experiences of different aspects of the procedure of ECT (Vergese et al. 1968; Freeman and Kendell 1980; Malcolm 1989; Walter et al. 1999; Taieb et al. 2001; Tang et al. 2002; Benbow and Crentsil 2004; Kershaw et al. 2007; Rajgopal 2008). The results of these studies suggested that waiting for ECT (8–75% of patients), being administered the anaesthetic agent (19–60% of patients) and waking up after the procedure (10–50% of patients), were the most distressing

Table III. Some aspects of the experience of ECT among patients.

No.	Study details	Proportion of patients anxious or fearful of ECT[1]	Proportion of patients with memory impairment	Severity, persistence, distress due to memory impairment[2]
1.	Crumpton et al. 1963 (n=96)	Some fear universal among patients	–	–
2.	Cronholm and Ottosson 1963 (n=35)	–	37%	–
3.	Vergese et al. 1968 (n=36)	75%	Memory disturbance commonest side effect	Memory loss highly disturbing
4.	Spencer 1968 (n=53)	54%	–	–
5.	Gomez 1975 (n=96)	75%	3%	Mild and short-lived in most
6.	Spencer 1977 (n=20)	28%	Memory impairment frequent	A principal area of concern for many
7.	Freeman and Kendell 1980 (n=166)	40% (46% not anxious)	64%	25% – severe; 30% – persistent loss; 50% – worst side effect
8.	Hughes et al. 1981(n=72)	44% pre-ECT19% post-ECT	44% memory loss	18% with persistent impairment
9.	Bagadia et al. 1980 (n=43)	–	63%	–
10.	Bagadia et al. 1981 (n=40)	–	25%	–
11.	Kerr et al. 1982 (n=60)	7–12%	30%	Persistent in all
12.	Squire and Slater 1983 (n=31)	In 3% more scary/stressful than expected	87% in the first week	55% with persistent impairment
13.	Baxter et al. 1986 (n=55)	–	Memory dysfunction common	–
14.	Benbow 1988 (n=26)	29%	–	–
15.	Malcolm 1989 (n=100)	60% pre-ECT55% post-ECT	31%	–
16.	Riordan et al. 1993 (n=37)	69%	75%	Mild impairment in most
17.	Tharyan et al. 1993 (n=200)	75%	–	–
18.	Pettinati et al, 1994 (n=78)	–	51%	Short lived impairment in most
19.	UKAN[3] 1995 (n=308)	–	73%	Many with persistent impairment
20.	Johnstone 1999 (n=20)	70%	Nearly 100%	Many found memory problems upsetting
21.	Walter et al. 1999 (n=26)	58% pre-ECT50% post-ECT	62%	50% found memory problems upsetting
22.	Ishimoto et al. 2000 (n=185)	–	Memory problems in 16%	–
23.	Pedler 2000 (n=418)	29%	42%	Permanent loss of past memories in all
24.	Taieb et al. 2001 (n=10)	60% pre-ECT50% post-ECT	60%	20% severe; 10% with persistent impairment
25.	Tang et al. 2002 (n=96)	44%	61 %	20% found memory problems upsetting
26.	Philpot et al. 2004 (n=44)	–	79%	41% with persistent impairment
27.	Benbow and Crentsil 2004 (n=70)	–	72%	10% – severe
28.	Sienaert et al. 2005(n=36)	47%	67%	Persistent impairment in many
29.	Rajkumar et al. 2006 (n=52)	Many	27%	12% found memory problems distressing
30.	Myers 2007 (n=148)	–	About 50%	Persistent and distressing in all
31.	Kershaw et al. 2007 (n=161)	14%	–	–
32.	Virit et al. 2007 (n=70)	36%	51%	11% with persistent impairment
33.	Rush et al, 2007 (n=51)	55%	94%	60% with persistent impairment
34.	Rajgopal 2008(n=50)	40%	50%	About 10% with persistent impairment
35.	Malekian et al. 2009(n=22)	52%	95%	59% -moderate to severe

[1]In addition, fear of ECT was reported as an important theme in qualitative studies by Johnstone 1999; Froede and Baldwin 1999; Koopowitz et al. 2003; Rajkumar et al. 2007.

[2]In addition, distress due to memory impairment appears as an important theme in qualitative studies by Johnstone 1999; Froede and Baldwin 1999; Koopowitz et al. 2003; Philpot et al. 2004; Rose et al. 2004; Rajkumar et al. 2007; Vamos, 2008.

[3]UKAN, United Kingdom Advocacy Network.

aspects of the procedure for most patients. Then again, these rates were not very different from that found among patients administered anaesthetics for routine surgical procedures (Clifton 1984).

Experience of side effects. Data on side effects was available from 36 reports. The proportion of patients reporting adverse effects following ECT varied from as low as 18% (Gomez 1975), to as high as 100% in some of the studies (Johnstone 1999; ECT Anonymous 1999; Taieb et al. 2001). On the average about two-thirds of the patients undergoing ECT reported adverse effects such as memory impairment, aches/pains, confusion, headache, nausea/vomiting, etc.

Memory impairment was the commonest side effect reported in virtually all the studies (Table III). It was also among the most persistent, distressing and troublesome problem reported by the patients. Rates of post-ECT memory loss varied widely from 3 to 100% across different studies; with persistent impairment occurring in 10% to nearly 100% of patients. Roughly about 60% of the patients in this review reported memory problems after ECT; in about 40% this persisted from several weeks to several years. The rates of memory loss derived from these studies were very similar to those reported by systematic reviews, which have estimated that rates of persistent memory loss to vary from 29 to 55% across the more methodologically sound studies (SURE 2002; Rose et al. 2003). In addition to the quantitative aspects of memory impairment, qualitative data and subjective accounts of patients also provided graphic accounts of memory difficulties and their psychosocial consequences (Froede and Baldwin 1999; Johnstone, 1999; Koopowitz et al, 2003; Philpot et al, 2004; Rose et al. 2004; Rajkumar et al. 2007; Vamos 2008). Then again, there is still considerable disagreement on both the extent of post-ECT memory loss, as well as the methods that should be employed to accurately estimate it.

Apart from memory impairment, other adverse effects of ECT were reported by patients in 15 studies (Vergese et al. 1968; Gomez 1975; Freeman and Kendell 1980; Malcolm 1989; Riordan et al. 1993; Tharyan et al. 1993; Walter et al. 1999; Ishimoto et al. 2000; Taieb et al. 2001; Tang et al; 2002; Benbow and Crentsil 2004; Philpot et al. 2004; Rush et al. 2007; Virit et al. 2007; Malekian et al. 2009). Other common side effects following ECT included muscle aches and pains (1–94%), confusion (20–65%), headache (1–55%) and nausea or vomiting (0.5–25%).

Attitudes towards ECT among patients

The efficacy and safety of ECT is evident from a substantial body of evidence derived from randomized controlled trials. This evidence consistently shows that, in the short-term, ECT is an effective treatment for depression, and to a lesser extent for schizophrenia and mania. At the same time reliable evidence regarding its long-term effects, particularly residual cognitive effects, is still limited (Carney and Geddes 2003). However, though clinical trials clearly show that ECT produces improvement in symptoms, patients' perceptions of benefit are usually based on broader considerations than mere relief from symptoms. Attitudes towards ECT among patients seem to involve a complex trade-off between its risks and benefits, as judged by the patients. Most studies on the subject have used essentially the same format of asking patients to respond to questions or complete checklists about their attitudes towards ECT. Many of them have attempted to elicit patients' views on perceived benefit of ECT and willingness to repeat the treatment, which are considered the most meaningful measures of attitudes towards ECT. In addition, patients have been asked to respond to a series of statements regarding ECT, e.g., its safety, usefulness, relative efficacy, etc. Such studies have often used simple response categories, global ratings and relatively simple techniques of analyzing data, and thus were more likely to represent an overly simplistic approach, which fails to address this complex trade-off between risks and benefits (Freeman and Cheshire 1986; Rose et al. 2003; Myers 2007). Despite these problems, these methods do provide a reasonably accurate estimate of the perspective of patients.

Information on attitudes of patients regarding ECT could be extracted from 54 reports (Table IV), four of which had only provided qualitative data (Froede and Baldwin 1999; Koopowitz et al, 2003; Rose et al. 2004; Rajkumar et al. 2007). In 37 of these studies a majority of patients found ECT to be helpful. Rates of perceived benefit in these studies ranged from 50 to 100% (average 71%). In the same vein, 53–98% of the patients (average 70%) in 21 studies were willing to have ECT again if required. Moreover, in 31 studies a majority of patients, ranging from 60 to 100%, had positive attitudes regarding other aspects of ECT. Subjective accounts of patients also reflected this favourable perception of ECT (Koopowitz et al, 2003; Rajkumar et al. 2007). Then again, in many of the same studies that had reported benefits from ECT, a significant proportion of patients, ranging from 4 to 40%, expressed clearly negative views concerning the treatment.

However, not all studies had endorsed such a positive outlook about ECT among patients. Accordingly, in nine other studies, the proportion of patients who perceived ECT to be helpful was much lower and ranged from 12 to 44% (average of 30%). Similarly,

Table IV. Attitudes of patients towards ECT.

No.	Study details	Proportion of patients who found ECT to be beneficial	Proportion of patients willing to have ECT again	Other aspects of attitudes towards ECT among patients[1,2]
1.	Hillard and Folger 1977 (n=53)	–	–	Significantly more positive attitudes among patients in the ward where ECT was used more frequently
2.	Freeman and Kendell 1980 (n=166)	78%	65%	Majority (30–80%) had positive attitudes; negative attitudes in 13–39%
3.	Hughes et al. 1981 (n=72)	83%	81%	–
4.	Kalayam and Steinhart 1981 (n=63)	–	–	Positive responses among most patients; negative responses in 7–22%
5.	Kerr et al. 1982 (n=60)	73 %	–	Generally positive attitudes; negative attitudes in 7–22%
6.	Squire and Slater 1983 (n=31)	61%	45%	Positive attitudes in about half the patients; negative attitudes in about 40%
7.	Aperia 1986 (n=27)	70%	63%	Two thirds of the patient group had positive attitudes towards ECT; negative attitudes in 13%
8.	Baxter et al. 1986 (n=55)	ECT helpful in most	–	Majority had positive attitudes
9.	Benbow 1988 (n=54)	73%	69%	Most patients' attitudes were favourable
10.	Dodwell and Goldberg 1989 (n=16)	81%	75%	Majority had positive attitudes
11.	Szuba et al. 19991 (n=25)	76%	72%	Highly positive attitudes toward ECT among majority of patients; negative attitudes in about 4%
12.	Riordan et al. 1993 (n=49)	60% pre-ECT; 56% post-ECT	67%	Patients usually had a positive or neutral attitude towards ECT; negative attitudes in 10–20%
13.	Rogers and Pilgrim 1993 (n=231)	43%	–	–
14.	Pettinati et al. 1994 (n=78)	–	98%	Majority of patients treated with ECT had favourable attitudes
15.	UKAN[3] 1995 (n=308)	30%	21%	Predominantly negative attitudes among (> 50%) patients
16.	MHF[4] 1997 (n=107)	30%	–	–
17.	ECT Anonymous 1999 (n=200)	29%	–	–
18.	Bernstein et al. 1998 (n=52)	83%	79%	–
19.	Sestoft et al. 1998 (n=113)	–	29%	–
20.	Goodman et al. 1999 (n=24)	–	82%	Majority (81–96%) had positive attitudes
21.	Johnstone 1999 (n=20)	55%	5%	Mixed, mainly negative attitudes; 40% wanted ECT banned
22.	Wheeldon et al. 1999 (n=150)	83%	77%	Vast majority of patients (93%) satisfied with staff & facilities
23.	Walter et al. 1999 (n=26)	50%	69%	Positive attitudes in the vast majority (about 90%) of patients; negative attitudes in 8–23%
24.	Pedler 2000 (n=418)	36%	–	Negative views among most (up to 43%) patients
25.	Taieb et al. 2001 (n=10)	100%	60%	Positive attitudes in the vast majority (70–100%) of patients; negative attitudes in 10%

(*Continued*)

Table IV. *(Continued)*

No.	Study details	Proportion of patients who found ECT to be beneficial	Proportion of patients willing to have ECT again	Other aspects of attitudes towards ECT among patients[1,2]
26.	Tang et al. 2002 (n=96)	75%	53%	Positive attitudes in the majority (59–83%) of patients; negative attitudes in up to 26%
27.	MDP Fellowship[5] 2002 (n=97)	30%	–	–
28.	Brodaty et al. 2003 (n=81)	69% post ECT (vs. 40% pre-ECT)	–	–
29.	Iodice et al. 2003 (n=64)	–	52–53% at 2 & 4 weeks post-ECT	–
30.	Benbow and Crentsil 2004 (n=70)	85%	–	–
31.	Philpot et al. 2004 (n=44)	44%	60%	–
32.	Sienaert et al. 2005 (n=36)	50%	36%	Majority (50–65%) had positive attitudes; negative attitudes in 25–56% of patients
33.	Kuruvilla et al, 2006 (n=100)	–	–	Elderly patients did not think highly of ECT, either in its effectiveness or acceptability
34.	Rosenquist et al. 2006 (n=77)	–	44%	–
35.	Rajkumar et al. 2006 (n=52)	20%	23%	Majority (83%) not unhappy about ECT
36.	Myers 2007 (n=148)	66%	–	Majority (67%) satisfied with ECT; negative views in 9–19% of the patients
37.	Kershaw et al, 2007 (n=389)	–	–	> 65% patients reported good standards of care
38.	Kopecek et al. 2007 (n=22)	68%	–	–
39.	Rush et al. 2007 (n=51)	69%	86%	High degree of satisfaction with ECT; all respondents gave positive responses
40.	Arshad et al. 2007 (n=190)	–	41%	Negative attitudes among a significant proportion (34–62%) of patients
41.	Virit et al. 2007 (n=70)	80%	69%	Majority of patients (60–90%) had positive attitudes; negative attitudes in up to 28%
42.	Bustin et al. 2008 (n=75)	12–15%	37%	Majority (up to 75%) had negative attitudes
43.	Felieu et al. 2008 (n=46)	ECT rated to be more effective than drugs.	–	Following ECT attitudes became more positive
44.	Rajgopal 2008 (n=50)	70%	56%	Mostly positive attitudes among patients; clearly negative attitudes in less than 10% of patients
45.	Malekian et al. 2009 (n=22)	59%	–	Majority (80%) of patients satisfied with ECT; positive attitudes in the majority (50–73%); negative attitudes in up to 32% of patients
46.	Sienaert et al. 2009 (n=48)	–	58%	Majority (58–73%) of patients satisfied with results of ultra-brief ECT

[1]Positive attitudes and efficacy also reported by Calev et al. 1991; Aggarwal et al. 1992;Battersby et al. 1993; Campos and Higa 1997; however, the proportion of patients with such attitudes is not mentioned in these studies.
[2]Qualitative data by Johnstone 1999; Froede and Baldwin 1999; Koopowitz et al. 2003; Philpot et al. 2004; Rose et al. 2004; Rajkumar et al. 2007, also have important insights regarding attitudes of patients towards ECT.
[3]UKAN, United Kingdom Advocacy Network.
[4]MHF, Mental Health Foundation.
[5]MDP, Manic Depression Fellowship.

in nine of these reports, the proportion of patients willing to repeat ECT was lower and varied from 5 to 45% (average of 31%). More than half of these studies which had reported low rates of perceived benefit from ECT were surveys conducted by consumer organizations (Rogers and Pilgrim 1993; United Kingdom Advocacy Network 1995; Mental Health Foundation 1997; ECT Anonymous 1999; Pedler 2000; Manic Depression Fellowship 2002; Philpot et al. 2004) These consumer surveys also found that dissatisfaction with ECT was more widespread than is often supposed, with up to 50% of the patients expressing negative attitudes regarding ECT. A high prevalence of negative views on ECT among patients (34–75%), was also found in three studies conducted by clinicians (Kuruvilla et al. 2006; Arshad et al. 2007; Bustin et al. 2008). Finally, qualitative data from several studies and accounts of patients also revealed that negative attitudes regarding ECT were quite common (Johnstone, 1999; Froede and Baldwin 1999; Philpot et al, 2004; Rose et al. 2004).

On the whole, these results provided considerable support for several earlier reviews on the subject which have all concluded that, in general, most patients have a positive view of ECT, and for most of them the benefits of the treatment outweigh the costs, in terms of apprehension, side effects or discomfort. However, a variable but significant proportion of them also have very negative views regarding ECT (Freeman and Cheshire 1986; Dowman et al. 2005).

"Consumer-led" studies of ECT

Consumer-led research, particularly surveys of ECT recipients carried out by consumer groups in the UK, has been highly critical of ECT. Differences between consumer-led and clinician-led research are greatest in the area of expressed satisfaction with ECT. As is evident from Table IV, the perceived benefit from ECT was much lower (29–55%) among consumer-led than clinician-led studies, as were the rates of patients willing to repeat ECT, while negative attitudes concerning ECT were far more common. Though consumer-led research is somewhat more likely to selectively include people antagonistic to ECT, systematic analysis of the evidence has attributed these differences to certain methodological variables (SURE 2002; Rose et al. 2003, 2005). These include the length of time that has elapsed since treatment; clinical studies that have taken place too soon after completion of ECT tended to report higher rates of benefit. These differences are further amplified by the tendency of clinician-led studies to rely on medical assessors to gather data, and their use of brief questionnaires with low complexity, both of which might have over-estimated perceived benefit from ECT. In other areas such as persistent memory loss, informed consent and perceived coercion, there was considerable overlap between results of clinician- and consumer-led research. Then again, even in these areas interpretations of the basic data often differed radically. Clinical research had typically judged problems to be insignificant or limited, whereas consumer research had generally chosen to focus more on the adverse impact that such problems had on lives of patients. Consequently, efforts to obtain a true picture of patients' perceptions appear to be severely hampered by such fundamental differences between clinical and consumer-led research. Not surprisingly, most authors have recommended replacing both these types of research by genuinely collaborative high-quality efforts, which can overcome the deficiencies of the two different strands of research (Carney and Geddes 2003; Philpot et al. 2004).

Other methodological considerations

Apart from the methodological limitations referred to earlier, a fundamental problem of ECT research is that it deals with people who are usually severely ill and often cognitively impaired, because of which they often choose not participate in research. The modest sample sizes and response rates in many of the studies suggest that such patients may have been excluded. Since this group may have more adverse attitudes towards ECT their exclusion biases the results in favour of more positive perceptions. Additionally, patients who are invested in their treatment may be selectively included, which artificially inflates the degree of satisfaction with ECT (Goodman et al. 1999; Walter et al. 1999; Kershaw et al. 2007). Moreover, the methods used to elicit patients' perceptions and awareness in these studies were far from perfect. The measures employed in a majority of the studies were also not properly validated, neither were they consistent or standardised across studies. Since treatment with ECT comprises of many stages, longitudinal investigations employing proper controls would be ideal, but such studies were few. The possibility that current mood state and cognitive impairment may alter response patterns is another problem that has been rarely overcome (Goodman et al. 1999; Walter et al. 1999; Rose et al. 2003; Benbow and Crentsil 2004). Clinician-led research is further hampered by the difficulty patients may have in expressing their true opinions to the very doctors who treated them (Freeman and Kendell 1980). On the other hand, the alternative methods of consumer-led research

have often suffered from the same problems of reliability, selection and response bias, etc.

Discussion

Not so long ago one of the leading practitioners of ECT stated that "Doctors who give ECT have shown remarkably little interest in their patients' views of the procedure and its effects on them" (Abrams1997). However, the large number of studies included in this review suggests that there is no dearth of data on these aspects of ECT. Moreover, despite the differences in origin and methodology, there is a certain uniformity in the results of most such reports, which makes it possible to obtain a reasonably accurate representation of knowledge and perceptions of ECT among patients.

Broad trends indicated that patients were generally not aware of several aspects of ECT, except for the very basic and elementary details. This lack of awareness could be due to several factors such as inadequate information offered prior to ECT (reported by about two-thirds of the patients in this review), post-ECT memory loss (prevalent among about 60% of patients in this review), or confounding effects of current psychopathology. Modifications in technique and procedures for consent have endeavoured to make ECT a relatively safe and less unpleasant procedure. This is often endorsed by patients in more than a few studies who find the procedure to be no more than mildly distressing or upsetting. However, certain parts of the experience still continue to generate considerable distress. About one-third of the patients in this review who volunteered to have ECT reported feeling coerced to accept the treatment. Fear of ECT causing permanent damage, which resulted in moderate to severe anxiety prior to treatment, was present in about half the recipients. Varying proportions of patients also found other parts of the procedure to be unpleasant. On the issue of patients' attitudes towards ECT there appeared to be a sharp divide between clinician-led and consumer-led research. Studies conducted by clinicians mostly (though not invariably) reported much higher rates of perceived benefit from ECT, as well as more favourable attitudes towards the treatment, whereas those performed by consumer organizations either reported lower rates or radically different and usually critical views regarding ECT. Such differences have been previously attributed to certain methodological variables (Rose et al. 2003). Nevertheless, such disagreement often makes it difficult to decide where the balance between the favourable and the unfavourable lies. However, going the sheer weight of evidence presented in this review it appears that a majority of patients believe ECT to be beneficial and feel positively about the treatment. At the same time, a sizeable proportion of the patients are extremely critical about ECT, though very little is known about the extent, nature, and reasons for such strong reactions against ECT (Johnstone 1999; Philpot et al. 2004).

Fortunately, despite divergent findings the implications of the evidence for the practice of ECT are much more certain. Briefly stated, given the general lack of awareness, problems with recall and dissatisfaction with consent procedures, it is essential that all patients undergoing ECT receive a detailed and comprehensive explanation of the treatment beforehand. Information needs to be disclosed in a graded, stepwise manner and if necessary, repeated till reasonable comprehension is achieved. Sufficient time is also required for patients to absorb the implications and express their fear and worries. Information leaflets and audiovisual aids may be of additional help. It is necessary that such information be provided by professionals, preferably by the patient's own doctor. Professionals should also ensure that no one signs the consent form for ECT under duress. Since a positive experience of the treatment is likely to have an enduring impact on patients' perceptions, ECT should be used only when indicated. Rather than banning the treatment, it is the excessive and unregulated use of ECT that has to be restricted. Recent evidence from accredited ECT clinics clearly demonstrates that the stress and discomfort associated with the procedure can be considerably lessened by adhering to certain minimum standards of care (Kershaw et al. 2007; Rush et al. 2007; 2008). It should not be too difficult to implement these standards which emphasise reduced waiting times, provision of clean, comfortable environments and practical and emotional support by dedicated staff. Families of patients should be involved in the treatment process, wherever possible, since this helps reassure both the patients and their relatives. Finally, the medical profession must take the responsibility of educating other professionals and the public about the true nature and effectiveness of ECT, more seriously.

If these measures do indeed ensure continued access to ECT for those patients who are likely to benefit from it, adopting them will be well worth the trouble.

Acknowledgements

None.

Statement of interest

No conflict of interest of any of the authors in connection with this article.

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The World Journal of Biological Psychiatry, 2010; 11: 538–549

ORIGINAL INVESTIGATION

Abnormal body perception and neural activity in the insula in depression: An fMRI study of the depressed "material me"

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Abstract

Objectives. In addition to affective-cognitive symptoms, patients with major depressive disorder (MDD) suffer from somato-vegetative symptoms, suggesting abnormal interoceptive awareness of their "material me". While recent imaging studies have extensively investigated affective-cognitive symptoms in MDD, the neural correlates of somato-vegetative symptoms and abnormal interoception remain unclear. Since the "material me" has been especially associated with the anterior insula in healthy subjects, we hypothesized abnormalities in this region during interoceptive awareness in MDD. *Methods.* We therefore investigated behavioural and neural correlates of interoception in healthy and depressed subjects using the Body Perception Questionnaire (BPQ) and a well established heartbeat perception task in fMRI. *Results.* MDD patients showed significantly higher scores in the BPQ and reduced neural activity during rest periods, particularly in the bilateral anterior insula. In contrast to healthy subjects, BPQ scores no longer correlated with activity during rest periods in the anterior insula. Both BPQ scores and left anterior insula signal changes correlated with depression severity. *Conclusions.* We demonstrate for the first time abnormal body perception and altered activity in the insula during rest in MDD. Our results suggest that these behavioural and neural abnormalities are closely related to these patients' somato-vegetative abnormalities and their abnormal "material me".

Key words: *Major depressive disorder, functional magnetic resonance imaging, awareness, insular cortex, self concept, mind-body relations*

Introduction

Patients with major depressive disorder (MDD) can be characterized by abnormalities in both mental and physical aspects of their self (Northoff 2007). Abnormalities in the mental self include abnormal emotions and cognitions like ruminations, self-blame, and increased association of their self with negative emotions (Ingram 1990; Treynor 2003; Rimes and Watkins 2005; Frodl et al. 2007; Northoff 2007); whilst alterations in the physical self are reflected in various persisting somato-vegetative symptoms, along with an apparent hyperawareness of bodily changes (Beck et al. 1961; Garcia-Cebrian et al. 2006; Nyboe Jacobsen et al. 2006). Patients with MDD can thus be described as suffering from major abnormalities in their "material me".

Recent imaging studies of MDD have indicated an association of the mental aspects of the self – i.e., its emotional and cognitive abnormalities – with altered neural activity in the medial cortical regions, particularly the dorsomedial prefrontal cortex (Northoff 2007; Grimm et al. 2009a). In contrast, the neural correlates of the abnormal physical aspects of the self in MDD remain to be explored.

The physical aspect of our self (Panksepp 1998; Damasio 1999; Gillihan and Farah 2005; Northoff et al. 2006, for the distinction between mental and physical aspects of the self) has been conceptualized

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(Received 1 September 2009; accepted 16 December 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903563794

as our "bodily or proto-self" (Panksepp 1998; Craig 2002, 2003, 2004). Craig (Craig 2002, 2003, 2004) characterizes the "bodily or proto-self" as perception and awareness of one's body, with him describing this interoceptive awareness as the "material me". Consistent with the basics of the James-Lange theory of emotion and Damasio's somatic marker hypothesis, he describes the representation of the interoceptive body in the anterior insula as essential for subjective feelings from the body and for emotional awareness. This "material me" is thought to be predominantly mediated by neural activity in the right anterior insula (Craig 2002, 2003, 2004, 2009). This has recently been further supported by imaging studies that have identified the anterior insula as a key region in interoceptive awareness (Critchley et al. 2004, 2005; Pollatos et al. 2007).

The neural correlates of abnormal somato-vegetative symptoms and interoceptive awareness in MDD remain unclear however. Early PET, and more recent MRI studies, do show alterations to the insula in MDD, but these studies have been concerned only with the resting state or exteroceptive perception (Mayberg 2002; Mayberg 2003; Fitzgerald et al. 2008). Emotional-cognitive stimulation has also been seen to induce abnormal neural activity in the insula in MDD (Mayberg 2002; Mayberg 2003; Phillips et al. 2003a,b; Keedwell et al. 2005; Paulus and Stein 2006; Fitzgerald et al. 2008). In contrast, studies targeting the insula specifically during interoceptive awareness, as distinguished from resting periods, exteroceptive awareness and affective components (as they are present in, for instance, pain perception; Bar et al. 2007; Strigo et al. 2008a,b) remain to be reported.

The aim of our study was to investigate the changes in neural activity in the insula during interoceptive awareness and their relation to abnormal body perception in MDD. Since previous findings demonstrated neural abnormalities in the insula during resting periods and exteroceptive, i.e. emotional-cognitive, stimulation (see above), we also investigated signal changes in the insula during both rest periods and exteroceptive stimulation. Our main focus was thus not on the activity of the rest period itself, but on the modulation of interoception and exteroception by the rest period. Based on the above mentioned findings, we hypothesized abnormal neural activity in specifically the anterior insula in MDD, as well as abnormal body perception, as measured by the Body Perception Questionnaire (BPQ; Porges 1993). To induce neural processing of interoceptive awareness, we applied a modified and well established heartbeat perception task and compared it with activity during rest periods and tone perception mirroring exteroceptive awareness (Critchley et al. 2004; Pollatos et al. 2007).

Methods

Depressed subjects and healthy controls

We studied 22 psychiatric in-patients suffering from major depressive disorder (MDD), diagnosed according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders (4th edition); American Psychiatric Association 1994), using functional magnetic resonance imaging (fMRI). Patients with MDD were recruited in an acute state from either the Department of Psychiatry at the University of Magdeburg or from the state hospital of Uchtspringe. Eligibility screening procedures included the 21-item Beck Depression Inventory (BDI; Beck et al. 1961) and the 20-item Beck Hopelessness Scale (BHS; Beck et al. 1974). Diagnoses of depression were made by the participants' treating psychiatrists. Inclusion criteria were a score of at least 16 on the BDI, while exclusion criteria were major medical illnesses, histories of seizures, metallic implants, a history of substance dependence, head trauma with loss of consciousness, pregnancy and criteria for any psychiatric disorder other than MDD.

The data for one depressed subject was excluded from the analysis due to structural abnormalities identified in their anatomical scan. A further four depressed subjects were excluded due to motion artefacts. Usable fMRI data was thus available for a total of 17 depressed subjects. Behavioural test results were not available for two depressed subjects.

The group of depressed subjects (11 female and six male subjects, all right-handed) revealed a mean age of 41.88 (± 12.1 SD) and mean educational years of 15.44 (± 2.84 SD). Mean scores for verbal intelligence (MWT-B; Lehrl 1995) were 111.35 (± 9.86 SD) and for nonverbal intelligence (LPS-3; Horn 1983) 108.77 (± 13.56 SD). The mean BHS score was 32.13 (± 4.45 SD), the mean BDI score 29.93 (± 8.56 SD), and the mean score for the clinician rated Montgomery–Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg 1979) was 26.07 (± 8.48 SD), indicating that patients were moderately depressed. Seventeen depressed subjects were taking one or more antidepressants from the following pharmacological classes: six subjects SSRIs, five subjects NaSSAs, 10 subjects NARIs/MAOI/others. None of the control subjects were taking any psychotropic medications at the time of the investigation.

Our healthy control group consisted of 17 subjects (11 female and six male subjects, all right-handed) with no psychiatric, neurological, or medical illness. They had a mean age of 37.59 (± 12.84 SD) and mean educational years of 15.44 (± 2.71 SD). Their mean score for verbal intelligence was 117.47 (± 14.28 SD) and for nonverbal intelligence 117.56 (± 16.93 SD). The healthy group was thus

well-matched to the patient group for group size, sex, age, years of education, and verbal and general intelligence. Groups did not differ significantly in gender distribution, age, verbal/ nonverbal intelligence or years of education.

The study was approved by the local ethics committee and all participants gave written informed consent before participating in this study.

Paradigm

The event related fMRI design was based on a paradigm introduced by Pollatos and Critchley (Critchley et al. 2004; Pollatos et al. 2007) which involves subjects counting intero- and exteroceptive stimuli in the form of heartbeats and tones. The paradigm was altered from the form originally described by Pollatos and Critchley in order to make it more suitable for use with a depressed population. A number of conditions were excluded (specifically, the presence or absence of a feedback delay, and a modulated tone) in order to make the paradigm less complicated and to reduce the time that patients spent in the scanner. Subjects were thus presented with three separate experimental conditions – an interoceptive task, an exteroceptive task, and rest periods – in a pseudo-randomised order.

During the interoceptive conditions, subjects were asked to silently count their own heartbeat for as long as the task-type indicator (a dark coloured heart on a light background) was displayed (9–13 s). After each interoceptive task presentation subjects were asked to report the number of heartbeats counted via a simple visual analogue scale (4 s). The indicator on the scale was moved by the subject to the labelled position representing the number of beats that they counted (left and right button presses corresponding to left and right on the scale). This feedback component allowed subject's attendance to the task to be monitored.

Exteroceptive conditions were indicated by a dark coloured musical note symbol on a light background (9–13 s). During such tasks subjects had to silently count the number of tones heard during the period that the task-type indicator was visible. Two different tones were presented, alternating with each of the fours scanning runs, each with a duration of 200 ms; a length that is comparable to the average duration of the sound of a heartbeat. In order to make the difficulty of both the intero- and exteroceptive tasks closely comparable, tones were presented at an individually determined volume that meant they were, like the heartbeat, just audible. The general presentation frequency of the tones was adapted to correspond to each subject's pulse-rate, with the individual onset time of the tones being jittered by 200 ms from this general frequency in order to control for habituation effects. As with the interoceptive task, subjects were asked to report after each exteroceptive trial the number of tones heard via a visual analogue scale (4 s).

Rest conditions were indicated by a dark cross on light background (9–13 s). Subjects were instructed to relax and reduce any cognitive work during these periods.

The total experiment consisted of 4 runs of 9.6 min (290 volumes), with each condition being presented 48 times in total. The paradigm was executed on an ordinary desktop personal computer running the software package "Presentation" (Neurobehavioral Systems, http://www.neurobs.com). Visual stimuli were projected via an LCD projector onto a screen visible through a mirror mounted on the headcoil. Auditory stimuli were presented via the scanner loudspeaker.

Behavioural tests

To assess body perception, we applied after fMRI measurement the Body Perception Questionnaire (BPQ; Porges 1993), which includes several factors: The awareness subscale (A) of the BPQ includes 45 items. Subjects should imagine how aware they are of their body processes and rate their awareness. In the second subscale (S: stress response, 10 items), subjects are asked to imagine being in a very stressful situation and rate their awareness of perceived changes due to stress. The third subscale, autonomic nervous system reactivity (ANSR), requires that subjects answer 27 items about their own autonomous nervous system reactions. Finally, the stress style (SS) subscale contains 12 items and evaluates the manner in which the subject responds to stress. All ratings are made on a five-point Likert scale.

fMRI data acquisition and analysis

Functional measurements were performed on a 3-Tesla whole body MRI system (Siemens Trio, Erlangen, Germany) with echo planar imaging (EPI) using an eight channel head coil. The slices were acquired parallel to AC–PC plane in an odd-even interleaved acquisition order. 32 $T2^*$-weighted echo planar images per volume with blood oxygenation level-dependent (BOLD) contrast were obtained (matrix: 64×64; 32 slices per volume; FoV: 224×224 mm; spatial resolution: $3.5 \times 3.5 \times 4$ mm; TE = 30 ms; TR = 2000 ms; flip angle = 80°). Functional data were recorded in four scanning runs, each containing 290 volumes. The first five volumes were discarded due to saturation effects.

The fMRI data were preprocessed and statistically analyzed according to the general linear model approach (Friston et al. 1995) using the SPM2 software package (spm2, http://www.fil.ion.ucl.ac.uk) running on MATLAB 6.5 (The Mathworks Inc., Natick, MA, USA). All functional images were slice time corrected with reference to the first slice acquired, corrected for motion artefacts by realignment to the volume taken nearest to the anatomical images, and spatially normalized to a standard T1-weighted SPM template (Ashburner and Friston 1999). Four MDD patients were excluded due to head-movements of more than 2 mm. The normalization was generated by warping the subject's T1-structural image to the T1-template provided by the MNI (Montreal Neurological Institute) and applying these parameters to all functional images. The images were resampled to $2 \times 2 \times 2$ mm and smoothed with an isotropic 6-mm full-width half-maximum Gaussian kernel. The time-series fMRI data were filtered using a high pass filter and cut-off of 128 s. A statistical model for each subject was computed by applying a canonical response function (Friston et al. 1998).

All three conditions (interoception, exteroception, and rest) were included in the SPM model as separate events. Regionally specific condition effects were tested by employing linear contrasts for each subject and each condition. The resulting contrast images were submitted to a second-level random-effects analysis by applying a one-sample *t*-test to the images created for all subjects in each condition. To control for the multiple testing problem we performed a false discovery rate correction (Nichols and Hayasaka 2003). The anatomical localization of significant activations was assessed with reference to the standard stereotactic atlas by superimposition of the SPM maps on the standard MNI brain template provided by SPM2.

Following the functional localizer approach (Saxe et al. 2006; Lamm and Decety 2008; Vul et al. 2008), we next determined the regions involved in interoception through the comparison between interoceptive and exteroceptive awareness (count heartbeat > count tones). In accordance with Goldstein and colleagues (Goldstein et al. 2007) we calculated this contrast for a combined group of healthy and depressed subjects (n=34). This was done to ensure that neither the group of healthy subjects nor the group of depressive subjects should be favoured and therefore have a dominant influence on the determination of the regions of interest (ROI). This contrast yielded significant signal changes in the bilateral anterior and middle insula. Spherical ROIs (radius 5 mm) were then located at the peak voxel within the left (x,y,z: –32,14,6) and right (36,16,6) anterior insula, and left (–42,12,-2) and right (43,8,0) middle insula. Signal changes in these ROIs during interoception, exteroception and rest were then extracted using the Marseille Region of Interest Toolbox software package (MarsBaR 1.86, Brett et al. 2002, http://www.sourceforge.net/projects/marsbar).

Mean normalized fMRI signal values from 4 to 10 s of the BOLD response for each condition were first compared between healthy and depressed subjects (two-sample *t*-test, two tailed) using SPSS 16.0 (SPSS inc., Chicago, IL). In a second analysis, intero- and exteroceptive trials that followed a rest period were identified. The signal changes during these trials were thus assumed to represent the change from the baseline state; as opposed to those events which followed another trial type, which would represent a change from a stimulus-induced state. This allowed the effect of differences in resting-state activity on subsequent stimulus-induced signal changes to be characterised. These so-called baseline corrected signal changes were then compared between healthy and depressed subjects (two-sample *t*-test, two tailed). Finally, the signal changes during the rest condition were correlated with the subscales of the Body Perception Questionnaire (Pearson's, two-tailed).

Results

Behavioural data

MDD patients showed significantly higher scores in the Body Perception Questionnaire (BPQ) when compared to healthy subjects (see Figure 1a,b). This is true for the total score (*t*-test $P = 0.03$), as well as the subscores for stress response (S) (*t*-test $P = 0.001$), autonomic nervous system reactivity (ANSR) (*t*-test $P = 0.0001$) and stress style (SS) (*t*-test $P = 0.0001$). MDD patients did not differ significantly from healthy subjects in the BPQ subscore for awareness (A).

The BPQ stress style subscale scores correlated positively with total BDI scores ($r = 0.61$, $P < 0.05$). The higher the BPQ for stress style is, the higher the BDI scores and hence depression severity.

Signal changes during intero- and exteroceptive processing and rest in the insula

In a first step, we identified the bilateral anterior and middle insula as being involved in interoception by investigating signal changes from the comparison between interoceptive and exteroceptive awareness in the contrast (count heartbeat > count tones) in all subjects, healthy and depressed (n=34), as described above (Methods). This is in accordance with the

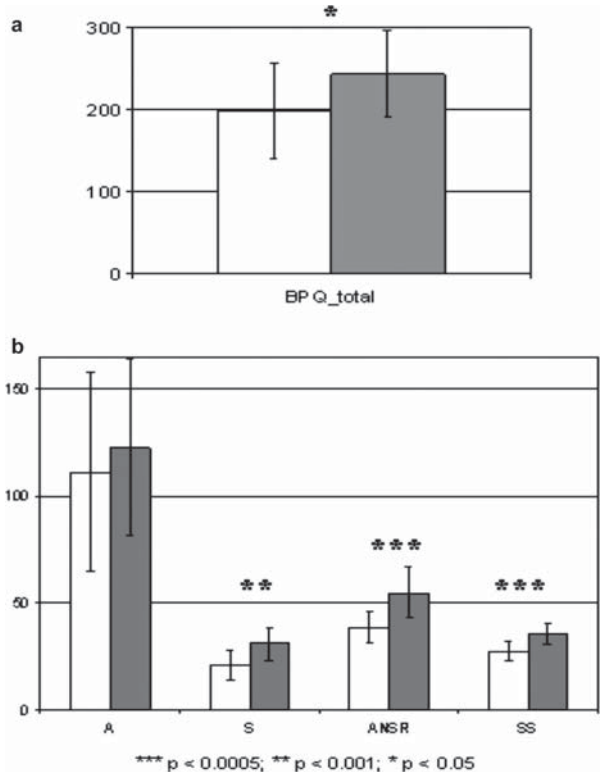

Figure 1. Results of the Body Perception Questionnaire (BPQ) for healthy (white bar) and depressive subjects (grey bar) (means ± SD). (a) Comparison of the total score of the Body Perception Questionnaire (BPQ_total); (b) comparison of the four subscores of the Body Perception Questionnaire. A, awareness; S, stress response; ANSR, autonomic nervous system reactivity; SS, stress style (1+2); total, total score.

regions obtained from the same contrast by Critchley et al. (2004), suggesting that our modified paradigm can be considered to be valid. Using the bilateral anterior and middle insula regions as functional localisers, we determined signal changes in these regions during interoceptive awareness (counting heartbeat), exteroceptive awareness (counting tones), and rest (fixation cross), and compared them between the two groups.

Signal changes during interoceptive processing - the heartbeat perception task - did not differ significantly between groups in the two relevant parts of the insula (see Figures 2a, b and c). This was independent of whether interoceptively associated signal changes were analyzed in relation to the preceding rest period or not (see Table I).

In contrast to interoceptive signal changes, signal changes during exteroceptive processing, the tone perception task, did differ significantly between both groups. Depressed subjects showed significantly reduced signal changes, with lower deactivation (i.e. smaller negative BOLD response), or even activation (i.e. switching to a positive BOLD response), in the left and right anterior insula when compared to healthy subjects (see Table I).

However, it cannot be excluded that such altered signal changes during exteroceptive processing might also be due to higher signal changes during the preceding rest period, which was rather long in our case (see above in the methods). This in turn might enhance the signal changes that are induced by subsequent exteroceptive stimulation. We therefore conducted a second analysis of the same data where we calculated exteroceptive signal changes as dependent on the level of signal change in the respectively preceding rest period (i.e. the fixation cross) for both healthy and depressed subjects. When these signal changes were then compared, depressed subjects no longer showed any significant difference from healthy subjects in the anterior and middle insula during exteroceptive processing (see Table I and Figure 2a–c). This suggests that the higher signal changes during exteroceptive processing yielded in the first analyses may be due to increased signal changes during rest periods.

Since the preceding rest period was shown to most likely affect signal changes during exteroceptive processing, we compared these signal changes themselves between both groups in the two relevant insula regions. MDD patients showed significantly lower deactivation (i.e. a reduced negative BOLD response) in the left anterior and middle (only marginally significant) insula, as well as in the right middle insula (see Figure 2a-c and Table I).

Taken together, our findings show abnormally reduced activity changes, i.e. a lower deactivation, during rest periods in the insula in MDD patients when compared to healthy subjects. In contrast, depressed patients showed no abnormalities in intero- and exteroceptive processing in the insula independent from rest periods.

Relationship between signal changes in the insula and body perception

Utilising the same regions of the insula as described above, we correlated signal changes during rest and intero- and exteroceptive processing with the scores in the BPQ (Body Perception Questionnaire) in healthy and depressed subjects.

Healthy subjects showed significantly positive correlations of rest signal changes in the right anterior insula with the BPQ total, BPQ awareness and BPQ stress response scores (see Figure 3b and Table II). The less deactivation during the rest periods in the right anterior insula, the higher the BPQ scores indicating abnormal body perception. This relationship was not obtained in MDD subjects, where decreased deactivation in the insula was no longer related to body perception scores (see Figure 3b).

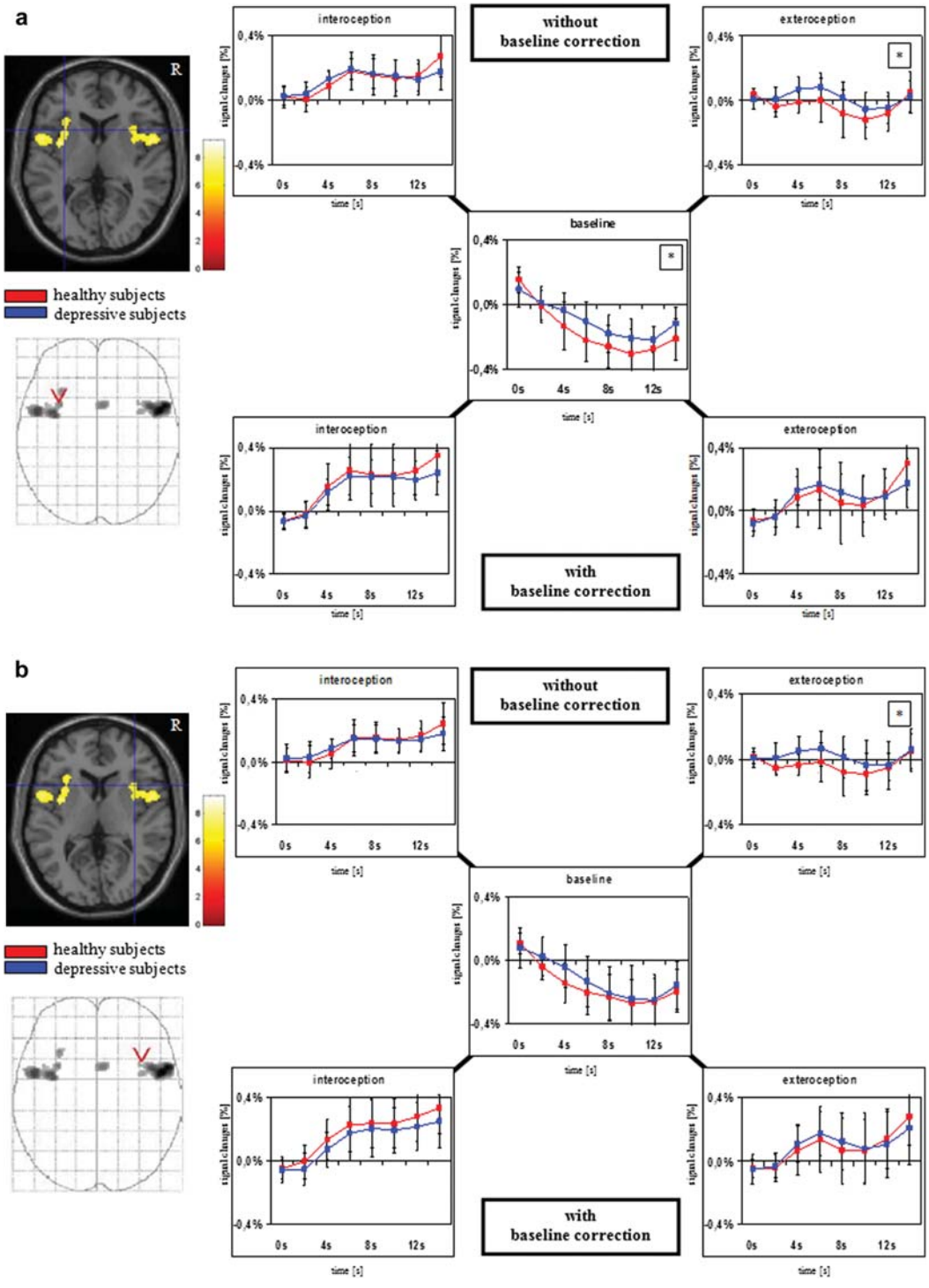

Figure 2. Comparison of interoceptive insular activity (SPM images) during intero- and exteroception (with and without baseline correction) and baseline between healthy (red lines) and depressive subjects (blue lines). SPM images show the comparison between interoceptive and exteroceptive awareness by the contrast (count heartbeat > count tones), $P < 0.01$, FWE corrected, $k>10$, $n=34$ subjects (17 healthy and 17 depressive subjects). BOLD curves (x axis: time in seconds, y axis: percent signal changes) are based upon regions of interest (ROIs) that are derived from this contrast. These are plotted separately for healthy (red lines, means ± SD) and depressive (blue lines, means ± SD) subjects and show baseline-corrected (i.e., signal intensities during interoception relative to the preceding baseline) and non-baseline-corrected (i.e., signal intensities during interoception independent of the preceding baseline) time courses for interoception and exteroception, as well as for rest periods. Figure 2a shows the results for the left anterior insula and Figure 2b for the right anterior insula.

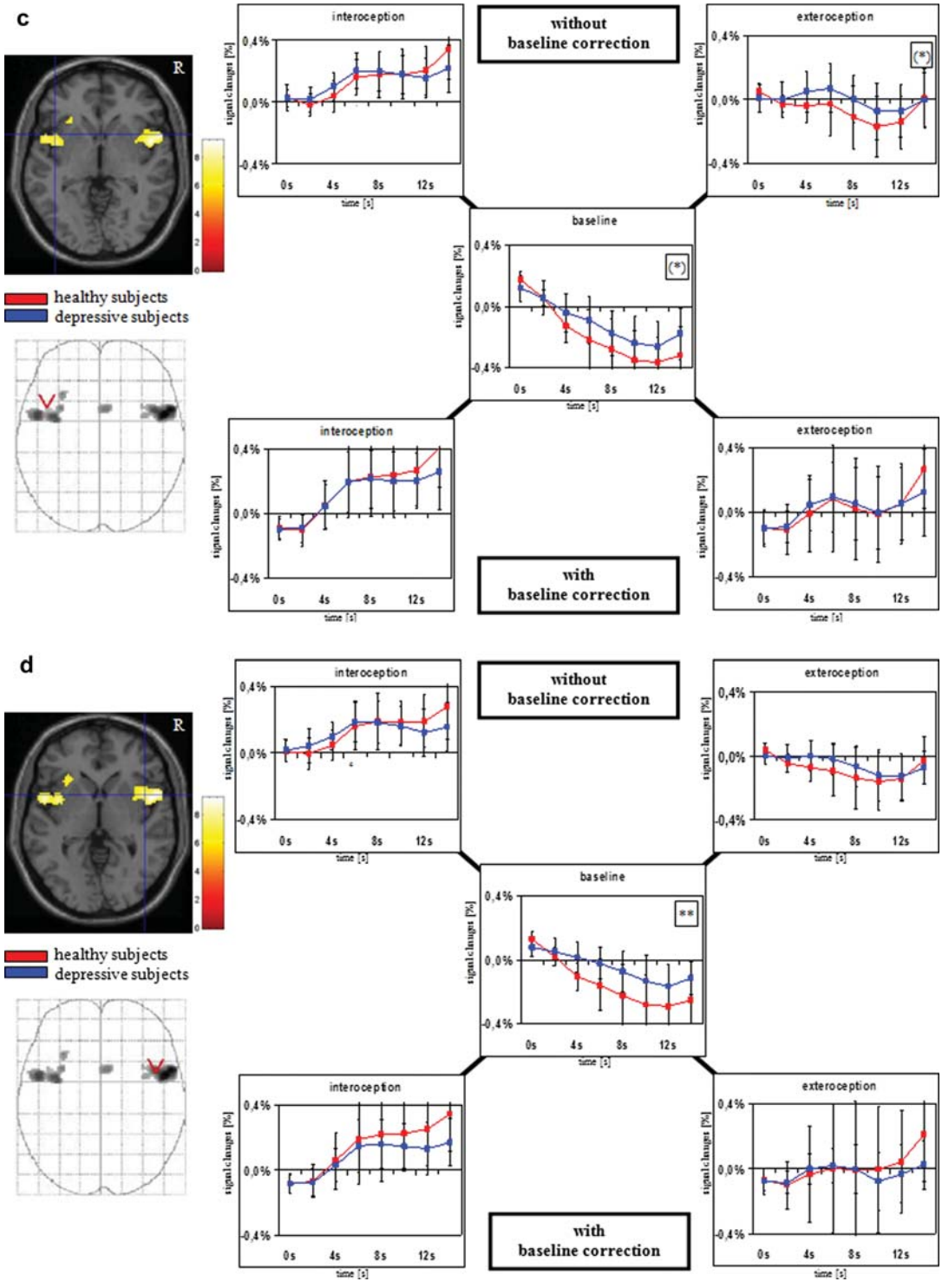

Figure 2 (*Continued*). Figure 2c shows the results for the left middle insula and Figure 2d for the right middle insula. Significant differences between both groups were obtained for exteroception without baseline-correction in the left and right anterior insula ($^{*}P<0.05$), while this difference was no longer seen when baseline-correction was done. (a) Activation of left anterior insula (x,y,z: –32,14,6) in healthy and depressive subjects ($n = 34$); (b) activation of right anterior insula (x,y,z: 36,16,6) in healthy and depressive subjects ($n = 34$); (c) activation of left middle insula (x,y,z: –42,12,–2) in healthy and depressive subjects ($n = 34$); (d) activation of right middle insula (x,y,z: 43,8,0) in healthy and depressive subjects ($n = 34$).

Table I. Percent signal changes of anterior and middle insula for healthy (n=17) and depressive (n=17) subjects. Interoceptive (Int.) and exteroceptive (Ext.) processes were analyzed in relation to the preceding rest period (with base correction) or not (without base correction).

	Healthy subjects Means ± SD	Depressive subjects Means ± SD	P value (two-tailed)
Anterior Insula, L			
Int. without base corr.	–	–	–
Ext. without base corr.	–0.054 ± 0.11	0.028 ± 0.08	0.02*
Rest	–0.231 ± 0.12	–0.13 ± 0.1	0.013*
Int. with base corr.	–	–	–
Ext. with base corr.	–	–	–
Anterior Insula, R			
Int. without base corr.	–	–	–
Ext. without base corr.	–0.057 ± 0.11	0.024 ± 0.097	0.03*
Rest	–	–	–
Int. with base corr.	–	–	–
Ext. with base corr.	–	–	–
Middle Insula, L			
Int. without base corr.	–	–	–
Ext. without base corr.	–0.085 ± 0.158	0.011 ± 0.123	0.055(*)
Rest	–0.242 ± 0.164	–0.137 ± 0.171	0.076(*)
Int. with base corr.	–	–	–
Ext. with base corr.	–	–	–
Middle Insula, R			
Int. without base corr.	–	–	–
Ext. without base corr.	–	–	–
Rest	–0.192 ± 0.132	–0.053 ± 0.111	0.002**
Int. with base corr.	–	–	–
Ext. with base corr.	–	–	–

**$P < 0.005$; *$P < 0.05$; (*) $P < 0.1$; – $P > 0.1$.

Signal changes during rest periods in the left anterior insula were also significantly positively correlated with BPQ stress response in healthy subjects; this no longer being the case in MDD patients (see Figure 3a and Table II). Instead, the reduced signal changes during rest periods correlated significantly with depression severity as measured with the BDI ($r = 0.57$, $P < 0.05$). The less deactivation during rest in the left anterior insula, the more severely patients experience their depressive symptoms (see Figure 3a). No significant correlation of BDI was observed with either the right anterior or middle insula.

Discussion

We here investigated body perception and neural activity in the insula as behavioural and neural measures of abnormal interoceptive awareness in depression. MDD subjects showed significantly higher body perception scores and lower signal changes during rest periods (i.e. reduced negative BOLD response) in the anterior and middle insula when compared to healthy subjects. It should be noted that a reduced negative BOLD-response may be due to either higher activity during rest periods or decreased neural activity. In contrast to healthy subjects, signal changes during rest periods in the anterior insula were no longer parametrically related to body perception scores in MDD. Most interestingly, both abnormal body perception scores and reduced signal changes during rest periods in the left anterior insula correlated with depression severity, as measured with the Beck Depression Inventory (BDI; Beck et al. 1961). Taken together, our findings demonstrate abnormal body perception and modulation of exteroceptive processing by the activity during rest in the anterior insula in MDD, mirroring these patients' abnormal "material me".

MDD patients showed significantly higher scores in body perception, as measured with the BPQ. Our observation of abnormal body perception is in accordance with previous findings of altered sensitivity and awareness of vegetative bodily changes in MDD (Stewart et al. 2001; Dunn et al. 2007; Strigo et al. 2008b). We were able to extend these findings by showing that different dimensions of body perception, such as stress response, autonomic nervous system reactivity and stress style, seem to be abnormally increased in depressed patients. This indicates abnormal body perception; although this may not concern awareness itself as we did not observe a significant difference in the awareness subscale of the BPQ. Future investigations of depressive subgroups may be needed to further detail their relationship with bodily awareness, with anxiety-dominated MDD patients,

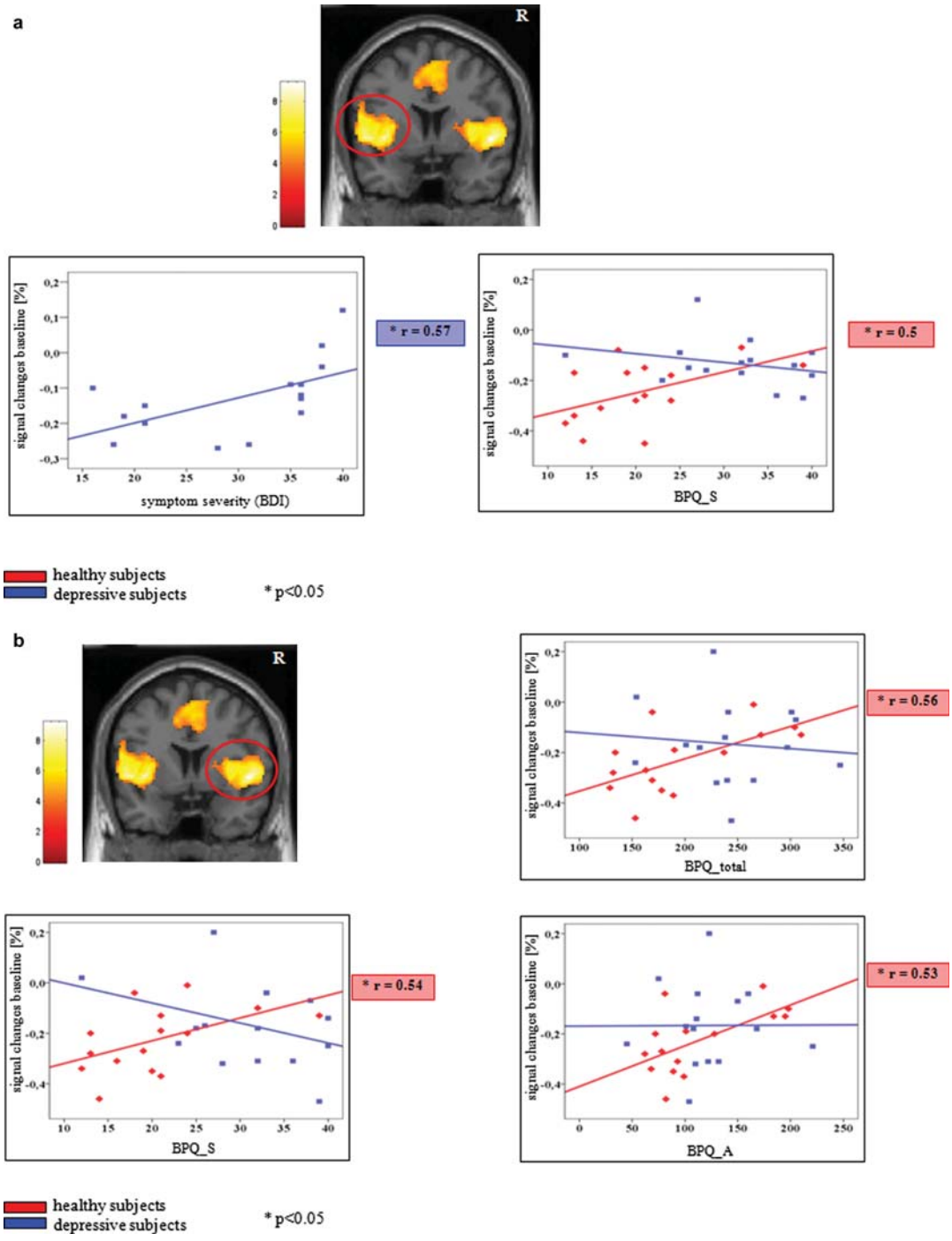

Figure 3. Correlation diagrams between left (a) and right (b) anterior insula activity during rest, Body Perception Questionnaire (BPQ) and Beck Depression Inventory (BDI). The SPM image shows the comparison between interoceptive and exteroceptive awareness (count heartbeat > count tones, n=34). The threshold of significance is set to $P < 0.01$ (FDR corrected, k>10). The diagrams show the correlation curves between percent signal changes of the left and right anterior insula during rest (y axis) and the scores of the Body Perception Questionnaire (BPQ, x axis). Healthy subjects (n=17, red lines) show significant correlations of the baseline signal changes in the right anterior insula (b) with the total score of the BPQ (BPQ_total), awareness (BPQ_A) and stress response (BPQ_S) (* P<0.05). Depressive subjects (n=15, blue lines), in contrast, showed no significant correlations between the BPQ in the right anterior insula (b). Baseline signal changes in the left anterior insula (a) show a significant correlation with stress response (BPQ_S) in healthy subjects, while the same regions correlated with depression severity in depressed subjects ($^*P$<0.05). The higher the signal changes in the left anterior insula during baseline, the more severe subjects scored their depressive symptoms.

Table II. Results of correlational analysis between signal changes in rest periods in different insular regions and subscores of the Body Perception Questionnaire (BPQ). white: healthy subjects, grey: depressive subjects.

	Awareness	Stress response	ANSR	Stress style (1+2)	Total score
Anterior Insula, L					
Rest, healthy	–	r=0.5	–	r=0.42	–
	–	P=0.044*	–	P=0.097(*)	–
Rest, depressive	–	–	r=–0.51	–	–
	–	–	P=0.054(*)	–	–
Anterior Insula, R					
Rest, healthy	r=0.53	r=0.54	–	–	r=0.56
	P=0.028*	P=0.027*	–	–	P=0.019*
Rest, depressive	–	–	–	–	–
	–	–	–	–	–
Middle Insula, L					
Rest, healthy	–	r=0.45	r=0.47	r=0.43	–
	–	P=0.068(*)	P=0.057(*)	P=0.087(*)	–
Rest, depressive	–	–	–	–	–
	–	–	–	–	–
Middle Insula, R					
Rest, healthy	–	–	–	–	–
	–	–	–	–	–
Rest, depressive	–	–	–	–	–
	–	–	–	–	–

*$P < 0.05$; (*)$P < 0.1$; – $P > 0.1$.
ASNR, autonomic nervous system reactivity.

for example, probably showing decreased awareness of the body (Pollatos et al. 2009).

Moreover, abnormal body perception correlated with depression severity as measured with the BDI. The more abnormally high body perception was, the more intensely and severely patients experience their depressive symptoms. This yields strong empirical support to the often made clinical observations of somato-vegetative symptoms, and of abnormal interoceptive awareness being an indicator of depressive symptoms and depression severity (Kirmayer 2001; Tylee and Gandhi 2005; Garcia-Cebrian et al. 2006; Nyboe Jacobsen et al. 2006).

MDD patients showed significantly reduced signal changes during rest in the insula, predominantly in the left anterior and middle insula. This is in accordance with early studies in MDD concerning rest periods that, using PET, also observed increased activity during rest in the insula (Mayberg 2002, 2003; Phillips et al. 2003a,b; Fitzgerald et al. 2008). In contrast, MDD patients did not show any abnormalities in this region during either intero- or exteroceptive stimulation when compared to healthy subjects. We were here able to extend these early observations by showing no changes during either intero- or exteroceptive stimulation independent of changes during rest periods. Although we did observe some abnormalities in the insula during the exteroceptive task, these could most likely be traced back to the reduced activity during rest rather than the exteroceptive stimulation itself, as revealed in our baseline corrected analysis. This underlines the proposed abnormal rest-stimulus interaction in depressed patients, with, it is suggested, an abnormally high activity during rest periods leading to a reduced stimulus-induced change in activity.

Furthermore, reduced neural changes during rest periods in the left anterior insula correlated with depression severity. The smaller the deactivation during rest was, the more severely MDD patients experienced their depressive symptoms. This is in accordance with previous findings concerning both the insula and other regions, such as the ventro- and dorsomedial prefrontal cortex (Brody et al. 2001; Milak et al. 2005; Perico et al. 2005; Paulus and Stein 2006; Simmons et al. 2006; Stein et al. 2007; Grimm et al. 2009a, 2009b). Taken together, our findings provide strong evidence of reduced activity during rest periods in the left anterior insula and the relation of this to depressive symptoms.

Most importantly, our findings indicate decoupling of body perception from neural activity changes in the insula in MDD. In accordance with Craig's hypothesis of the right anterior insula mediating the "material me", rest activity in this region correlated with body perception, as measured with the BPQ, in healthy subjects. This was no longer the case in depressed subjects, where activity during rest periods in both right and left anterior insula no longer correlated with BPQ scores. Body perception and activity during rest thus seem to be dissociated or, better, decoupled from each other in MDD.

MDD patients' abnormal body perception and its relation to altered activity in the insula may be

interpreted as the inability of depressed subjects to shift their focus of perception/awareness from the own body to their environment. This could lead to increased interoceptive awareness, mirroring the abnormal "material me" of MDD patients.

Several limitations of our study need to be considered. Our patients were all medicated and therefore we cannot exclude medication effects. Hence, the same study may need to be conducted again in unmedicated MDD patients.

One may criticize that we did not include a true resting state period with scanning for about 5–10 min in the mere resting state. Instead, we only included 4–10-s long periods with fixation cross. This was done because our main purpose was to clearly separate signal changes associated with rest from those related to intero- and exteroceptive processing within the regions of interest. In order to do this we analysed the relative signal changes induced by intero- and exteroceptive processing in relation to the respectively preceding rest period, something that is not possible with a separate, long rest period. Future studies with a separate and longer resting state period will be necessary to investigate fully the relationship between the resting state network, the default-mode network (Raichle et al. 2001; Raichle and Gusnard 2005), and the interoceptive network and body perception. One may also argue that body perception itself does not account for what is called the "material self". The "material self" could only be investigated by explicitly testing for self-relatedness of one's body, which we did not do here. Hence, future studies are necessary that include both body perception and self-relatedness as implicit and explicit measures of the self with regard to the (inner and outer) body.

In conclusion, we here demonstrate the crucial relevance of the anterior insula to abnormal body perception and depression severity in MDD. MDD patients showed differing body perception scores and reduced activity during rest periods in the anterior insula specifically. Signal changes during rest in the anterior insula no longer correlated with BPQ scores in MDD patients, while they were related to depression severity. Taken together, our findings demonstrate abnormal body perception and reduced activity in the insula during rest periods, with the latter being decoupled from the former. This may account for the often observed somato-vegetative symptoms in MDD patients.

Acknowledgements

We thank the staff from the state hospital of Uchtspringe, the Department of Neurology and Lilly Germany for their skilful assistance as well as Björn Enzi for his helpful comments on the manuscript. The study was made possible by financial contributions from Lilly Germany (to GN), the Salus Foundation (to GN), the Hope of Depression Research Foundation (HDRF, to GN) and the German Research Foundation (DFG, Sonderforschungsbereich 779-A6, to GN and CW).

Statement of interest

The authors report no conflict of interests.

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The World Journal of Biological Psychiatry, 2010; 11: 550–555

ORIGINAL INVESTIGATION

Serum brain-derived neurotrophic factor level is reduced in antidepressant-free patients with late-life depression

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Abstract

Objectives. The aim of the present study is to investigate serum BDNF levels in older depressed patients as compared to healthy elderly controls. *Methods.* Twenty-nine elderly subjects with major depression and 42 healthy older adults were enrolled to this study. All depressed patients were antidepressant-free for at least 1 month prior clinical and laboratorial assessments. Serum BDNF levels were determined by sandwich ELISA. *Results.* BDNF levels were lower in elderly depressed patients as compared to controls ($P=0.034$). Patients with late-onset depression had the lowest BDNF level (median 478.5, interquartile range 373.5–740.9 pg/l) when compared to early-onset depression (median 620.7, interquartile range 366.1–971.9 pg/l) and healthy controls (median 711.3, interquartile range 534.7–1181.0 pg/l) ($P<0.03$). *Conclusions.* Reduced serum BDNF level may be a state marker of late-life depression in non-medicated elderly patients. Our findings provide further evidences that reduced neurotrophic support may have an important role in the physiopathology of late-life depression.

Key words: *Brain-derived neurotrophic factor, geriatric depression, late-onset depression, physiopathology, cognition*

Introduction

Depression is the most common psychiatric disorder in geriatric practice and has a chronic and disabling course (Alexopoulos 2005). Geriatric depression is a heterogeneous disorder, and the age of onset of the first depressive episode may help identify subgroups of patients with distinct pathophysiological mechanisms. Several relevant studies have focused on the vascular hypothesis of geriatric depression (Alexopoulos et al. 1997), according to which late-onset depression is strongly associated with vascular cerebral burden, whereas in early-onset depression other pathophysiological mechanisms including hippocampal degeneration appear to play a major role (Janssen et al. 2007; Herrmann et al. 2008).

Few studies addressed the role of neurotrophic cascades in geriatric depression. Brain-derived neurotrophic factor (BDNF) is one of the most important and widely distributed neurotrophic factors in the brain (Murer et al. 2001). In adults, BDNF plays important roles in synaptic plasticity, neuronal resilience to insults, neurorestorative functions and neurogenesis (Hu and Russek 2008; Schindowski et al. 2008). Genetic studies showed that polymorphisms in the BDNF gene (e.g., Val66Met) are associated to an increased risk of depression in elderly patients (Taylor et al. 2007). Nevertheless, a recent study failed to find a significant difference in BDNF serum levels between community-dwelling elderly depressed subjects and healthy controls (Ziegenhorn et al. 2007).

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(Received 20 July 2009; accepted 24 November 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903544620

The aim of the present study was to investigate serum levels of BDNF in a clinical sample of older depressed patients as compared to age-matched controls. We hypothesize that serum BDNF levels are reduced in anti-depressant free late-life depression patients as compared to elderly controls.

Methods

Patient's assessment and diagnosis

Elderly subjects with evidence of current major depressive episode were recruited to this study ($n=29$). All participants were outpatients and underwent comprehensive clinical, psychiatric and cognitive assessments and were interviewed with the Structured Clinical Interview for DSM-IV disorders (SCID) (First et al. 2002). The diagnosis of major depressive disorder (first or recurrent episode) was made according to DSM-IV criteria (American Psychiatric Association 2000). Severity of the current depressive episode was evaluated by the scores on the 21-item Hamilton Depression Scale (HAM-D) (Hamilton 1960). Cognitive assessment was carried out with the CAMCOG (Roth et al. 1986; Nunes et al. 2008) and the Mini-mental state examination (MMSE) (Folstein et al. 1975).

The diagnosis of the current depressive episode was established for each patient upon recruitment, which means that these patients were not undergoing any clinical treatment. Therefore, all patients were antidepressant-free and not on use of any other psychotropic drug for at least 1 month prior to assessments. Patients with age of onset of the first depressive episode above 60 years old were regarded as with late-onset depressive disorder (LOD); otherwise, patients with one or more depressive episodes observed under the age of 60 years of age were regarded as early-onset cases (EOD).

Forty-two healthy elderly subjects, no evidence of current psychiatric or cognitive disorders (score of 0 on the HAM-D), were included in this study as a comparison group. These subjects are a subsample of healthy elderly subjects enrolled in a prospective clinical study on cognitive ageing (Diniz et al. 2008). The elderly subjects in the comparison group were not on antidepressant treatment at the time of blood sampling. There was no *a priori* matching strategy for gender and age for inclusion of controls in this study.

All depressed patients and controls had no evidence of relevant or uncontrolled clinical and neurological disorders, or prior history of clinically relevant cerebrovascular events at the time of assessment. This study was carried out according to the Helsinki Declaration and was approved by the local ethics committee. All patients and controls signed an informed consent form prior to psychiatric, cognitive and laboratorial assessments.

Serum BDNF determination

Following the clinical and cognitive assessment, blood samples were collected aseptically in the morning and the patients fasting for 10 h. Serum was then prepared and stored at −70°C until BDNF analysis. The BDNF assays of all patients were done at the same time.

The concentration of BDNF in serum was measured using sandwich ELISA kits for BDNF according to the procedure supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed on duplicate. The detection limit for this assay was 10 pg/l. In brief, the capture antibody (concentration provided by the manufacturer) was diluted in phosphate-buffered saline (PBS), added to each well and left overnight at 4°C. The plate was washed four times in PBS with 0.05% Tween 20 (Sigma, St Louis, MO, USA). The plate was blocked with 1% bovine serum albumin and incubated for 2 h at room temperature before washing four times with PBS and 0.05% Tween 20. The samples and standards were added and the plate incubated overnight at 4°C. After washing the plate, detection antibody (concentration provided by the manufacturer) diluted in PBS was added. The plate was incubated for 2 h at room temperature. After washing the plate, streptavidin (DuoSet R&D Systems) was added and the plate incubated for 30 min. At last, color reagent *o*-phenylenediamine (Sigma) was added to each well and the reaction was allowed to develop in the dark for 15 min. The reaction was stopped with the addition of 1 M H_2SO_4 to each well. The absorbance was read on a plate reader at 492-nm wavelengths (Emax, Molecular Devices, Minneapolis, MN, USA).

Statistical analysis

Mann–Whitney non-parametric tests were carried out to assess median differences of socio-demographic, clinical, cognitive variables, and BDNF serum levels between depressed patients and controls. Chi-square with Fisher exact test analyses were carried out to assess differences in the frequency of dichotomous variables between depressed patients and controls. Afterwards, depressed patients were divided into LOD and EOD patients and Kruskal–Wallis analysis with pos-hoc Dunn's multiple comparison tests was done to assess median serum BDNF levels differences among EOD, LOD and controls. This analysis was secondary to the main study hypothesis and

Table I. Socio-demographic data and scores on cognitive and psychopathological scales in elderly depressed patients (early-onset and late onset) and matched controls.

	Controls (n=42)	LOD (n=15)	EOD (n=14)	P
Gender (M/W)	6/36	3/12	3/11	0.774
Age (years)	69.5 [64.0–72.25]	72.0 [66.0–74.0]	70.0 [66.75–72]	0.511
HAM-D 21*	–	18.0 [14.0–22.0]	18.5 [8.5–26.25]	0.813
MMSE	29 [29–30]	26 [25–29]	27 [24.5–29]	<0.001
CAMCOG	99 [96–102]	86 [83–93]	85.5 [81.5–95.75]	<0.001
Episode duration (months)*	–	12 [6–24]	6 [5.5–30]	0.505
Number of depressive episodes*	–	1.0 [1.0–1.0]	3.0 [2.0–3.5]	>0.001
Age of the first depressive episode (years)*	–	66.5 [64.5–69.5]	54 [43–57.5]	0.01

*Mann–Whitney test of LOD vs. EOD. Values displayed as median [25th–75th quartile].
HAM-D 21, Hamilton Depression Scale 21 items; CAMCOG, Cambridge Cognition; MMSE, Mini-mental State Examination; EOD, early-onset depression; LOD, late-onset depression.

exploratory. Multivariate analyses, with type III sum of squares, were carried out to address the potential interaction of gender and diagnosis in serum BDNF levels. Spearman correlation analyses were carried out to assess for correlation between BDNF levels and socio-demographic, severity depressive symptoms and cognitive performance in the whole sample. All statistical analyses were done with the Software Package for Social Science v. 14.0 for Windows (SPPS, Chicago, IL).

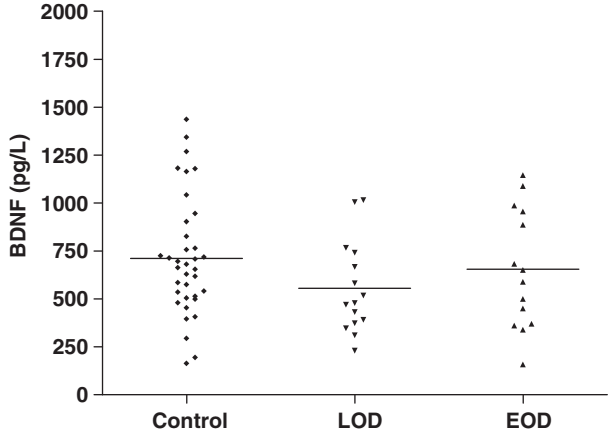
Results

Socio-demographic data and psychometric scores of 29 depressed patients (14 EOD and 15 LOD) and 42 non-depressed healthy controls are presented in Table I. There were no differences in age and gender distribution between depressed patients and controls. BDNF levels were significantly lower in depressed patients as compared to controls (depression: median 518.0 pg/l [interquartile range 372.5–827.0]; controls: median 711.3 pg/l [interquartile range 530.0–1179.9], P=0.013).

After grouping patients in EOD and LOD, the latter had the lowest BDNF levels (Figure 1). Dunn's multiple comparison tests showed a statistically significant difference in BDNF levels between LOD and controls (P<0.05). There was no significant difference in BDNF levels between either EOD and LOD or EOD and controls. The results remained unchanged after controlling for the number of depressive episodes and age of onset of the first depressive episode. There was no significant difference in gender distribution and age among the diagnostic groups; however, depressed patients (EOD and LOD) had significantly worse performance on cognitive tests as compared to controls. There were no significant differences between EOD and LOD patients in disease severity, duration of depressive episodes, and cognitive performance.

In the multivariate analysis, serum BDNF levels was significantly reduced in the elderly depressed patients as compared to controls (F=9.91, df=1, P=0.002). There was no significant differences in serum BDNF for gender (F=2.42, df=1, P=0.125) and no significant interaction between diagnosis and gender (F=3.32, df=1, P=0.08).

We found a significant negative correlation between BDNF levels and the severity of the depressive episode as measured by the scores on the HAM-D in the whole sample (ρ=–0.266, P=0.025). BDNF levels did not correlate with age, MMSE and CAMCOG scores, or episode duration.



	Controls (n=42)	LOD (n=15)	EOD (n=14)	P
BDNF (pg/l)	711.3 [524.7–1181.0]	478.5 [373.5–740.9]*	620.7 [366.1–971.9]	0.03

Horizontal line represents median values. Dunn's test EOD vs. LOD P <0.05. Values displayed as median [25th–75th quartile]. BDNF, brain-derived neurotrophic factor; EOD, early-onset depression; LOD, late-onset depression.

Figure 1. Scatter plot of BDNF plasma levels (pg/L) in elderly depressed (early-onset and late-onset depression) patients and controls.

Discussion

In the present study, antidepressant-free elderly depressed patients had lower serum BDNF levels as compared to age-matched controls. Also, BDNF levels had a negative correlation with the severity of depression. These results are in agreement with previous studies with younger depressed patients (Brunoni et al. 2008; Grassi-Oliveira et al. 2008; Sen et al. 2008), although a recent study did not find significant difference in BDNF levels between elderly depressed subjects and healthy controls (Ziegenhorn et al. 2007). Methodological differences such as study setting (community-based vs. tertiary clinic), severity of the depressive symptomatology, mean age of subjects, sample size, use of antidepressants, and the classification of the depressed patients according to the age of onset of the illness (not done in the latter study) may account for the different results observed. In particular, the lack of control for antidepressant treatment and the severity of depressive symptomatology might be of greater importance to explain for the differences observed between the latter study and ours. Community-based studies of depression generally include patients with mild depressive symptomatology and, thus, small reductions in serum BDNF might be expected in these patients. On the other hand, the use of antidepressants is associated with increased serum BDNF levels in depressed patients (Matrisciano et al. 2008). These factors altogether might have precluded finding significant differences in the Ziegenhorn et al. (2007) study. In addition, the role of circulating serum BDNF and its regulatory mechanisms are still poorly understood (Fujimura et al. 2002) what may also be a reason for these inconsistent results for serum BDNF levels in major depression found in the literature. Our findings should be viewed in light of the small number of subjects recruited and the lack of *a priori* matching for gender in the inclusion of patients and controls which are potential limitations of this study. Thus, the present findings need to be replicated in larger samples and other clinical settings.

The reduction in serum BDNF levels was more pronounced in patients with late-onset as compared to early-onset depression; albeit there was no statistical difference in serum BDNF levels between these two groups. To the best of our knowledge, this is the first study to report a distinct pattern of serum BDNF reduction according to the clinical classification of geriatric depression with respect to the age of onset of the depressive disorder. In the past decade, the pathophysiology of late-onset geriatric depression has been extensively discussed in the light of the "vascular hypothesis" (Alexopoulos et al. 1997), in which long-lasting ischemic changes in the brain secondary to underlying cardiovascular risk factors play a decisive role in the etiology of symptoms (Sachdev et al. 2008). More recently, evidence of internal validity of this concept in clinical samples supports the notion that vascular depression may be regarded as a unique diagnostic subtype in late life depression (Sned et al. 2008), rendering early- and late-onset geriatric depression biologically distinct. In this case, the vascular burden observed more often in late-onset depression may implicate a specific mechanism of homeostatic disruption, in which the availability of BDNF may be a state marker of a brain-based pathological process.

Accordingly, mRNA expression of BDNF was shown to be reduced after chronic ischemic insult in the hippocampus and cerebral cortex of hypertensive rats (Lee et al. 2006), and treatment with BDNF protects against acute cerebral ischemia (Almli et al. 2000; Müller et al. 2008). Also, a recent neuroimaging study with a large cohort of depressed and non-depressed elderly subjects showed that the presence of the Met66 allele of BDNF gene is associated to greater white matter hyperintensities volume in this cohort (Taylor et al. 2008). Therefore, the present results, along with the aforementioned neuroimaging and animal data, may also suggest that impaired BDNF signalling, and as a consequence the lack of neurotrophic support (Duman and Monteggia 2006), may play a role in the physiopathology of geriatric depression, particularly in patients with late-onset geriatric depression.

Although BDNF is mostly expressed in the nervous system, it is also found in platelets, lymphocytes and endothelial cells. Platelets bear most of the content of circulating BDNF, and they appear to bind, store and release, but not produce, BDNF (Karege et al. 2002). The biological functions of circulating and platelet-stored BDNF are poorly understood, but it may serve as a buffer system that supply the brain with additional BDNF when necessary (Fujimura et al. 2002). Another important issue is whether peripheral BDNF correlates to central BDNF. This correlation is poorly understood and recent studies with animal models of depression and humans showed a poor correlation and an inverse correlation between serum/plasma and CSF BDNF levels (Laske et al. 2007; Elfving et al. 2009). These issues in conjunction may weaken the interpretation of the involvement of reduced peripheral BDNF in depression and related conditions.

On the other hand, a large body of evidence demonstrated that pathological processes associated with several neuropsychiatric disorders produce disease-specific molecular changes in the blood and

the disruption of the signalling network between the brain and the body, which are manifested by pathological changes peripheral cytokines, chemokines and growth factors (Britschgi and Wyss-Coray 2009). In such case, BDNF levels are reduced in several psychiatric and neurologic disorders. Medication-free, acutely ill bipolar patients in manic or depressed states present with low BDNF levels, which resumes levels similar to those observed in control subjects after successful treatment (Cunha et al. 2006). In addition, patients with Alzheimer's disease also display lower BDNF levels as compared to cognitively unimpaired controls (Laske et al. 2006), which are sensitive to long-term treatment with cholinesterase inhibitors (Leyhe et al. 2008). Taken together, these data suggest that low BDNF level is an unspecific marker of several neuropsychiatric disorders and a surrogate marker of brain-based pathological processes associated with the disruption of neurotrophic cascades. In addition, the increments in BDNF levels observed after successful treatment of these conditions suggest that the partial or complete restoration of adequate neurotrophic support underlies clinical recovery.

Acknowledgements

This work was funded by grants from Rede Instituto Brasileiro de Neurociência (IBN Net/Finep), FundaÇão de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant no. 02/12633-7), AssociaÇão Beneficente Alzira Denise Hertzog da Silva (ABADHS). The funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Statement of interest

None to declare.

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The World Journal of Biological Psychiatry, 2010; 11: 556–566

ORIGINAL INVESTIGATION

Reduced density of ADAM 12-immunoreactive oligodendrocytes in the anterior cingulate white matter of patients with schizophrenia

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Abstract

Objectives. Abnormalities of brain white matter and oligodendroglia are replicated findings in schizophrenia research. The largely oligodendroglia-associated enzyme ADAM (A disintegrin and metalloprotease) 12 might be involved in the pathophysiology of schizophrenia, because the gene coding for human ADAM12 is located on chromosome 10q26.3, a gene locus which has been linked to schizophrenia, and some of its putative substrates are altered in schizophrenia. *Methods.* We studied the numerical density of ADAM12 expressing oligodendrocytes in post-mortem prefrontal brains of patients with haloperidol treated, chronic schizophrenia and matched controls. *Results*. A significantly reduced numerical density of ADAM12 immunoreactive oligodendrocytes was found in the white matter of the anterior cingulate cortex of schizophrenic patients. *Conclusions*. Although the pathophysiological implications of this finding are currently unknown, it is well conveyable that reduced ADAM12 protein contributes to a deviant metabolism of some of its substrates. These substrates are either parts of important signalling cascades (EGF, betacellulin, TGF-beta) or chemical components of myelin (neurofascin-ankyrin) known to be compromised in schizophrenia.

Key words: *ADAM (A disintegrin and metalloprotease) 12; schizophrenia; oligodendroglia; prefrontal cortex; immunohistochemistry; morphometry*

Abbreviations: ACC, anterior cingulate cortex; ADAM, A disintegrin and metalloprotease; BMI, body mass index; DAPI, 4′,6′-diamidin-2′-phenylindol-dihydrochloride; DLPFC, dorsolateral prefrontal cortex; EGF, epidermal growth factor; HB-EGF, heparin binding-epidermal growth factor like growth factor; ICD, International Classification of Diseases; IGF, Insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IgG, Immunoglobulin G; LI, liver-intestine; OL, oligodendrocyte(s); OLN, oligodendroglial cell line; PBS, phosphate-buffered saline; P-LAP, placental leucine aminopeptidase; RT-PCR, real time polymerase chain reaction; SNP, single nucleotide polymorphism; SPSS, statistical product and service solutions; TGF, transforming growth factor; Wnt, Wingless and Int.

Introduction

Schizophrenia is increasingly conceptualised as a disease of disturbed functional circuitry. A wide range of white matter abnormalities have been revealed in schizophrenia. They include volume reductions, hypodensities, reduced fractional anisotropy, myelination defects (Hakak et al. 2001), and alterations in the density, morphology and gene expression profiles of oligodendrocytes (OL; Hof et al. 2002, 2003; Uranova et al. 2001, 2004; Davis et al. 2003; Byne et al. 2006; Bernstein et al. 2009; Höistad et al. 2009; Schmitt et al. 2009). Reduced OL densities are also found in the cortical gray matter in schizophrenia (Vostrikov et al. 2007).

ADAM12, aka meltrin alpha, is a member of the family of multidomain metalloprotease-disintegrins which possess cell-binding, cell-signaling and proteolytic properties. Curiously, while its normal function in the CNS is still poorly understood (Edwards et al. 2008; Kveiborg et al. 2008), ADAM12 has been implicated in neurological and psychiatric disorders (i.e. Multiple Sclerosis, Alzheimer's disease and bipolar

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(Received 27 August 2009; accepted 17 November 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903497936

disorder; Malinin et al. 2005; Moon et al. 2006; Harold et al. 2007; Nadri et al. 2007; Kveiborg et al. 2008).

ADAM12 might also play roles in schizophrenia, because (1) this enzyme is mainly expressed in human white matter and gray matter OL (Bernstein et al. 2004), (2) the gene coding for human ADAM12 is located on chromosome 10q26.3, a gene locus which has been linked to schizophrenia (Ewald et al. 2002; Park et al. 2004; Bulyaeva et al. 2007; Chissoe et al. 2008), (3) some of the substrates of the enzyme are altered in schizophrenia (Futamura et al. 2002; Häninnen et al. 2007; Wu et al. 2007; Iossifov et al. 2008; Cruz et al. 2009; Kalkman 2009, see Table I), and (4) long-term application of methamphetamine alters ADAM12 expression in rat striatal tissue (Cadet et al. 2002). For these reasons we felt encouraged to look for ADAM12 immunoreactivity in post-mortem brains of patients with schizophrenia. We have concentrated on the dorsolateral prefrontal and the anterior cingulate cortex because structural abnormalities in these prefrontal brain areas may represent a neurobiological basis for many of the clinical manifestations of schizophrenia (reviewed in Fornito et al. 2009; Kompus et al. 2009; Schmitt et al. 2009).

Material and methods

Human brain material

All brains were obtained from Magdeburg brain bank. Case recruitment, acquisition of personal data, performance of autopsy, and handling of autoptic material were conducted in accordance with the Declaration of Helsinki, and have been approved by the responsible Ethical Committee of Magdeburg. Brains of nine individuals with clinically confirmed schizophrenia (four females and five males, aged 52.2 ± 7,1 years) and seven sex- and age-matched controls without neurological or psychiatric disorders (three females and four males, aged 56.4 ± 12.0 years) were studied. A lifetime psychiatric diagnosis of schizophrenia was established according to ICD 10. Eight patients had suffered from the paranoid subtype, one from the residual subtype of the illness.

Only patients with well preserved clinical records were selected for the present study. Demographic and histological data are summarized in Tables II and III. All patients with schizophrenia had received long-term medication with haloperidol (15–20 mg/day).

The tissue preparation was as described earlier (Bernstein et al. 1999). Briefly, brains were removed

Table I. Potential substrates and interaction partners of ADAM12 and their possible impact in schizophrenia (after Kveiborg et al. 2008, modified and extended).

Substrate/Interaction partners	ADAM12splice variant	Functions ofthe substrates	Findings in schizophrenia
IGFBP-3,-5(Loechel et al. 2000)	Soluble	Release of IGF, IGFR signalling, cell growth, cell differentiation	Lower serum levels of IGFBP-3 in obese schizophrenia patients after physical activity (Wu et al. 2007), normal blood levels (Akanji et al. 2007)
HB-EGF(Asakura et al. 2002; Kurisaki et al. 2003)	Transmembrane	EGFR Transsctivation/ Signaling, cell growth, cell differentiation	HB-EGF normal in schizophrenia(Futamura et al. 2002)
EGF (Horiuchi et al. 2007)	Transmembrane	EGFR signaling cell growth, cell differentiation	Decreased in post-mortem prefrontal cortex in schizophrenia (Futamura et al. 2002); EGF gene polymorphism associated with age of onset in male schizophrenics (Häninnen et al. 2007)
Betacellulin(Horiuchi et al. 2007)	Transmembrane	EGFR signaling	Betacellulin: genetic linkage(Iossifov et al. 2008).
TGF-beta (Atfi et al. 2007)	Transmembrane	Wnt signaling cell proliferation, differentiation, and apoptosis	TGF-beta signalling hyperactive in schizophrenia (Kalkman 2009); via cyclin D1 influence on abnormal OL cell cycle (Katsel et al.2008)
Delta-like 1 (Dyczynska et al. 2007)	Transmembrane	Notch-signaling, cell fate determination	Gene locus linked to schizophrenia(Moon et al. 2006)
P-LAP, Oxytocinase(Ito et al. 2004)	Transmembrane	Oxytocin cleavage	Normal expression in postmortem hypothalamus in schizophrenia (in preparation)
Neurofascin-155(Maier et al. 2006)	Transmembrane	Neuron-glia interaction, myelin integrity	Decreased density of neurofascin binding partner ankyrin in schizophrenia (Cruz et al. 2009)

within 48 h after the death. Brain volumes were calculated by the weight method using fresh whole brain weight and brain density (Yamada et al. 1999). Volume shrinkage was determined for each brain. Volume shrinkage factors were calculated using the formula: $VF=(A1/A2)^{3/2}$ (VF=volume shrinkage factor; A1=cross-sectional area before processing of tissue; A2=cross-sectional area after processing of tissue). Sections were taken at intervals of 400 μm (according to Cavalieri's principle, Mayhew, 1992).

There were no significant differences (Student's *t*-test, χ^2 analysis) between patients with schizophrenia and controls with regard to age, gender, and storage delay. Additionally, quantitative neuropathological changes due to neurodegenerative disorders were ruled out by an experienced neuropathologist (Danos et al. 2003). In order to reveal a possible influence of diagnosis-independent obesity on the cerebral expression of ADAM12 (Kurisaki et al. 2003; Rankinen et al. 2006), individual body mass indices (BMI) were calculated whenever possible (Bernstein et al. 2007; Tables II and III). The brains were then fixed *in toto* in 8% phosphate-buffered formaldehyde (pH 7.0) for 2 months. After embedding of the brains in Paraplast®, serial coronal 20-µm thick sections were cut on a microtome and mounted on slides. Every 50th section was stained for morphological orientation (cresyl violet and myelin staining according to Nissl and Heidenhain-Woelcke).

For Western blot analysis and RT-PCR samples of the left prefrontal cerebral cortex of were used. The brains of two male individuals without signs of neurological or psychiatric disorders (54 and 58 years old) were removed 9 and 15 h after death, dissected into small pieces, snap-frozen in liquid nitrogen and stored at −80°C.

ADAM12 immunohistochemistry

A well-characterized polyclonal ADAM12 antiserum produced in rabbits against the appropriate peptide sequence SA-378 was used (BIOMOL Hamburg, Germany). For immunohistochemical staining, sections from the prefrontal cortex were selected at intervals of about 1.0 cm (Bernstein et al. 2004). At least three sections per case were immunostained. After dewaxing of the paraffin sections with xylol the sections were preincubated with methanol/H_2O_2 to depress endogenous peroxidases. After repeated washing with phosphate-buffered saline (PBS, 2× for 10 min each wash), goat serum at a dilution of 1:10 in PBS was added to the sections. Then, the sections were placed in a humidified chamber for 1 h at room temperature. The primary antibody was applied at a dilution of 1:100 in PBS in a humidified chamber for 48 h in the cold. The specificity of the antiserum was tested (for details, see Bernstein et al. 2004). This step was followed by repeated washings with phosphate-buffered saline (PBS, 2 times for 10 min each wash). Further immunocytochemical protocol involved the incubation with goat anti-rabbit IgG serum, diluted 1:100 in PBS (DAKO, Wiesentheid, Germany) and the application of the avidin–biotin technique (avidin–biotin complex from Amersham, Freiburg, Germany). Then, repeated washing with PBS (2× for 10 min each wash) followed. The immunoreaction was visualized with 3,3′-diaminobenzidine and washed in distilled water (2× for 5 min each wash), 60% alcohol for 5 min 70% alcohol for 5 min 96% alcohol for 5 min absolute alcohol for 5 min and xylol (2× for 15 min each wash). To visualize the reaction product, 3,3′-diaminobenzidine was used. The colour reaction was enhanced by adding 2 ml of 0.5% ammonium nickel sulphate to yield a purplish-blue reaction product (Bernstein et al. 1999). As a control, the primary antibody was replaced by PBS or normal rabbit serum. Furthermore, we performed preabsorption of the antiserum by the peptide which was used to generate the antiserum (peptide SA-378, Biomol). The antiserum and the blocking peptide were mixed at a ratio of 20 µg peptide per 1 µg antibody. This mixture was incubated overnight in the cold, diluted into blocking solution and added to the sections. Then the immunohistochemical protocol was continued as usual. The sections without the specific primary antibody or with blocked antiserum did not show any immunostaining (Figure 1D).

Table II. Demographical data for the control subjects.

Control subjects	Age	Gender	BMI	Cause of death
1	61	M		sudden cardiac death / coronary thrombosis
2	66	M	26.5	myocardiac infarct, ventricular fibrillation, Cardiac respiratory insufficiency
3	63	F	21.2	acut heart failure
4	31	M		left cardiac insufficiency
5	39	F		pneumonia
6	61	F		sudden cardiac death
7	66	F	29.0	right heart failure

Immunolocalization of ADAM12 in OLN-93 cells

OLN-93 is a permanent OL cell line that was derived from spontaneously transformed cells in a primary rat brain glial culture. This well-characterized cell line was kindly provided by Dr Richter-Landsberg (University of Oldenburg, Germany). Handling of the cell culture was as recently described (Steiner

Table III. Demographical data for the schizophrenic patients.

Individuals with schizophrenia	Age(years)	gender	BMI	Cause of death	Duration of illness (years)
1	51	M	20.7	Cardiac insufficiency past large intestine ileus	28
2	40	F	36.0	Septic-toxic cardiac and circulatory failure	19
3	48	M		Bolus death with signs of asphyxia	32
4	54	M		Cardiac respiratory insufficiency	34
5	45	M	22.6	Cardiac and circulatory failure	20
6	54	F	37.0	Pulmonary embolism	18
7	55	F		Suicide with tabletts	6
8	61	M	29.8	Sudden cardiac death	35
9	62	F	19.3	Cardiac respiratory insufficiency	16

et al. 2008). Briefly, cryopreserved OLN-93 were defrosted, resuspended in DMEM supplemented with 10% foetal calf serum, 50 U/ml penicillin and 50 μg/ml streptomycin (growth medium), and transferred to culture flasks. After 1 week, cells were removed from the flasks by mild trypsinization (5 min; trypsin/EDTA: 0.05%/0.002%) and replated on ∅35-mm Petri dishes (30th passage, 50.000 cells/dish). After 3 days, the foetal calf serum concentration of the growth medium was reduced to 0.5%, and the respective experiments were performed 72 h later. All cultures were plated on poly-d-lysine-coated dishes and maintained at 37°C in a humidified atmosphere under 5% CO_2 in air, and were fed twice per week by changing 1 ml of medium. All cultures were kept at 37°C in a humidified atmosphere under 5% CO_2 in air (normoxic conditions) for the duration of the experiment. OLN-93 cultures were thoroughly washed twice with phosphate-buffered saline (pH 7.4), then fixed for 30 min in 4% buffered paraformaldehyde and incubated at room temperature with ADAM12 antiserum diluted in PBS

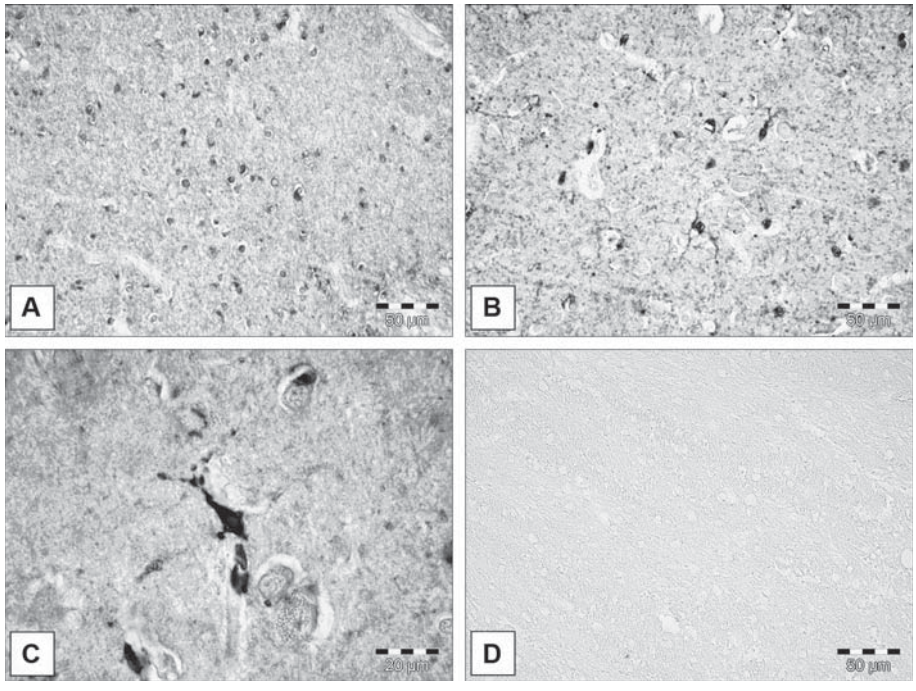

Figure 1. Expression of ADAM12 protein in human prefrontal cortex OL. (A) Multiple ADAM 12 immunoreactive white matter ACC OL (Control case). (B) Gray matter DLPFC OL showing ADAM12 immunoreactivity (schizophrenic patient). (C) Single ADAM12-ir OL in the gray matter at higher magnification (control case). (D) Lack of specific ADAM12-ir after omission of the primary antiserum.

with 0.3% Triton X-100 and 1% normal goat serum for 3 h. Following incubation with the primary antiserum, the cultures were washed in phosphate-buffered saline (3×5 min) and incubated for 3 h with the secondary antibody (Molecular Probes, Göttingen, Germany) at a 1:500 dilution: Alexa Fluor 546 (goat anti-rabbit-IgG; yielding red fluorescence). The specimens were examined using a fluorescence microscope (AxioImager, Zeiss, Germany) equipped with phase-contrast, fluorescein, rhodamine and 4′,6′-diamidin-2′-phenylindol-dihydrochloride (DAPI) optics.

Stereology and statistical analysis

ADAM12 expressing OL were counted in different prefrontal brain areas: left and right gray and white matter dorsolateral prefrontal cortex (DLPFC) and left and right gray and white matter anterior cingulate cortex (ACC). The ACC and the DLPFC (Brodmann's areas 8, 9, 10. 46), were delineated as proposed by Mai et al. (2003); see Steiner et al. (2006)). Sections were situated at least 1 cm in front of the corpus callosum. The section thickness after the histological procedures was 18.9 ± 1.0 μm (mean ± SD). A counting grid (dimensions 25 × 25 μm = 625 μm^2) was used to define a three-dimensional box within the thickness of the section as described earlier (Bernstein et al. 1998) allowing at least 4-μm guard zones at the top and bottom of the section, and to apply a direct, three-dimensional counting method. All immunostained OL were counted separately in a linear probe of stacked counting boxes extending from the pial surface to the cortical layer VI (gray matter fractions according to Selemon et al. 2003) and within 2.25 mm of the gray-white border (white matter fractions according to Kirkpatrick et al. (2003)) using ×40 objectives during the analysis. Since the actual thickness of the sections was between 18 and 20 μm, two well-defined optical planes within the section were used (distance 16 μm) and immunostained cells were counted that come into focus as one passes from the upper to the lower optical plane.

Normal distribution of demographic data was demonstrated by Kolmogorov–Smirnov tests. A stepwise multiple regression analysis was performed to control for possible influences on OL densities of age, gender, duration of disease, storage delay, whole brain volume, duration of disease, BMI and medication. Regional effects were analyzed by separate analyses of variance with repeated measurements and post hoc Tukey tests. Significance was defined as $P < 0.05$. Statistical analyses were performed with the SPSS package version 11.0 (Statistical Product and Service Solutions, Chicago, IL, USA). Investigators were blind for diagnosis of the cases. To establish inter-rater reliability, measurements of five brains were performed by three investigators (N.F., H.-G.B., S.F.). The inter-rater reliability was 0.82. Test–retest (intra-rater) reliability (N.F.) was 0.95.

Western blot

Frozen tissue samples from the prefrontal cortex were pulverized in liquid nitrogen and subsequently homogenized in lysis buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100. 0.1% SDS), containing a protease inhibitor cocktail (Roche Diagnostics, Heidelberg, Germany). Tissue homogenates were centrifuged and the resulting supernatant was stored at −80°C until further use. Aliquots representing 30 μg protein were subjected to SDS–PAGE (gradient gels from 5 to 25%), followed by transfer to nitrocellulose membranes (NEN Life Science, Boston, MA, USA). Membranes were blocked with 5% milk (Roth, Karlsruhe, Germany) in TBS-Tween and then incubated with either the polyclonal antiserum (diluted in TBS-Tween 1:500). Anti-rabbit horseradish peroxidase conjugated antibodies (Cell Signalling, Frankfurt/Main, Germany) diluted 1:5 000 in TBS-Tween were applied after washing the blots three times in TBS-Tween. For chemoluminescence detection, the SuperSignal West Dura substrate (Pierce, Bonn, Germany) was used. The expression of beta-actin was used as internal standard.

RT-PCR

Total RNA was prepared from frozen samples of human prefrontal cortex and transcribed into cDNA (Arndt et al.2002). Quantitative PCR was performed using the iCycler (Bio-Rad, Munich, Germany). All samples were analysed in triplicate. A 25-μl reaction mixture consisted of 12.5 μl HotStart Tag Master Mix (Qiagen Hilden, Germany), 1 μl cDNA, and 0.5 μmol/l of the specific primers for ADAM12 (5′-gCTgATgAAgTTgTCAgTgC-3′ and 5′-gAgCTgACTgCTg AATgCAg-3′). Initial denaturation at 95°C for 15 min was followed by 40 cycles with denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 60 s. Amplified ADAM12 cDNA fragments were applied to 1.6% agarose gel electrophoresis and visualized by ethidium bromide staining.

Limitations of the study

Constraints of this study include the small cohort size and the limited power to detect changes in the OL-associated protein ADAM12 by determining the number of immunolabelled cells. Hence, some of our results must be regarded as preliminary and should be substantiated in further investigations.

Results

Qualitative observations

Numerous OL in human postmortem cortical gray and white matter of DLPFC and ACC were found to express ADAM12 immunoreactivity (Bernstein et al. 2004). Control sections were free of specific immunostaining (Figure 1A–D). Qualitatively, there were no differences in the staining patterns between schizophrenic patients and controls.

The presence of ADAM12-immunoreactive material in OL was further confirmed by experiments with cultured OL (cell line OLN-93). Cultured OL were found to differently express the enzyme protein: a subset of cells was characterized by very strong immunostaining, whereas a majority of cells showed only a moderate red immunofluorescence. Among the ADAM12-immunoreactive OL were some dividing cells (Figure 2). The immunoreaction was confined to the cell bodies of the OL.

Quantitative observations

In human postmortem brains we found significantly reduced densities of ADAM12-immunoreactive white matter OL of the ACC of schizophrenics in comparison to controls (both hemispheres taken together, $P=0.021$), as well as for the right hemisphere ACC alone ($P=0.013$). A tendency towards reduction was estimated for white matter ACC of the left hemisphere ($P=0.058$) and for the white matter of both hemispheres of the DLPFC (right side: $P=0.257$; left side: $P=0.139$; Figures 3 and 4).

No significant differences were found between schizophrenics and controls with regard to the density of immunopositive OL in the gray matter of the ACC (right side: $P=0.997$; left side: $P=0.988$) and the DLPFC (right side: $P=0.880$; left side: $P=0.450$; Figures 5 and 6).

Variables which could influence OL densities, such as brain weight, BMI, age at time of death, post-mortem delay, and neuroleptic medication did not correlate with the parameters measured. The latter aspect is of relevance, since it is known that chronic antipsychotic treatment may slightly reduce OL number in monkey brains (Konopaske et al. 2008). Importantly, a significant negative correlation was revealed between time of fixation and density of ADAM12-immunopositive OL ($r=-0.563$; $P=0.063$), which was taken into account when calculating numerical cell densities.

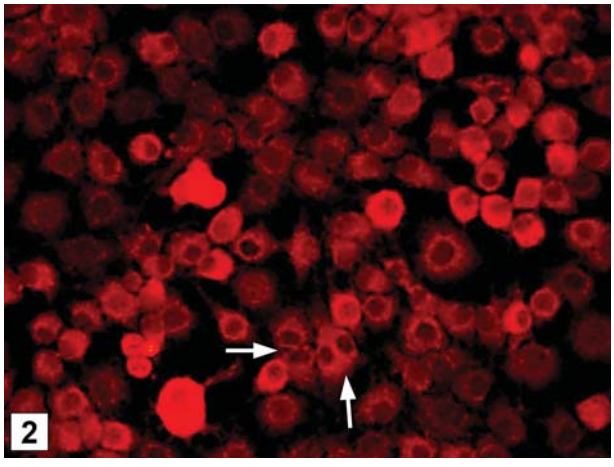

Figure 2. ADAM12 expressing OL cultured in cell line OLN-93. Red immunofluorescence indicates that a fast majority of cells is immunoreactive for the metalloprotease, though to a different degree. ADAM12 is even expressed in dividing cells (arrows).

Western blotting

The results of the Western blot analysis are shown in Figure 7. When probing representative samples (20 μg of total protein per lane) from prefrontal cortex of controls with our ADAM12-specific antibody (lanes A and B) we detected a main protein band with a molecular mass of about 84 KDa, corresponding to the processed form of the enzyme. The observed pattern is similar to what was reported by Kodama et al. (2004) for ADAM12 expression in brain tumours. The lower molecular forms of ADAM12 with masses between 60 and 25 kDa probably represent cleavage products of the enzyme (for details on various ADAM12 species, (see: www.genwaybio.com/product_info.php?products).

RT-PCR

ADAM12 mRNA was detected in both human postmortem brain tissue samples. A single PCR product of the expected size (280bp) could be demonstrated (lanes A and B, Figure 8).

Discussion

Numerous findings have been published in support of this myelin hypothesis of schizophrenia. In the endeavour to identify cellular correlates of the observed white matter changes, OL have come into focus of schizophrenia research. Imaging and morphometric studies demonstrated decreases in prefrontal cortical white matter volume and OL density of schizophrenic patients, while gene expression microarray and immunohistochemical investigations showed a downregulation of several myelin transcripts. In rats, downregulation of oligodendrocyte transcripts is associated with impaired prefrontal cortex functions (Gregg et al. 2009) However, the extent to which in humans individual reduced or

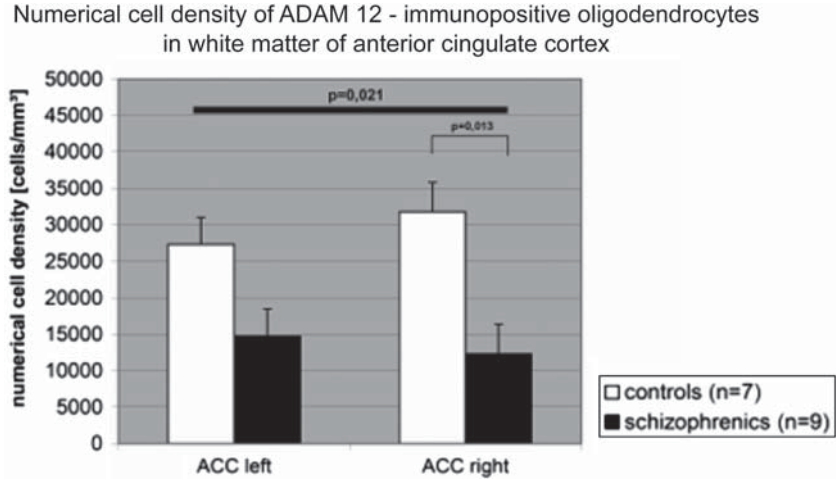

Figure 3. Significantly reduced numerical cell density in the white matter of the anterior ACC (P=0.021).

elevated OL markers contribute to pathophysiological domains of schizophrenia is not well known (Gregg et al. 2009; Martins-de-Souza et al. 2009). We here provide evidence for reduced cell densities of ADAM12 expressing OL in the white matter ACC in schizophrenia. Cellular changes in the white ACC in schizophrenia are in good accordance to findings from diffusion tensor imaging studies demonstrating disease-related, reduced microstructural integrity in the anterior cingulum bundle (Manoach et al. 2007). Recently, some of us could reveal that this brain region shows metabolic abnormalities in patients with chronic schizophrenia (Schmitt et al. 2009).

ADAM12 is predominantly expressed in subsets of mammalian and avian white and gray matter OL (Bernstein et al. 2004; Lin et al. 2009). Numerous studies on OL in schizophrenia report disease-related reductions in OL densities (Hof et al. 2003; Uranova et al. 2001, 2004; Stark et al. 2004; Vostrikov et al. 2007; Schmitt et al. 2009 vs. Segal et al. 2009). Hence, the question may arise, if reduced numerical density of ADAM12 immunopositive OL does simply reflect the overall decline of OL density in schizophrenia, or if the reduction of this particular OL protein may be of relevance for our understanding of the disease. Firstly, we found a significant reduction of the density of ADAM12 immunopositive OL only in the white matter anterior cingulate cortex in schizophrenic patients. Recently, Segal et al. (2009) have shown that in schizophrenia there is no general reduction of OL density in the cingulum bundle of this region. Since more than half of our counting boxes in the white matter anterior cingulate cortex contained the cingulum bundle, a reduction of ADAM12 immunoreactive OL because of a general reduction of this cell type is not very likely, although it cannot be fully excluded. Secondly, linkage analyses point to an association of schizophrenia with chromosome locus 10q26.3,

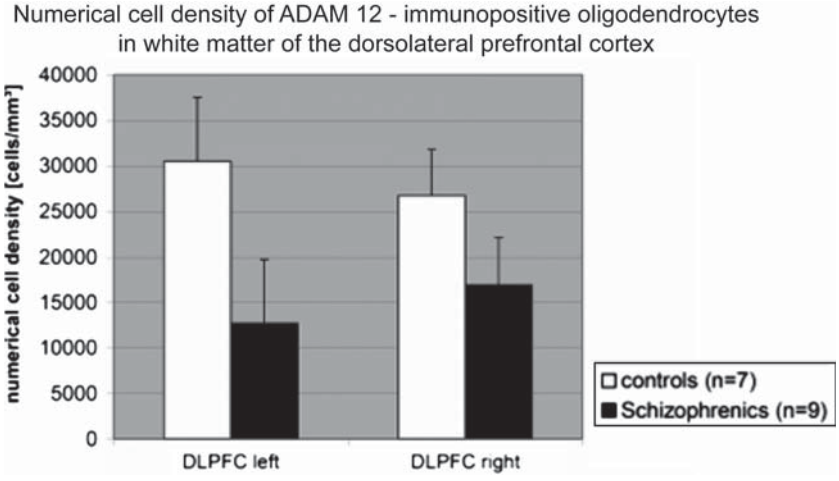

Figure 4. A tendency towards a reduction of numerical cell density is found in the white matter of the DLPFC of schizophrenic patients.

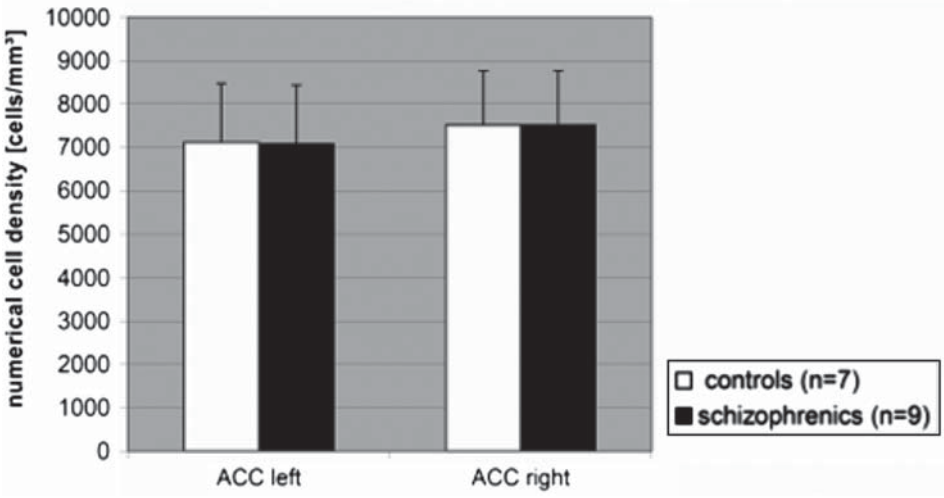

Figure 5. Numerical cell density of ADAM 12 immunopositive oligodendrocytes in the gray matter of the ACC; no significant differences were found.

where the ADAM12 gene is encoded (Ewald et al. 2002; Park et al. 2004; Bulyaeva et al. 2007; Chissoe et al. 2008). Moreover, it has been shown that at least one SNP within the human ADAM12 gene is associated with schizophrenia (Chissoe et al. 2008). However, gene polymorphisms would eventually help explain only a small percentage of the cases. Hence, a closer look at properties of ADAM12 might be fruitful in attempting to get more conclusive answers.

ADAM12 is a transmembrane multidomain protein. Besides, an alternatively spliced secreted form, ADAM12-S (short) is known to exist (Wewer et al. 2005; Kveiborg et al. 2008). In addition to its protease activity, ADAM12 possesses cell binding and cell signalling properties. This functional diversity of ADAM12 is reflected in its complex structure (i.e. possessing head, body, and tail; Jacobsen and Wewer 2009). The head of the ADAM12 molecule mediates processing of growth factors and cytokines. The body (consisting of the disintegrin, cysteine-rich, and EGF-like domains) is involved in contacts with the extracellular matrix and other cells through interactions with integrins and syndecans, whereas the tail of the protein interacts with intracellular signalling molecules (reviewed in detail by Jacobsen and Wewer 2009). Thus, ADAM12 is a multitalented enzyme involved in many aspects of normal and pathological catabolism as well as cell signalling events (Kveiborg et al. 2008). ADAM12 expression has been correlated with quite different human diseases, including osteoarthritis, tumour growth and progression, bipolar disorder, and Down syndrome (Laigaard et al.

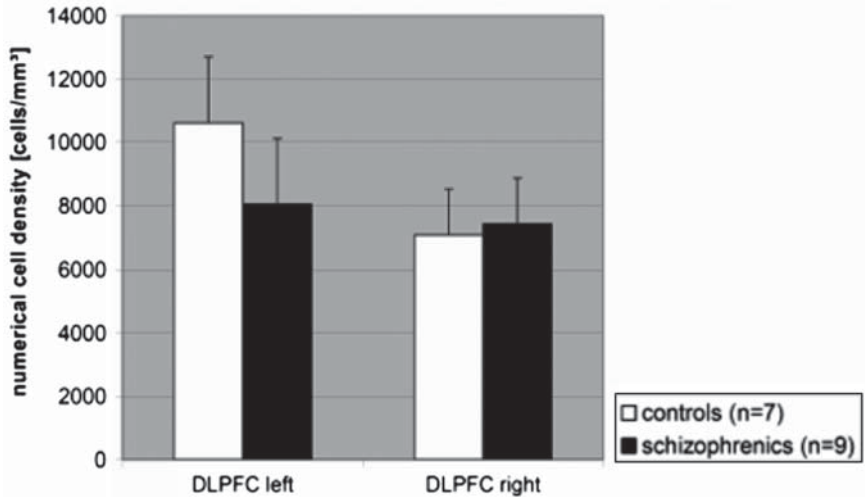

Figure 6. Numerical cell density of ADAM 12 immunopositive oligodendrocytes in the gray matter of the DLPFC; no significant differences were found.

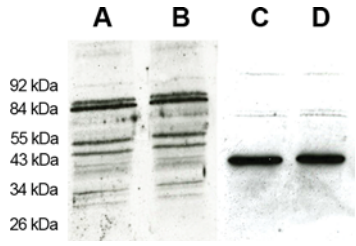

Figure 7. Western blot analysis of ADAM12 expression in lysates of in human prefrontal cortex of a control case. Lanes A and B show blots of ADAM12; lanes C and D show blots of beta-actin (reference protein). A major ADAM 12 protein band of about 84 kDa and some bands of lower molecular masses (probably cleavage products of the enzyme protein) were identified.

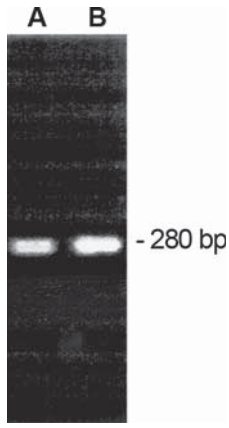

Figure 8. Detection of ADAM12 mRNA in samples of human prefrontal cortex from two control cases (lanes A and B). A single PCR product of the expected size (280 bp) could be demonstrated for both cases.

2003; Nadri et al. 2007; Jacobsen and Wewer 2009). Further, genetic linkage studies point to a role of the enzyme in late-onset Alzheimer's disease (Malinin et al. 2005; Harold et al. 2007). As indicated above (table) several putative substrates and interaction partners of ADAM12 have been shown to be altered in schizophrenia. Almost all of them interact with the membrane-bound molecular form of ADAM12, which is detected by immunohistochemistry. Amongst these potential substrates are EGF, beta-cellulin and delta-like 1 (Moon et al. 2006; Horiuchi et al. 2007; Jacobsen et al. 2008), which have been associated with schizophrenia by genetic linkage analyses (Häninnen et al. 2007; Iossifov et al. 2008). Further, EGF protein levels were found to be decreased in prefrontal cortex in schizophrenia. This reduction was not a result of haloperidol treatment (Futamura et al. 2002). Delta-like 1 (Drosophila homologue) acts through binding to Notch receptors. However, an involvement of Notch signalling in schizophrenia is controversial (Ivo et al. 2006; Wang et al. 2006). Another potential substrate of ADAM12 is TGF-beta (Atfi et al. 2007). This compound, which is involved in Wnt signalling and other signal transduction events, was found to be hyperactive in schizophrenia (Kalkman 2009). Remarkably, ADAM12 protein is even detectable in just dividing OL (as shown in our cell culture experiments). Since a role of the enzyme in cell cycle control has been established (Cao et al. 2003), it is possible that ADAM12 is also involved in the abnormal OL cell cycle in schizophrenia (probably through the TGF-beta-cyclin D1 axis; Katsel et al. 2008). Lastly, ADAM12 is known to interact with neurofascin 155. Neurofascin is involved in neurite outgrowth and migration and myelination. ADAM12 is thought to be the metalloprotease for ectodomain shedding of neurofascin, which is important for these functions (Sherman et al. 2005; Maier et al. 2006). A reduction of ADAM12 expressing OL could bring about decreased ectodomain shedding of neurofascin, which could in turn lead to a decrement of myelination as observed in schizophrenia. There is recent evidence for decreased density of neurofascin binding partner ankyrin in the cortex of patients with schizophrenia (Cruz et al. 2009). However, the aforementioned substrates are also susceptible to other metalloproteases, and the relative contribution of ADAM12-mediated cleavage of, or molecular interaction with, these substrates *in vivo* is still poorly understood (Horiuchi et al. 2007; Jacobsen et al. 2008). Finally, ADAM12 is inhibited by the tissue inhibitor of metalloproteinases 3 (TIMP-3; Loechel et al. 2000; Jacobsen et al. 2008, and others), which has genetically been linked to schizophrenia (Hung et al. 2001). In sum, it can be stated that OL-associated ADAM12 may in many ways be linked to schizophrenia, and further studies using larger cohorts and using other methods to measure the protein are clearly warranted to learn more about the potential role of the enzyme in the pathophysiology of this disease.

Acknowledgements

None.

Statement of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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The World Journal of Biological Psychiatry, 2010; 11: 567–578

ORIGINAL INVESTIGATION

Ultrastructural damage of capillaries in the neocortex in schizophrenia

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Abstract

Objectives. Neuroimaging studies showed lowered blood flow, glucose metabolic rates and hypoactivation of the prefrontal cortex (PFC) in patients with schizophrenia. The aim of the study was to clear up whether there are abnormalities in the microvasculature in the neocortex in schizophrenia. *Methods.* Capillaries were studied in PFC (BA 10) and visual cortex (VC) (BA 17) by electron microscopy and morphometry in 26 schizophrenia cases and 26 normal controls. Capillary diameter and areas of capillaries and of pericapillary astrocytic end-feet were estimated in layers I–II of the prefrontal and visual cortices. *Results.* Ultrastructural abnormalities of capillaries in schizophrenia included thickening, deformation of basal lamina, vacuolation of cytoplasm of endothelial cells, basal lamina and astrocytic end-feet, swelling of astrocytic end-feet, of pericapillary oligodendrocytes and signs of activation of microglial cells in both PFC and VC. Capillary diameter and area did not differ significantly between the groups. Area of astrocytic end-feet was significantly higher in PFC (+49%, $P<0.001$) and in VC (+29%, $P<0.01$) in schizophrenic group and in different clinical subgroups as compared to controls. *Conclusions.* Ultrastructural abnormalities of capillaries and of pericapillary cellular environment found suggest that blood–brain barrier dysfunction might contribute to the pathogenesis of cortical lesions in schizophrenia.

Key words: *Schizophrenia, neocortex, capillaries, ultrastructure, morphometry*

Introduction

Functional neuroimaging studies consistently showed that core symptoms of schizophrenia are associated with local changes of cerebral blood flow, particularly in the frontal cortex. Patients with schizophrenia were unable to increase the frontal regional blood flow while performing cognitive tests (Weinberger et al. 1986; Parellada et al. 1998; Ragland et al. 1998; Moreno-Iñiguez et al. 2005). Reduced frontal blood flow is associated with negative symptoms (Gonul et al. 2003; Wang et al. 2003; Li et al. 2005; Zhao et al. 2006). Lowered blood flow (Andreasen et al. 2008), lower glucose metabolic rates (Buchsbaum et al. 2002; Potkin et al. 2002), hypoactivation, (Semkovska et al. 2001), resting hypofrontality (Hoshi et al. 2006) were detected in the prefrontal cortex of schizophrenia patients. However, it remains unclear whether these changes are associated with abnormalities of microcirculation in the disease. The theory proposed by Hanson and Gottesman (2005) suggests that abnormalities of blood flow lead to altered neuronal-glial function that, in turn, leads to psychopathology.

Some data point out that microcircuitry might be abnormal in schizophrenia. Ultrastructural alterations of capillaries in human embryos from schizophrenia mothers (Orlovskaia and Solovyeva 1976), an atypical simplified angioarchitecture and abnormal arborisation of the brain vessels (Senitz and Winkelmann 2001), high nailfold plexus visibility (Gooding and Miller 1998; Curtis et al. 1999), reduced niacin skin flush response

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(Received 28 May 2009; accepted 7 June 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903414188

(Buretić-Tomljanović et al. 2008), reduced vasodilatory response to methylnicotinate (Ross et al. 2004) have been reported in schizophrenia patients. However, capillary length density was not changed in the prefrontal cortex in schizophrenia (Kreczmanski et al. 2005, 2009).

Blood–brain barrier (BBB) impairment has been reported in schizophrenia (Muller and Ackenheil 1995; Shcherbakova et al. 1999). BBB is formed by a complex cellular system of endothelial cells, astroglia, pericytes, perivascular macrophages, and a basal lamina (Bradbury 1979). Pericapillary oligodendrocytes and microglial cells contribute to pericapillary cell microenvironment. Astrocytes project their endfeet tightly to the endothelial cells, influencing and conserving the barrier function of these cells and being involved in regulation of local blood flow (Simard et al. 2003). Brain water homeostasis is controlled by astrocytes at the level of BBB (del Zoppo and Hallenbeck 2000). Astrocytic end-feet make close contacts with neuronal synapses and blood vessels, and they have been recognized as key intermediaries in the neurovascular response (Filosa and Blanco 2007). Neuroimaging study provides evidence for brain swelling during exacerbation of psychosis in schizophrenia (Garver et al. 2000), increased water diffusivity in the fronto-temporal regions and in the occipital cortex of schizophrenia patients (Shin et al. 2006; Andreone et al. 2007). Swelling of astrocytes in upper layers of the prefrontal cortex (Oĭfa and Uranova 1991), decreased GFAP labelling of astrocytes adjacent to blood vessels (Webster et al. 2001) and lowered number of pericapillary oligodendrocytes (Vostrikov et al. 2008) have been reported in postmortem prefrontal cortex in schizophrenia. The data suggest that these abnormalities might be associated with some abnormalities of microvasculature in the prefrontal cortex in schizophrenia.

The aim of the study was to study the ultrastructure of capillaries and of pericapillary cellular environment in upper layers of postmortem prefrontal and visual cortices in schizophrenia and normal controls.

Material and methods

Tissue samples from the brain collection of Mental Health Research Center (MHRC) (Moscow) were used in this study. All brains were obtained within short postmortem delay (about 7 h). Ethical considerations in obtaining and using human autopsy material were informed by the Ethic Committee of the MHRC, Russian Academy of Medical Sciences. Twenty-six subjects with schizophrenia and 26 normal control subjects matched by age and postmortem delay were used. Demographic data are given in Table I. Clinical records were obtained and ICD-10 diagnoses were made by psychiatrists from the MHRC. The Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS) were used by psychiatrists to rate negative and positive symptoms during the last hospitalization in schizophrenia subjects. Summary scores of negative and positive symptoms were determined on the basis of the ratio of the percentage of negative and positive scores in summary scores. Schizophrenic group included 17 cases of chronic paranoid schizophrenia and nine cases of chronic nonparanoid schizophrenia: undifferentiated schizophrenia ($n=4$), catatonic schizophrenia ($n=2$), residual ($n=2$), unspecified schizophrenia ($n=1$).

Table I. Demographic characteristics of subject groups (Mean±SD)

		Schizophrenia group ($n=26$)	
		SPPS ($n=14$)	SPNS ($n=12$)
	Control group ($n=26$)	Paranoid schizophrenia ($n=17$)	Nonparanoid schizophrenia ($n=9$)
Age (years)	52.3±14.8	53.3±16.2	
		54.7±16.6	52.3±14.8
Postmortem interval (h)	5.6±0.9	5.9±2.1	
		6.2±2.6	5.6±1.4
Gender Female/Male	5F/21M	15F, 11M	
		10F, 4M	5F, 7M
Causes of death	Cardiovascular disease, myocardial infarction, blood vessel rupture, pneumonia	Cardiovascular disease, myocardial infarction, pulmonary embolism, aspiration asphyxia	
Duration of disease (years)	–	21.3±11.6	
		20.7±9.3	22.0±14.5

SPNS, schizophrenia with predominantly negative symptoms; SPPS, schizophrenia with predominantly positive symptoms.

For electron microscopy small tissue pieces from gray matter of BA 10 (left hemisphere) obtained perpendicular cortical surface were fixed by immersion with mixture of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer for 1 week, then postfixed in 1% osmium tetroxide for 1 h, stained with uranyl acetate for 1 h, dehydrated in ethanol series and embedded in Araldit epoxy resin. Sections were cut using Reichert ultramicrotome, and semithin 1-μm sections stained with toluidine blue were used for orientation in cortical layers. Small pyramids were trimmed on layers I–II and ultrathin sections were cut, counterstained with lead citrate and viewed with the electron microscope Philips EM 420.

Quantitative data collection

Cases were coded, and the study was performed blindly. EM photographs were made from all transversally cut blood capillaries using magnification 4000×. Capillaries were identified according to criteria described by Peters (1976). Only microvessels <10 μm in diameter were included in the study as capillaries. Capillary diameter and cross-sectional area of capillaries and of pericapillary astrocytic end-feet were estimated in two upper layers of BA10 and BA17 in control and schizophrenia groups. Three tissue blocks from each cortical field per case were used.

The parameters of capillaries were estimated using transversally cut capillaries. A total of 25–30 capillaries per case in each brain structure were estimated. Cross-sectional area of astrocytic end-feet was estimated using a transparent grid (square lattice with 1.6-cm intervals). The grid was superimposed upon the electron microscopic films at a final magnification ×32,000 using the enlarger ("Minolta"), and the number of test points falling on the profiles was counted. Area of profiles was calculated using formula: $A=a/p\times P$, where a/p is the area associated with each point, P is the number of points hitting the object.

Statistical comparisons

Statistical analysis was performed using STATISTICA software package. Differences between control group, schizophrenic group and schizophrenic clinical subgroups were analyzed. Both group and subgroup differences were examined using one-way ANCOVA with diagnosis as between-subject factors, and age and postmortem delay as covariates, followed by *post hoc* test, taking the Bonferroni' correction. Influence of age, duration of disease, onset of disease and neuroleptic exposure (expressed as chlorpromazine equivalents according to Davis (1974)) were examined by Pearson correlation analysis.

Results

Ultrastructural abnormalities of capillaries

In control brains both in the prefrontal and visual cortices the capillaries looked well preserved. They were round with open lumina, endothelial cells and pericytes appeared with normal ultrastructure. The basal lamina of capillaries was rather thin. Pericapillary astrocytic end-feet looked as thin processes with electron lucent cytoplasm ensheathing capillary basal lamina (Figures 1A and 2A). Astrocytic somata were rarely seen in close apposition to capillary wall. Oligodendrocytes and microglial cells with normal ultrastructure sometimes were located in close apposition to pericapillary astrocytic end-feet (Figure 2A).

Ultrastructural alterations of capillaries in schizophrenia were detected in both the prefrontal and visual cortices, and they were similar in both brain structures. The most important structural changes were found in the basal lamina and cytoplasm of endothelial cells: thickenings, deformation and vacuolation in specific points and protrusion towards astrocytic end-foot (Figures 1B, 2B,D and 3D). Also osmiophilic membrane-like inclusions were occasionally seen within the basal lamina (Figure 2B). Cytoplasm of some endothelial cells was vacuolated (Figure 1B). The intercellular tight junctions between capillary endothelial cells appeared to remain intact. Pericytes showed an increase in the number of lipofuscin granules and lysosomes in the cytoplasm (Figures 1C and 2C). Some pericytes contained dark nucleus with chromatin clumping (Figure 2C). Extracellular spaces looked intact. Astroglial foot processes expressed extensive vacuolization, and membranous debris was often seen inside the processes (Figures 1B,C and 3C). Some pericapillary oligodendrocytes looked well preserved, other were swollen and vacuolated and contained dark nucleus with chromatin clumping (Figure 3B). Few pericapillary oligodendrocytes demonstrated shrunken cytoplasm, chromatin clumping and osmiophilic dispersed material in euchromatin (Figure 3C). Microglial cells were located in close apposition to swollen and vacuolated astrocytic end-feet, had enlarged cytoplasm, irregular contours of nucleus, were vacuolated and contained lysosomes in the cytoplasm (Figure 3D) in contrast to controls where microglial cells were rarely vacuolated and contained lysosomes in cytoplasm.

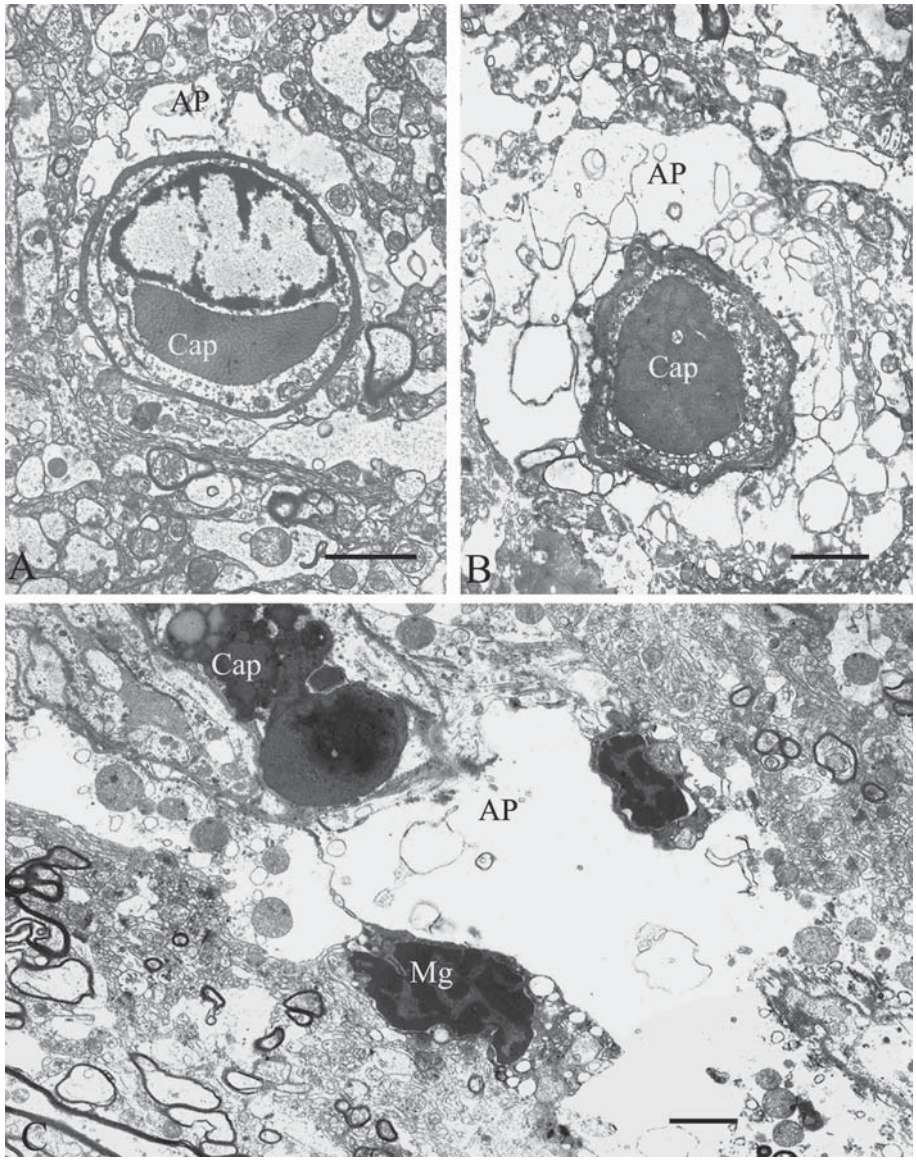

Figure 1. Electron micrographs of capillaries in the prefrontal cortex from control brain (A) and schizophrenic brain (B,C). Note thickening and deformation of basal lamina, swelling of peicapillary astrocytic end-feet and vacuolation of cytoplasm of endothelial cell, basal lamina and astrocytic foot process in schizophrenia (2B), dark inclusions of lipofuscin-like material and lysosomes in pericyte, and microglial cells apposed to astrocytic end-foot process (2C). Cap, capillary; AP, astrocytic process; Mg, microglial cell. Scale bars: 1 μm.

Morphometry of capillaries

Capillary diameter and area did not differ between the groups in either prefrontal or visual cortices (see Tables II and III). One-way ANCOVA with diagnosis as between subject factor and age and postmortem delay as covariates revealed that area of astrocytic end-feet was significantly higher in the prefrontal cortex: $F(1,50)=71.6$, $P<0.001$ and in the visual cortex: $F(1,50)=11.0$, $P<0.01$ in schizophrenic group vs. control group (see Tables II and III, Figure 4). *Post hoc* test showed that schizophrenic group had significantly higher area of astrocytic end-feet than the control group both in the prefrontal cortex (+49%, $P<0.001$) and in the visual cortex (+29%, $P<0.01$). One-way ANCOVA with two diagnostic subgroups (with predominantly positive and predominantly negative symptoms) and control group with diagnosis as between subject factor and age and postmortem delay as covariates revealed the effect of diagnosis on the parameter in the prefrontal cortex: $F(2,49)=33.1$, $P<0.001$ and in the visual cortex: $F(2,49)=5.4$, $P<0.01$ (Tables II and III). The subgroup of cases with predominantly negative symptoms demonstrated more pronounced changes in the prefrontal cortex than in visual cortex in schizophrenia Area of pericapillary astrocytic end-feet was

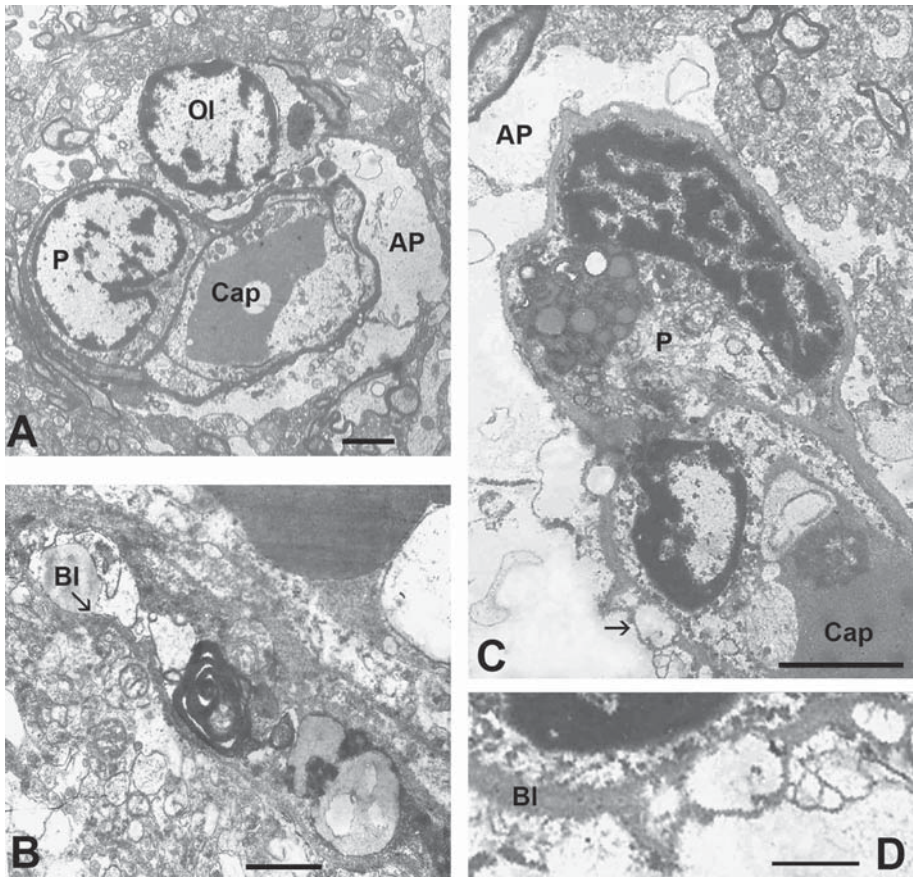

Figure 2. Ultrastructure of pericytes and of basal lamina in capillaries in the visual cortex from control brain (2A) and from schizophrenic brain (2C–D). Accumulation of lysosomes in basal lamina (2B) and in the cytoplasm of pericyte (2C); prominent vacuolation of basal lamina (2C, arrow, 2D) and membranous inclusion inside basal lamina (2B) in schizophrenic brain. Cap, capillary; Bl, basal lamina; P, pericyte; AP, astrocytic foot-process; Ol, pericapillary oligodendrocytes. Scale bars: A, μm; B, μm; C, μm; D, μm.

significantly higher in the subgroup of cases with predominantly negative symptoms as compared to the control group only in the prefrontal cortex (+48%, $P<0.001$) and nonsignificantly in the visual cortex (+23%). Mean values of the parameters in the subgroup of cases with predominantly positive symptoms was significantly higher in prefrontal cortex (+50%, $P<0.001$) and in the visual cortex (+35%, $P<0.01$) than in the control group (see Tables II and III, Figure 4).

ANCOVA with the control group and two schizophrenic subgroups (paranoid and nonparanoid) and age and postmortem delay as covariates showed significant effect of diagnosis on area of astrocytic end-feet both in the prefrontal cortex: $F(2,47)=37.6$, $P<0.001$ and in the visual cortex: $F(2,49)=6.5$, $P<0.01$ (see Tables II and III, Figure 5). *Post hoc* demonstrated that in the prefrontal cortex both subgroups differed significantly from controls ($P<0.001$) (see Tables II and III, Figure 5) but in the visual cortex only the subgroup of paranoid schizophrenia differed significantly from the control group ($P<0.01$) (Figure 5). When the subgroup of paranoid schizophrenia was separated into the subgroups of continuous and episodic course of the disease, ANCOVA confirmed a significant effect of the diagnosis on this parameter. *Post hoc* showed that both subgroups differed significantly from the control group in the prefrontal cortex and only the subgroup of continuous course of schizophrenia differed significantly from controls in the visual cortex (see Tables II and III, Figure 5). The highest value of area of astrocytic end-feet was detected in the prefrontal cortex in episodic course of schizophrenia. There were no significant differences between schizophrenic subgroups studied.

No gender effects on the parameters measured were found. There were no correlations between the parameters of capillaries measured and duration of schizophrenia, onset of the disease or chlorpromazine equivalents.

Discussion

The present study provides evidence for microvascular abnormalities in the neocortex in schizophrenia.

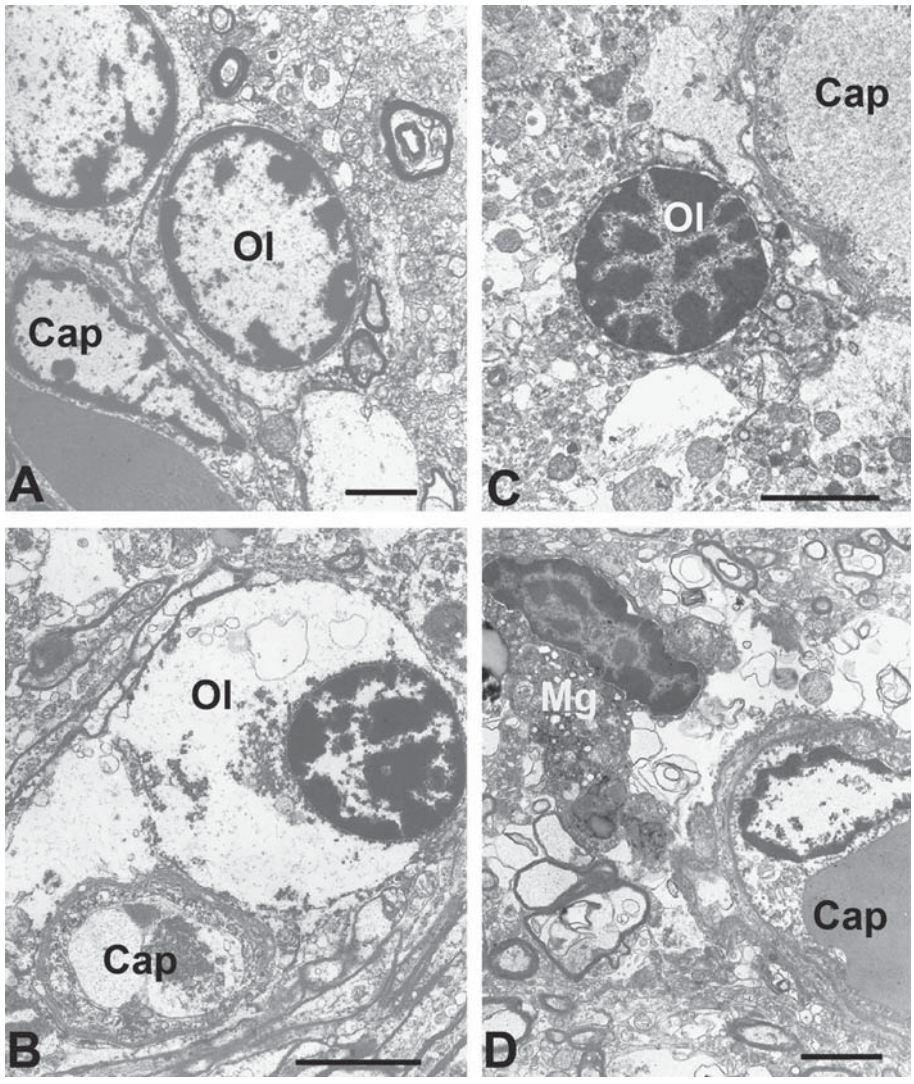

Figure 3. Electron micrographs of pericapillary oligodendrocytes and of microglial cell in the prefrontal cortex from control brain (A) and from schizophrenic brain (B–D). (A) oligodendrocyte has normal ultrastructure. (B) Swollen and vacuolated oligodendrocyte containing dark nucleus with chromatin clumping. (C) Oligodendrocyte with cytoplasm shrinkage, chromatin clumping and osmiophilic euchromatin. (D) Microglial cell apposed to swollen vacuolated astrocytic end-foot. Cap, capillary; Ol, oligodendrocyte; Mg, microglial cell. Scale bars: μm.

Qualitative study revealed ultrastructural alterations of capillaries in both brain structures in schizophrenia as compared to normal controls. Abnormalities of capillaries and of their microenvironment in schizophrenic brain included thickening, deformation, vacuolation of basal lamina, prominent swelling and vacuolation of astrocytic end-feet, alterations of pericapillary oligodendrocytes and signs of activation of microglial cells, such as irregular contours of nucleus, enlarged, vacuolated cytoplasm and increased number of lipofuscin-like granules. The results are consistent with previously reported dystrophic alterations and swelling of astrocytes in upper layers of the prefrontal cortex (Oĭfa and Uranova 1991), decreased GFAP labelling of astrocytes adjacent to blood vessels in the prefrontal cortex (Webster et al. 2001), dystrophic and degenerative alterations of pericapillary oligodendrocytes in our previous studies of layers V–VI of the prefrontal BA10 (Uranova et al. 2001; Vostrikov et al. 2008) in schizophrenia. Though Kreczmanski et al. (2005, 2009) found no changes in capillary length density in the prefrontal cortex in schizophrenia, the authors proposed that the cerebral microvasculature in schizophrenic brain might be altered at a molecular, rather than structural level. The present study demonstrated that the cerebral microvasculature dysfunction in schizophrenia might be at the ultrastructural level.

Morphometric study demonstrated significant swelling of capillary astrocytic end-feet and significant effect of diagnosis on this parameter in both the prefrontal and visual cortices. When schizophrenic

Table II. Ultrastructural parameters of capillaries in the prefrontal cortex in the control group, schizophrenic group and in schizophrenic subgroups (mean±SEM)

Parameters	BA 10 Control group (n=26)	BA 10 Schizophrenic group (n=26)	BA 10 SPNS (n=12)	BA 10 SPPS (n=14)	BA10 Nonparanoid schizophrenia (n=9)	BA10 Paranoid schizophrenia (n=17)	BA10 Continuous paranoid schizophrenia (n=11)	BA10 Episodic paranoid schizophrenia (n=6)
Capillary diameter (μm)	4.9±0.06	4.8±0.06	4.9±0.06	4.8±0.06	4.9±0.09	4.8±0.05	4.9±0.06	4.8±0.06
Capillary cross-sectional area (μm^2)	18.8±0.4	18.7±0.3	18.8±0.5	18.6±0.4	19.06±0.6	18.5±0.3	18.9±0.4	17.8±0.5
Cross-sectional area of astro-cytic end-feet (μm^2)	13.5±0.5	20.2±0.6***	20.04±1.2***	20.23±0.7***	19.1±0.9***	20.7±0.8***	19.8±0.9***	22.3±1.5***

**P<0.01; ***P<0.001, differences from the control group.
SPNS, schizophrenia with predominantly negative symptoms; SPPS, schizophrenia with predominantly positive symptoms

group was subdivided into different subgroups based on predominance of negative or positive symptoms or paranoid and nonparanoid types of schizophrenia or different course of paranoid schizophrenia, each of these subgroups differed significantly from the control group in the prefrontal cortex. There were no significant differences between the subgroups. Taken together, the data suggest that swelling of astrocytic end-feet is a common feature of cortical capillary abnormalities in different schizophrenic subtypes. However, the most prominent swelling of astrocytic end-feet was detected in episodic paranoid schizophrenia. These data are in line with the *in vivo* data of brain swelling during exacerbation of psychosis (Garver et al. 2000) and higher water diffusion coefficient in frontal, temporal and occipital lobes (Shin et al. 2006; Andreone et al. 2007) reported in schizophrenia, since astrocytes are involved in the permeability of BBB and regulation of brain water homeostasis (del Zoppo and Hallenbeck 2000), and alterations of BBB in schizophrenia have been repeatedly reported (Muller and Ackenheil 1995; Shcherbakova et al. 1999).

Our results have some similarities with ultrastructural changes of capillaries in aging brain, such as thickenings of basal lamina, accumulation of dark osmiophilic inclusions in pericytes (Peters et al. 1991; Alba et al. 2004). Interestingly, thickenings of the basal membrane, protrusions towards the astrocytic end-foot; membrane-like inclusions within the basal lamina and pericytes have been reported in senescence-accelerated mice with age-related deficits in learning and memory and damaged BBB (Ueno et al. 2001). However, extensive swelling and vacuolization of astrocytic end-feet detected in schizophrenia and vacuolization of basal lamina revealed in our study are not characteristic feature of capillary changes during aging and they might be associated with the disease.

Though the present study does not provide direct evidence that BBB is damaged in schizophrenia as compared to controls, prominent vacuolation and thickenings of basal lamina, vacuolation of endothelial cells, swelling of astrocytic end-feet detected in schizophrenia as compared to controls suggest that BBB might be altered in cortical brain structures in schizophrenia. Endothelial cells are known to be the principal barrier cells controlling cerebral microvascular permeability, however, abnormalities of pericytes, astroglial cells found might play important roles in the regulation of brain microvascular function. Prominent swelling of astrocytic end-feet found in the neocortex in the present study was not accompanied by changes in volume of extracellular space, and they resemble those found in cerebral ischemia. In the ischemic oedema, the initial cytotoxic

Table III. Ultrastructural parameters of capillaries in the visual cortex in the control group, schizophrenic group and in schizophrenic subgroups (mean±SEM)

Parameters	BA 17 Control group (n=26)	BA 17 Schizophrenic group (n=26)	BA 17 SPNS (n=12)	BA 17 SPPS (n=14)	BA17 Nonparanoid schizophrenia (n=9)	BA17 Paranoid schizophrenia (n=17)	BA17 Continuous paranoid schizophrenia (n=11)	BA17 Episodic paranoid schizophrenia (n=6)
Capillary diameter (μm)	4.2±0.06	4.4±0.05	4.3±0.06	4.4±0.08	4.3±0.08	4.4±0.06	4.3±0.08	4.4±0.12
Capillary cross-sectional area (μm^2)	18.7±0.4	14.9±0.3	14.9±0.4	14.9±0.5	14.7±0.5	15.04±0.4	14.8±0.5	15.4±0.8
Cross-sectional area of astro-cytic end-feet (μm^2)	11.8±0.6	15.4±0.8**	14.6±1.1	16.01±1.2**	13.4±1.5	16.4±0.9**	17.01±1.3**	15.2±0.7

**P<0.01; ***P<0.001, differences from the control group.
SPNS, schizophrenia with predominantly negative symptoms; SPPS, schizophrenia with predominantly positive symptoms.

intracellular accumulation of water is related to an acute tissue deprivation of glucose and oxygen, and the basic event here is a disturbance of cellular osmoregulation, originating from brain metabolic disturbances. The cell membranes become permeable to previously non-penetrating ions such as sodium, solutes like lactate accumulate and cellular swelling occurs (Mchedlishvili et al. 1986). Similar mechanisms might occur in schizophrenic brain.

On the other hand, the ultrastructural damage of capillaries and pericapillary microenvironment detected in the present study might be due to chronic hypoperfusion and BBB damage, since experimental chronic cerebral hypoperfusion leads to astrocytic foot process vacuolation and BBB disruption (Ueno et al. 2002; Wu et al. 2006), white matter injury and microglial activation (Farkas et al. 2006), and hypoperfusion is consistently reported in the prefrontal cortex in schizophrenia (Vita et al. 1995; Suzuki et al. 2005). Ultrastructural capillary abnormalities and BBB alterations might be induced by oxidative stress (Haorah et al. 2007; Kamada et al. 2007; Theoharides et al. 2007) playing an important role in the pathogenesis of schizophrenia (Prabakaran et al. 2004), by proinflammatory substances (Persidsky et al. 2006). Circulating TNF-alpha may involve the astrocytic end-feet: it induced capillary ultrastructural damage and breakdown of the BBB through the release of nitric oxide in the rat brain (Farkas et al. 2006). Acute exacerbation of schizophrenia is associated with increased TNF-alpha plasma concentrations (O'Brien et al. 2008), higher serum level and increased mRNA expression for TNF-alpha (Song et al. 2009) and increased levels of TNF mRNA in the prefrontal cortex (Paterson et al. 2006) have been reported in schizophrenia subjects. Also, alteration of BBB integrity by retroviral infection has been reported (Afonso et al. 2008), and retroviruses may contribute to the pathogenesis of schizophrenia (Yolken et al. 2000; Karlsson et al. 2001).

Swelling of pericapillary oligodendrocytes found in the present study and ultrastructural dystrophic and degenerative alterations of pericapillary oligodendrocytes detected in our previous study in the prefrontal BA10 in schizophrenia (Vostrikov et al. 2008) suggest that these cells might be involved in alteration of BBB in schizophrenia brain. Pericapillary oligodendrocytes contain transporters for lipids and glutamate (Navarro et al. 2004), they also provide regulation of iron movement from the plasma to the brain. Increased content of lipid transporters (Dean et al. 2003), increased expression of the astrocytic glutamate transporter GLT-1 in the prefrontal cortex of schizophrenics (Matute et al. 2005), elevated levels of kynurenic acid in the CSF (Erhardt et al. 2001) and in the prefrontal cortex (Schwarcz

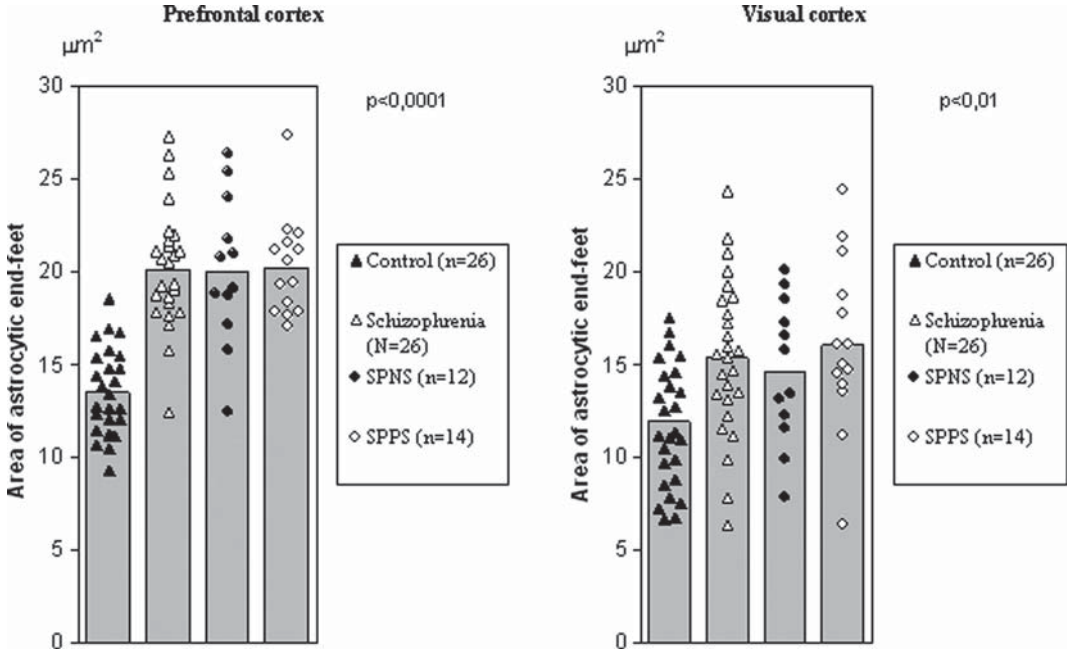

Figure 4. Area of astrocytic end-feet in the prefrontal cortex and visual cortices in control group, schizophrenic group and in subgroups of schizophrenics with predominantly negative symptoms (SPNS) and with predominantly positive symptoms (SPPS) (individual values and mean of means; *P* values between schizophrenic and control groups).

et al. 2001) have been reported in schizophrenia. Immunoreactivity of kynurenine aminotransferase, the biosynthetic enzyme for kynurenic acid, was found in astrocytic processes encircled capillaries and surrounded axospinous synapses (Roberts et al. 1992).

The damaged BBB might induce the increase of water diffusivity, the penetration of infectious agents, immunoglobulins, cytokines, autoantibodies and other serum macromolecules into the brain parenchyma. As a consequence of these events, damage of cellular membranes, and of myelin membranes in particular, might occur followed by activation of phagocytosis by microglia. Damage of myelin membranes and swelling of periaxonal oligodendrocyte processes have been reported in postmortem studies of the prefrontal cortex in schizophrenia (Uranova et al. 2001, 2007). *In vivo* data provide some evidence for myelin swelling during exacerbation of psychosis (Garver et al. 2000). MRS studies of patients with chronic schizophrenia as well as at first episode prior to treatment showed alterations in neuronal membrane biochemistry, including the prefrontal cortex, supportive of the membrane hypothesis of schizophrenia (Reddy and Keshavan 2003). The results of both metabolic and proteomic studies pointed to energy metabolism and lipid biosynthesis being impaired in schizophrenia (Buretć-Tomljanović et al. 2008), as well as alterations of free fatty acids, phosphatidylcholines, and ceramides (Schwarz et al. 2008). Taken together, the data suggest that capillary damage and altered BBB might be involved in the pathogenesis of schizophrenia. However, the present study did not answer the question of whether cytological parameters of microglial cells and numerical density of pericapillary microglial cells are changed in the brain in schizophrenia. Further morphometric studies are needed to test the hypothesis of a genetic–inflammatory–vascular theory of the pathogenesis of schizophrenia (Hanson and Gottesman 2005).

The origin of capillary damage in schizophrenia is unknown. No effects of duration of the disease, age at onset of the illness or of medication were found in the present study. study. *In vivo* studies demonstrated that cerebral blood flow abnormalities in patients with chronic schizophrenia are not due to chronicity of illness or the effects of medication. Lower flow in prefrontal regions is present at the early stage of schizophrenia illness, suggesting that a similar neural dysfunction occurs in both first-episode and chronic schizophrenia (Andreasen et al. 1997; Schultz et al. 2002). Since some capillary abnormalities have been reported in schizophrenia patients, such as ultrastructural alterations of capillaries in human embryos from schizophrenia mothers (Orlovskaya and Solovyeva 1976) an atypical simplified angioarchitecture and abnormal arborisation of the brain vessels (Senitz and Winkelmann 2001), reduced vasodilatory response to methylnicotinate (Ross et al. 2004), we believe that capillary abnormalities might occur during

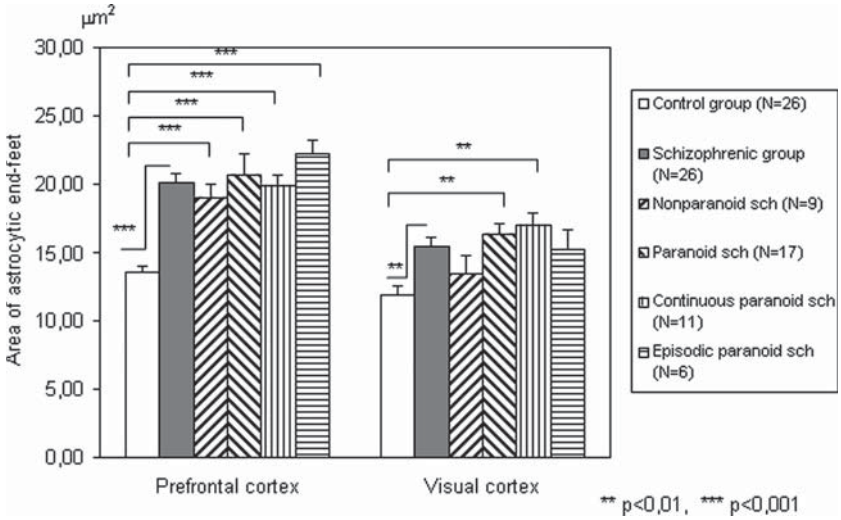

Figure 5. Area of astrocytic end-feet in the prefrontal cortex and in the visual cortex in control group, schizophrenic group and in the subgroups of nonparanoid schizophrenia, paranoid schizophrenia, continuous paranoid schizophrenia and episodic paranoid schizophrenia.

brain development and continue in the course of illness in schizophrenia patients.

The results of the present study suggest that ultrastructural alterations of capillaries described might be crucial in pathophysiology of schizophrenia. Both capillary damage and swelling of astrocytic end-feet in the prefrontal cortex in schizophrenia might contribute to reduced frontal blood flow during task performance and to incapacity to increase blood flow velocity during cognitive activation as control subjects do (Moreno-Iñiguez et al. 2005; Weinberger et al. 1986), hypoperfusion and hypofrontality (Andreasen et al. 1997; Li et al. 2005; Suzuki et al. 2005; Vita et al. 1995), altered metabolic rates (Buchsbaum et al. 2002) reported in the prefrontal cortex in schizophrenia. However, capillary abnormalities described in the prefrontal cortex in schizophrenia might only contribute to the alterations of blood flow and metabolism in the prefrontal cortex and appear to be not a causal for these alterations, since blood flow and metabolic abnormalities were found mainly in the frontal cortex, and we found ultrastructural abnormalities in both the prefrontal and visual cortices in schizophrenia.

We found more prominent swelling of pericapillary astrocytic end-feet both in prefrontal cortex than in the visual cortex in schizophrenia where only the subgroup of cases with predominantly positive symptoms and with continuous paranoid schizophrenia differed significantly from the control group. The data are in line with the notion that prefrontal cortex is more severely affected in schizophrenia than visual cortex. However, the deficits in early visual processing (Butler and Juvitt 2005), reduced modulation of visual cortex by salient stimuli (Taylor et al. 2005), a reduction in total neuron number and volume of BA17 have been reported in the schizophrenia relative to the normal subjects (Dorph-Petersen et al. 2007). These data suggest that ultrastructural abnormalities of capillaries found in the visual cortex in the present study might contribute to these functional abnormalities in patients with schizophrenia.

Capillary abnormalities found in the present study appear to be nonspecific for schizophrenia. BBB breakdown or alterations in transport systems play an important role in the pathogenesis of HIV-1 encephalitis, Alzheimer's disease, ischemia, tumours, multiple sclerosis, and Parkinson's disease (Persidsky et al. 2006). Since blood capillary distribution correlates with hemodynamic-based functional imaging in cerebral cortex (Harrison et al. 2002), the development of *in vivo* high-resolution imaging methods combined with functional MRI and vascular and BBB genomics technologies of human brain (Pardridge 2007; Shusta et al. 2005) will increase our knowledge of the role of vascular abnormalities in pathophysiology of schizophrenia and other mental disorders.

Acknowledgments

The present study was supported by Russian Academy of Medical Sciences. We also thank N. Matiatova and A. Teodorovich for expert technical assistance.

Statement of interest

None to declare.

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The World Journal of Biological Psychiatry, 2010; 11: 579–585

ORIGINAL INVESTIGATION

Gender differences in antidepressant use-related seasonality change in suicide mortality in Hungary, 1998–2006

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Abstract

Objectives. Studies show that the seasonality of suicide (spring/early summer peak, winter low) is mainly the consequence of the seasonal incidence of depression-related suicides. The aim of the present study was to analyse the relationship between increasing antidepressant utilization and national suicide rate of Hungary between 1998 and 2006, with particular regard to seasonal patterns and gender differences. *Methods*. Time trend analysis (ARIMA) had been applied to investigate the correlation between the trend of antidepressant prescription and both of suicide rates and seasonality index. *Results*. During the 9 years of the study period there was a significant ($P < 0.001$) correlation between the steadily increasing antidepressant prescription (113%) and continuous decline in total national suicide rate (23%) as well as both in females and males (21 and 23%, respectively), but this relationship was 8-fold stronger in males. Increasing antidepressant utilization was associated with significantly decreased seasonality of suicides only among males. *Conclusions*. The results suggest that decreasing seasonality of suicides could be a good marker of lowering rate of depression-related suicides in the population particularly among males.

Key words: *Antidepressants, gender, suicide, suicide rates, seasonality*

Introduction

A statistically significant correlation between increasing antidepressant (AD) utilization and decreasing national suicide rates have been reported recently from several countries (Ludwig and Marcotte 2005), including Sweden, Denmark, Finland, Norway (Isacsson 2000; Sondergard et al. 2006; Bramness et al. 2007), the United States (Grunebaum et al. 2004) Japan (Nakagawa et al. 2007) and from Hungary (Rihmer 2004; Berecz et al. 2005; Kalmar et al. 2008). Although ecological association does not mean causality considering that (1) there is a strong relationship between untreated major depression and suicide (Moller 2006; Rihmer 2007); (2) the appropriate acute and long-term treatment of patients with major depressive and bipolar disorders markedly reduces the suicide mortality even in this high-risk patient population (Baldessarini et al. 2006; Moller 2006; Rihmer 2007) and initially suicidal depressives become nonsuicidal with antidepressant treatment (Tondo et al. 2008); and (3) the annual prevalence of major depressive episode in the population is around 6–8% (Szadoczky et al. 1998; Rihmer and Angst 2005), it is logical to assume that more widespread treatment of depression is *one of the* mean causes of declining suicide rates in countries where AD utilization recently raised markedly.

However, the raise in antidepressant usage, as a reflexion of AD prescriptions, is only a proxy marker of greater access of patients to appropriate care and

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(Received 21 May 2009; accepted 1 October 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903397722

higher population density of doctors in general (Rihmer et al. 1993; Tondo et al. 2006) and psychiatrists and psychotherapists in particular (Rihmer 2004; Tondo et al. 2006; Kapusta et al. 2009; Pirkola et al. 2009) are negatively associated with national and regional suicide rates. It is also very likely that a fraction of patients receiving ADs also receive a prescription oflithium and other mood stabilizers as well as they receive more frequently supportive psychotherapy. Therefore, the decrease of suicide rates could reflect a general improvement in mental health care rather than being caused by increasing AD sales alone.

On the other hand, however, as national suicide rates are affected by many known (unemployment, divorce rate, alcohol consumption, living standard, etc.) and unknown factors (Gunnell et al. 2003; Preti 2002) the isolation ofthe result ofbetter treatment of depression in declining suicide rates is not easy. Studies show that the seasonality of suicide (spring/early summer peak, winter low) is very likely the consequence of the seasonal incidence of depression-related suicides (Eastwood and Peacocke 1976; Morken et al. 2002; Kim et al. 2004; Postolache et al. in press; Reutfors et al. in press). The peak of suicide mortality relates to the rapid increase of environmental light in spring and the number of suicides, particularly violent suicides, increase parallel to the duration of daily sunshine (Rocchi et al. 2007). The seasonal variation of suicide also well corresponds to the annual fluctuation of some indices of central serotonergic metabolism, including brain serotonin transporter binding capacity, indicating significantly lower brain serotonergic activity in spring and summer (Praschak-Rieder et al. 2008) and it is well demonstrated that low central serotonin metabolism is a strong biological correlate of several pathological behaviours, like depression, impulsivity and violent suicidal acts (Lindstrom et al. 2004; Wasserman et al. 2007; Praschak-Rieder et al. 2008). Based on the early findings suggesting that sea-sonality of suicides might be the consequence of the seasonal incidence of depression-related suicides (Eastwood and Peacocke 1976), previously we found that decreasing seasonality of suicides might be a good marker of lowering rate of depressive suicides in the population (Rihmer et al. 1998). However, studies examining very long-term (125 years) time series of suicides found that the decrease in the seasonality of suicides is an ongoing process that started around the end of the 19th century (Ajdacic-Gross et al. 2005) indicating that seasonality of suicides (at least for long-term perspective) are also affected by changing living conditions (lighting, heating, air conditioning, etc.) and by changing meteorological environment (Preti 2002). Moreover, a recent Italian study found that independently of better treatment possibilities of depression the seasonality of suicides was positively related to the global national suicide rates, with decreasing amplitude associated with lower suicide rates and vice versa (Rocchi et al. 2007).

The aim of the present study was to replicate our preliminary findings on the significant relationship between increasing antidepressant utilization and declining seasonality of suicides (Rihmer et al. 1998) on a large sample of suicide victims. Therefore we have analysed the relationship between increasing AD prescription and national suicide rates in Hungary between 1998 and 2006, with particular regards to gender differences and seasonal patterns.

Methods

Using the Anatomical Therapeutic Chemical (ATC) Classification System (WHO Collaborating Centre for Drug Statistics Methodology) AD prescription data for the period of 1998 to 2006 were obtained from the National Health Insurance Fund. The non-selective monoamine reuptake inhibitors (ATC-N06AA), selective monoamine reuptake inhibitors (ATC-N06AB), MAO inhibitor (ATC-N06AG), and other antidepressants (ATC-N06AX) had been investigated. The database contained the amount of AD prescribed by gender, age groups (0–19; 20–39; 30–59; 60+ years of age), and ATC codes for every studied year. These prescription data had been converted into Defined Daily Dose (DDD), which is the assumed average maintenance daily dose for a given drug. The advantage of the DDD is that it provides a fixed unit of measurement enabling the researcher to assess trends in drug prescription and to perform comparisons between population groups. Finally, the age, gender and year specific AD prescription had been worked out as DDDs per 1000 inhabitants per day as summary measure.

Source of population and mortality data was the Hungarian Central Statistical Office. The suicide rates had been calculated for the same subgroups of sex and age as the antidepressant prescription, and the age and gender standardized suicide rates had been computed as well. The population of Hungary at the beginning of 1998 was applied in standardization. These measures had been expressed as suicides/100,000 inhabitants/year (suicide rate).

Utilizing the monthly number of suicides, the within year variation coefficient (within year standard deviation over within year average of daily number of cases in a month) had been calculated as seasonality index of suicide. The actual numbers of days in a month were applied to avoid the bias from variable length of the months, considering the years of 2000 and 2004 as leap years.

Time trend analysis using the Auto-Regressive Integrated Moving Average (ARIMA) regression method (Box et al. 1994) had been applied to investigate the association between the trend of drug prescription and both of suicide rates and seasonality index. Because our intention was to describe the independent influence of drug consumption's change by handling the autoregression problem (e.g., the observed outcome parameters are not independent of their past values), 1 year lag has been applied in the computation. The statistical analysis had been carried out by SPSS 11.0 for Windows software. The results have been considered statistically significant in case of $P < 0.05$.

Results

There were 26,290 cases of completed suicides (20,096 males and 6194 females) in Hungary between 1 January 1998 and 31 December 2006. Tables I and II show the main results. During the nine years of the study period (1998–2006) there was a highly significant ($P < 0.001$) correlation between the steadily increasing AD prescription and continuous decline in suicide rate of the whole country as well as both in females and males. However, this relationship was about 8-fold stronger in males (ARIMA coefficient: males –1.660, females –0.215, Table I). Analysing the relationship between increasing AD utilization and season-ality of suicides we found a significant relationship only for males: increasing AD prescription was associated with significantly decreased seasonal index (Table II). Figure 1 shows what is really beyond the figures presented in Tables I and II. Suicide mortality shows more pronounced seasonal pattern among males and the spring/early summer peak, present in both genders at the late 1990s, gradually vanishes and disappears around 2005/ 2006 but this decreasing tendency is significant only among males.

Discussion

During the nine years studied, the 113% increase in the utilization of ADs in Hungary was paralleled by

Table I. Gender specific association between antidepressant prescription and suicide rates in Hungary between 1998 and 2006 by ARIMA (Auto-Regressive Integrated Moving Average) regression method.

	Male		Female		Both genders	
Year	AD consumption*	suicide rate**	AD consumption*	Suicide rate**	AD consumption*	Suicide rate**
Observed data by years:						
1998	6.32	50.05	16.22	14.40	11.50	31.41
1999	7.91	51.69	20.53	14.45	14.50	32.25
2000	7.30	50.16	19.02	15.02	13.42	31.81
2001	8.72	46.61	22.48	13.02	15.91	29.06
2002	9.92	45.56	25.14	12.27	17.88	28.15
2003	11.56	44.58	28.92	12.04	20.64	27.56
2004	11.24	43.14	28.18	12.35	20.10	27.04
2005	12.84	41.97	31.97	11.19	22.84	25.87
2006	13.72	38.56	34.37	11.32	24.52	24.32
Relative observations by years***:						
1998	1	1	1	1	1	1
1999	1.25	1.03	1.27	1.00	1.26	1.03
2000	1.16	1.00	1.17	1.04	1.17	1.01
2001	1.38	0.93	1.39	0.90	1.38	0.93
2002	1.57	0.91	1.55	0.85	1.56	0.90
2003	1.83	0.89	1.78	0.84	1.79	0.88
2004	1.78	0.86	1.74	0.86	1.75	0.86
2005	2.03	0.84	1.97	0.78	1.99	0.82
2006	2.17	0.77	2.12	0.79	2.13	0.77
ARIMA coefficient *(P)*	−1.660	(<0.001)	−0.215	(<0.001)	−0.623	(<0.001)
AR1**** *(P)*	−0.568	(0.131)	0.016	(0.972)	−0.405	(0.297)
r^2	0.462		0.438		0.462	

All the investigated variables have normal distribution (Kolmogorov-Smirnov test: P >0.05).

*Antidepressants (non-selective monoamine reuptake inhibitors, selective monoamine reuptake inhibitors, MAO inhibitor, other antidepressants) consumption by year (DDD/1000 persons/day).

**Suicide rate (/100,000).

***1998 as reference year.

**** Autoregressive, 1 year lae.

Table II. Gender specifiic association between antidepressant prescription and seasonality index of suicides in Hungary between 1998 and 2006 by ARIMA (Auto-Regressive Integrated Moving Average) regression method.

	Male		Female		Both sexes	
Year	AD consumption*	Seasonality index**	AD consumption*	Seasonality index**	AD consumption*	Seasonality index**
1998	6.32	0.160	16.22	0.188	11.50	0.148
1999	7.91	0.161	20.53	0.185	14.50	0.150
2000	7.30	0.165	19.02	0.161	13.42	0.156
2001	8.72	0.181	22.48	0.225	15.91	0.183
2002	9.92	0.116	25.14	0.145	17.88	0.114
2003	11.56	0.180	28.92	0.190	20.64	0.172
2004	11.24	0.110	28.18	0.169	20.10	0.112
2005	12.84	0.152	31.97	0.195	22.84	0.154
2006	13.72	0.135	34.37	0.169	24.52	0.132
ARIMA coefficient (*P*)	−0.005	(0.017)	0.001	(0.559)	−0.002	(0.086)
AR1*** (*P*)	−0.772	(0.012)	−0.742	(0.021)	−0.743	(0.019)
r^2	0.347		0.303		0.309	

All the investigated variables have normal distribution. (Kolmogorov-Smirnov test: $P>0.05$).
*Antidepressants (non-selective monoamine reuptake inhibitors, selective monoamine reuptake inhibitors, MAO inhibitor, other antidepressants) consumption by year (DDD/1000 persons/day).
**Seasonality index within year standard deviation over within year average of daily number of cases in a month.
***Autoregressive, 1 year lag.

23% decline in the total national suicide rate. A recent ecological analysis of trends in suicides and in the use of ADs in 27 countries showed that 13% increase in the use of ADs was on average associated with a decrease of 2.5% in the suicide rates (Ludwig and Marcotte 2005). This implies that the 113% increase in the use of ADs in Hungary during our study period (1998–2006) would be paralleled by a 21.7% decrease in suicide mortality, which is almost the same as the observed 23% decrease in our present study.

The significant role of more widespread use of ADs in reducing suicide rates has been supported by the findings from the USA and The Netherlands (Gibbons et al. 2007) as well as from Canada (Katz et al. 2008) showing that a recent marked decline in use of ADs in children and adolescents has been

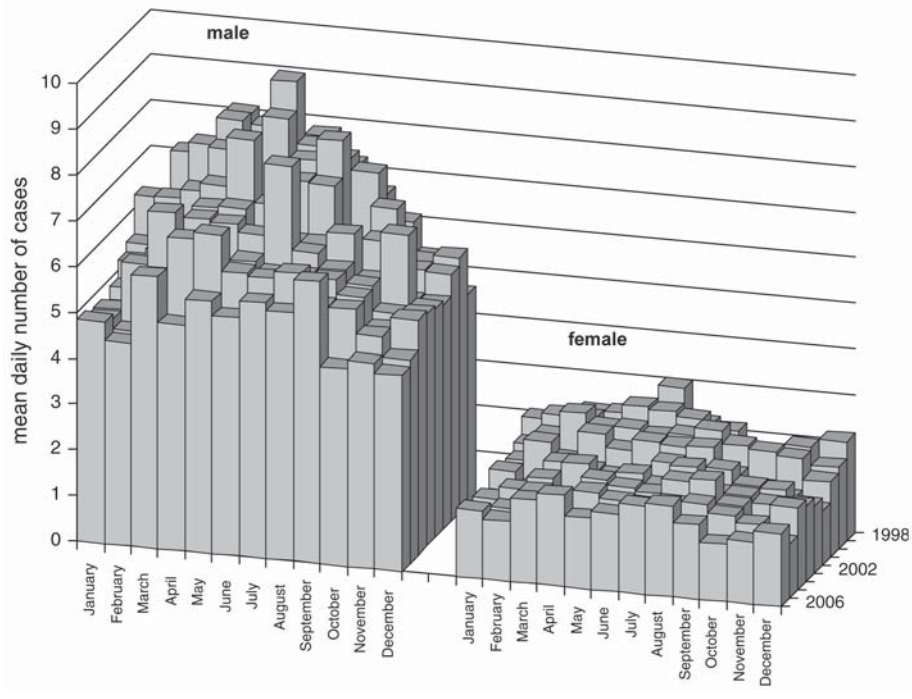

Figure 1. Seasonal distribution of suicide mortality (mean daily numbers) by year and gender.

accompanied by a sharp increase of suicide mortality in that age-groups while suicide rates further decreased in older people where the utilization of ADs further increased or declined only slightly. Studying all the suicides, natural deaths and accidental deaths, which had been investigated by forensic toxicological survey between 1995 and 2005 and looking at the blood levels of all ADs and some control medications (including tramadole), Isacsson et al. (2009) clearly demonstrated that the increased use of ADs had been a substantially contributing cause of the decrease in suicide mortality in Sweden, but it was not the case for the control medication (tramadole) that also showed sharply increasing utilization during the study period.

In agreement with international (Isacsson 2000; Grunebaum et al. 2004; Ludwig and Marcotte 2005; Sondergard et al. 2006; Bramness et al. 2007; Nakagawa et al. 2007) and with previously published Hungarian studies covering different time periods between 1980 and 2005 (Rihmer 2004; Berecz et al. 2005; Kalmar et al. 2008) we also found, as expected, a significant negative correlation between AD prescription and declining national suicide rate in Hungary between 1998 and 2006. However, in spite of the facts that this negative association was highly significant in both genders ($P < 0.001$) and the changes in AD utilization and in suicide rates were very similar in females and males (+ 112 and –21% in females and +117 and –23% in males, respectively) the effect of increasing AD consumption was about 8-fold stronger in males (Table I). The explanation of this could be that although the current and 1-year prevalence ofmajor depression in Hungary is two-times more frequent in females (Szadoczky et al. 1998) about 80% of suicide victims are males (Berecz et al. 2005; Kalmar et al. 2008). This indicates that depressed males are particularly vulnerable for suicide and therefore the same increase in AD pharmacotherapy could show greater anti-suicidal effect in males. The similar drop in suicide rates in both genders, on the other hand, may suggest that the traditional suicide protective factors, like good family and social support, children at home, religiosity are more common and/or work better among females. It should be also noted that the effect of a given intervention is greater if the baseline condition is more pathological (in this case, if the baseline suicide rate is higher). The suicide rate of males at the beginning of the investigational period was almost 4-times higher as compared to females (50.05 vs. 14.40 per 100,000 in 1998) which can also explain the stronger and more attractive effect of increasing AD utilization on suicide rate in males.

In line with the literature, we also found that the seasonal pattern of suicides was more pronounced among males (Meares et al. 1981; Maes et al. 1993; Rihmer et al. 1998; Preti 2002; Postolache et al. in press; Reutfors et al. in press). The seasonality of suicide in Hungary decreased significantly during a 20-year long period, i.e. between 1980 and 1999 (Voracek et al. 2004), while during the same time the utilization of ADs increased by more than 6-fold (Rihmer 2004; Kalmar et al. 2008). During the relatively shorter (9 year) study period ofour present investigation, we did not find a significant decline of seasonality of suicides in the whole sample (males and females combined, Table II). However, in spite of the same amount of change in AD consumption and suicide rate in both genders, we found a significant decline in seasonality of suicides, as reflected in the decreased seasonal index, only in males (Table II). One explanation of this could be the higher base rate of suicide mortality in males, as discussed above, particularly if we consider the fact that seasonality of suicide is more pronounced among males and among those who use violent suicide methods, methods that are characteristic primarily for males (Meares et al. 1981; Maes et al. 1993; Rihmer et al. 1998; Preti 2002; Kim et al. 2004; Lin et al. 2008; Postolache et al. in press; Reutfors et al. in press). Another explanation could be that males are more likely than females to benefit from increased use of appropriate mental health services, because they are less likely to use them (Rihmer and Rutz 2009).

As we have no information on the psychiatric diagnoses of suicide victims it should be mention as a limiting factor. In addition, the possibility of misclassification of non-suicidal deaths as suicides (particularly among those who used non-violent methods) is no possible to rule out by 100%. However, because this possible misclassification should be constant over time this bias could be negligible.

In summary, our result suggests that decreasing number of suicides for any reason (independently from better care of psychiatric patients including better treatment of depression) is not sufficient for fully explain the decreasing seasonality of suicides (Rocchi et al. 2007). In agreement with the prior study of us (Rihmer et al. 1998) and Oravecz et al. (2006) these findings also indicate that decreasing seasonality of suicides could be a good marker of lowering rate of depression-related suicides in the population, particularly in males who use much more frequently violent suicide methods (Maes et al. 1993; Kim et al. 2004; Lin et al. 2008). As gender distribution, method preference, psychiatric morbidity and seasonality of suicides in Hungary

(Murphy 2000; Voracek et al. 2004; Berecz et al. 2005; Kalmar et al. 2008) is the same as in other European and North American countries (Murphy 2000; Maes et al. 1993; Voracek et al. 2004; Oravecz et al.

2006; Rocchi et al. 2007) our present findings could be valid also for these countries. Whether our findings on the gender differences in antidepressant use-related seasonality change of suicide mortality are independent of the type of suicide methods (violent vs. non-violent) or restricted only to violent suicides, which have been shown to have more marked seasonality (Maes et al. 1993; Lin et al. 2008) remains an open question and is the subject of further studies.

Acknowledgements

None.

Statement of interest

There was no funding received for the work described. Dr. Rihmer is a member of the speakers bureaus or advisory boards of AstraZeneca, BMS, Egis, GSK, Lundbeck, Lilly, Organon, Pfizer, Rich-ter, Schering-Plough, Sanofi-Aventis and Servier. Dr. Gonda has received travel grants from Richter, GSK, Lilly, Krka, Organon and Schering-Plough. The other authors report no competing interests.

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The World Journal of Biological Psychiatry, 2010; 11: 586–593

ORIGINAL INVESTIGATION

Altered cardiovascular adaptability in depressed patients without heart disease

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Abstract

Objectives. Despite its clinical importance and relevance for health care policy, the pathways between depression and stress regulation remain poorly understood. The objective of our study was to compare cardiovascular and autonomic responses to brief psychosocial stress in a group of severely depressed subjects without heart disease and a non-depressed control-group. *Methods*.We recorded cardiovascular and autonomic reactions to two different stress tasks including anger recall and mental arithmetic in a sample of 25 severely depressed and 25 non-depressed subjects. Aggregated data were compared with repeated-measures MANOVA. We used contrasts to evaluate different response patterns concerning cardiovascular and autonomic reactivity vs. recovery. *Results*. Depressed subjects showed overall reduced high-frequency heart rate variability and an altered cardiovascular adaptability concerning heart rate, blood pressure, cardiac output, and, on a trend level, peripheral resistance. With few exceptions, we found no differences between reactivity vs. recovery response patterns. *Conclusions*. Our results provide further evidence for altered cardiovascular reactivity and impaired cardiac autonomic functioning in depression. Further research is needed on psychophysiological response to either more disease-oriented or more personality-oriented stressors.

Key words: *Depression, stress, heart rate variability, cardiovascular, reactivity*

Introduction

Depression can be considered a statistically independent risk factor concerning development, course and outcome of different forms of heart disease and other medical conditions (Rugulies 2002; Barth et al. 2004). Over the last years, a growing body of literature concerning risk-mechanisms, bio-psychological pathways linking depression and heart disease, screening procedures and intervention strategies has emerged (Rozanski et al., 2005; Lichtman et al. 2008). Most existing theories can be conceptualized within the framework of allostatic load, describing the "wear and tear" resulting from constant activation, insufficient down-regulation or inadequate functioning of physiological emergency systems, as well as their possibly deleterious impact on counter-regulatory mechanisms (McEwen 1998).

Most theories concerning development, course and consequences of depressive symptoms contain ideas about stress and stress regulation (Hammen 2006). At a cognitive level, worry and rumination are some of the key symptoms related to depressive episodes. Following the model proposed by Brosschot and colleagues (2005), these symptoms are also of central importance in the process of chronic or repeated activation of the cognitive representation of stressrelated content, thereby leading to a state of prolonged activation of elevated physiological stress response. Delayed recovery of cardiovascular parameters on the other hand is predictive of adverse outcome concerning cardiovascular health (Carroll et al. 2001).

A well-established and economical method for testing the proposed connections between stress, depression and cardiovascular adaptability within the framework of allostatic load is being provided by the research paradigm of cardiovascular reactivity (CVR) and recovery (Lovallo and Gerin 2003; Pieper and Brosschot 2005). Considering a variety of known

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(Received 12 May 2009; accepted 1 October 2009)

ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903397714

risk pathways and biological markers of depression (Mössner et al. 2007), one area of special interest deals with alterations in autonomic nervous system (ANS) function, heart rate variability (HRV) in particular (Carney et al. 2005). One method for assessing HRV is the use of different components derived from power spectral analysis in the frequency domain method (Task Force 1996). Especially the high-frequency (HF-HRV; 0.15–0.4 H_2) domain can be interpreted as a specific measure of cardiac parasympathetic control. Reduced HRV is a known risk factor for course and outcome of different forms of heart disease as mentioned above. Finally, lowHRV can also be interpreted as a marker of prefrontal brain hypoactivity, which is relevant for mental disorders such as depression (Thayer and Brosschot 2005).

Only a few studies have compared cardiovascular function in depressed and non-depressed subjects without heart disease. In a review Kibler and Ma (2004) found small to medium effects of depression on heart rate (HR) and blood pressure (BP) reactivity, influences of depressive symptoms on systemic vascular resistance for baseline as well as stress reactivity were reported elsewhere (Matthews et al. 2005). Salomon et al. (2009) found a mixture of less reactivity in HR, BP, cardiac output and less HR recovery for two tasks. Concerning the impact of depression on indices of the ANS, Hughes and Stoney (2000) showed an effect of mild depressive symptoms on the reactivity of parasympathetic cardiac control during stressors in healthy subjects. Findings of an overall decreased HRV were presented by Mueck-Weymann et al. (2002) and Nahshoni et al. (2004). A more recent study reported increased sympathetic and decreased parasympathetic activity in a homogeneous, never treated sample of depressed patients (Udupa et al. 2007).

The purpose of this study was to examine cardiovascular and autonomic responses to short-term, controlled cognitive-emotional stress in depressed subjects without heart disease. Adding to already existing data mostly dealing with the impact of mild forms of depression, we were particularly interested in using a sample of moderately to severely depressed, hospitalized individuals, with the aim of increasing external validity. More specifically, we expected altered cardiovascular reactivity and recovery on the one hand, and reduced HF-HRVon the other hand in our sample of depressed patients.

Methods

Participants

The study was approved by the local ethics committee. For the depressed group we asked psychotherapy inpatients with a main diagnosis of depression at the Department of Psychosomatics and Psychotherapy, University Hospital of Göttingen, Germany, for their participation. It should be noted that these patients were neither bed-ridden nor in a closed ward, but participated in a large variety of therapies and activities. For the non-depressed group, healthy volunteers were recruited via advertisements at the university campus. They were given a smallmonetary reward after participation. Both groups were matched according to age and sex.

Patients were excluded if they suffered from heart or other somatic diseases, comorbid anorexia nervosa or borderline personality disorder, for in these conditions altered autonomic and cardiovascular functioning has been reported (Battaglia et al. 1995; Koo-Loeb et al. 2000; Casu et al. 2002; Vigo et al. 2008). Controls were excluded following the same rules. We did not exclude patients with psychopharmacological treatment but tried to keep the number of people being treated with tricyclic antidepressants (TCA) as low as possible considering their documented cardiotropic effects (Roose and Miyazaki 2005).

A total of 60 participants were recruited and gave their written informed consent. One control turned out to be suffering from a psychiatric disorder; four patients had to be excluded because of evidence for heart disease or missing data. In another five patients rapid improvement in their depressive state as measured by questionnaire at time of our investigation was found, leaving a total of 50 participants to be studied, 25 in each group.

Psychological and health-related measures

Psychiatric diagnosis through clinical rating and information about medical condition of the patients were provided by the department's psychotherapists. Controls were assessed with the Mini-Mental State Examination (Mini-DIPS; Margraf 1994). Basic demographic data and information concerning smoking and exercise were collected by self-report.

General symptom load was measured using the global severity index (GSI) of the Symptom Check-List 90 R (SCL-90-R; Derogatis 1992; Franke 2002). The SCL-90-R is a widely used self-report questionnaire providing an overview over a broad range of psychiatric complaints on 90 items. Results from factor analysis suggest the use of the global severity index of this questionnaire for general research (Carpenter and Bittner 1995). The Beck Depression Inventory (BDI) has been developed specifically for targeting depression on 21 items (Beck et al. 1961). Advantages can be seen in its 2 J.C. Ehrenthal et al.

international use, shortness, internal consistency and content validity (Richter et al. 1998).

Physiological measures

All cardiovascular measures were assessed with the Task Force® Monitor (CNSystems, Graz, Austria). The Task Force† Monitor provides a stable, noninvasive, computer-supported, beat-to-beat monitoring system measuring continuous blood pressure corrected for oscillometric blood pressure, impedance cardiography and high resolution threechannel electrocardiogram (ECG), leading to a wide range of relevant indices to be recorded and computed (Fortin et al. 1998, 2006). The Task Force® Monitor is a monitoring system certified by the Food and Drug Administration and other regulatory authorities. For this study we report data on HR, mean blood pressure (MBP), cardiac output standardized to body surface area (CI), total peripheral resistance standardized to body surface area (TPRI) and autonomic function, especially HF-HRV. HF-HRV is computed by the Task Force® Monitor software package following the frequency method mentioned above (Gratze et al. 1998).

Procedure and tasks

The study took place in a well-tempered room at the university hospital. The time of the experiments was kept constant in the afternoon. Participants were instructed to apply only if in a healthy physical state and to stop caffeine intake and smoking 2 h before the appointment. The first part was spent on assessment of the cardiovascular data; afterwards the short interview and the questionnaire were administered. Assessment of the cardiovascular data followed a standardized procedure. For inducing stress we used a mental arithmetic subtraction task and the Ironson anger recall task (Prkachin et al. 2001). For the mental arithmetic, which was introduced as an achievement test, subjects were asked to sequentially subtract the number 13 from a given four-digit number, working as quickly and correctly as possible. For the anger recall, subjects were asked to recall and report an interpersonal experience related to anger, while the investigator kept asking questions with the aim of maximizing the representation of angerrelated thoughts and feelings. The procedure was as follows: (1) baseline (5 min), (2) mental arithmetic (5 min), (3) rest (5 min), (4) anger recall (5 min), (5) rest (10 min). Before and after inducing stress we asked the participants to rate their subjective stress level on a scale from 0 (not stressed at all) to 10 (totally stressed) as a manipulation check.

Analytical strategies

Following the suggestions made by Kamarck and Lovallo (2003) as well as Moseley and Linden (2006) for increasing external validity, we combined the mean values of the stress tests (phases 2 and 4) to a general stress value. Likewise, a general pre-stress value (phases 1 and 3) and a general relaxation value (phases 3 and 5) were computed. For verifying the feasibility of this procedure we related combined values for all three phases with their associated single phase values. Correlations were generally ranging from 0.94 to 0.99. Only the pre-stress values showed slightly reduced correlations between 0.83 and 0.89 for the baseline values of MBP, CI, TPRI and HF-HRV.

As a manipulation check concerning perceived stress we computed a mean pre-stress score and a mean post-test score and compared them using a 2×2 (depression (high vs. low) by time (before vs. after stressor)) repeated-measures ANOVA. For this analysis two patients were excluded due to missing data. Mean values can be found in Table I. Cardiovascular and autonomic parameters were visually inspected and checked for normal distribution. As HF-HRV values were skewed, log-transformed values were used for all analyses.

The main hypothesis concerning differences in cardiovascular and autonomic functioning between depressed and non-depressed subjects was tested using a 2×3 repeated-measures MANOVA (depression (high vs. low) by stress (pre-stress vs. stress vs. relaxation)) on the dependent variables HR, MBP, CI, TPRI and HF-HRV. Mean values can be found in Table II.

Table I. Descriptive data.

Variable (unit)	High depression (*N*=25)	Low depression (*N*=25)
Age (M [SD])	28.52 (7.08)	26.72 (4.56)
BDI (M [SD])	26.12 (6.36)	4.60 (3.86)
GSI (M [SD])	1.35 (.56)	.43 (.26)
Smoking (cigarettes/day)	7.24 (7.99)	.66 (1.60)
Body mass index (BMI)	22.97 (3.02)	21.28 (2.25)
Subjective stress		
before stressor	4.11 (1.85)	3.18 (1.64)
after stressor	6.80 (1.38)	5.94 (1.72)
Sex		
Male	32%	32%
female	68%	68%
Occupation		
student	36%	88%
working	44%	4%
other	20%	8%

BDI, Beck Depression Inventory; GSI, general severity index of the SCL-90-R.

Results

The recruitment and classification into two groups showed a good match concerning sex and age and sufficiently discriminating BDI scores (see Table I). Differences between the two groups can be found in some demographic variables: the highly depressed group reported more smoking, a higher body mass index (BMI) and contained a lower percentage of students. All patients had a diagnosis of some kind of depressive disorder. Eighty-eight percent suffered from major depression, including 16% who fulfilled criteria for recurrent major depression, 12% had "double depression" (major depression and dysthymic disorder), 4% dysthymic, and 8% reactive depressive symptoms in the context of an adjustment disorder. Eighty percent of the patients had one and 60% had two additional diagnoses of mental disorders. Most common comorbid conditions were anxiety disorders, personality disorders, adjustment disorders and bulimia nervosa. Ninety-two percent of the non-depressed group had no history of a psychiatric condition at all, two subjects reported mild symptoms of agoraphobia and obsessivecompulsive disorder. Thirty-three percent of the depressed group did not take any medication. Concerning psychotropic medication, seven patients received SSRIs, one of them in combination with a TCA. Three patients took monoamine oxidase inhibitors (MAOIs), one of them in combination with neuroleptic medication. Two subjects from the depressed group were given alpha-2 receptor blockers. Other medication included mainly oral contraceptives and antihistamines. 60% of the nondepressed group was free of any kind of medication. Existing medication mainly included contraceptives.

Manipulation check

A 2×2 (depression (high vs. low) by time (before vs. after stressor)) repeated-measures ANOVA on the combined scores of subjective stress revealed a significant main effect of time: $F(1,46)=101.60$, $P<0.001$, $\eta^2=0.69$, and depression: $F(1,46)= 5.14$, $P=0.028$, $\eta^2=0.10$. No interaction effect for depression by time was found: $F(1,46)=0.01$, $P=0.906$.

Differences in cardiovascular and autonomic parameters between depressed and non-depressed subjects

For the main hypothesis, 2×3 repeated-measures MANOVA (depression (high vs. low) by stress (prestress vs. stress vs. post-stress)) on the dependent variables HR, MBP, CI, TPRI and HF-HRV revealed a significant main effect for the factor stress, $F(10,39)=23.53$, $P<0.001$, $\eta^2=0.86$, no main effect, but a trend for the factor depression, $F(5,44)=2.24$, $P=0.067$, $\eta^2=0.20$, and a significant depression by stress interaction effect, $F(10,39)=2.62$, $P=0.015$, $\eta^2=0.40$. Subsequent univariate ANOVAs can be used for further differentiation of the results. As shown in Table II, there are significant differences in all of the dependent variables for the main within-subject effect of stress. For the depression by stress interaction effect differenceswere found for all the variables except HF-HRV, and a trend for differences in TPRI. When looking at the (non-significant) main effect for depression, the only overall differences between the groups can be found for HF-HRV and, on a trend level, CI, both being generally lower in the depressed group.

When entering smoking as measured by cigarettes per day, BMI and psychotropic medication as a dichotomous variable as covariates, the interaction effect depression by stress remains significant ($P=0.036$), the main effects for depression ($P=0.632$) and stress ($P=0.757$) fail to reach significance level. The pattern in the univariate ANOVAs stays mostly the same.

Contrasting each pre- and post-stress phase with the stress phase, we observed no reactivity but recovery for the main effect stress for HF-HRV. In other words, there were no differences in parasympathetic control between the preceding rest and stress, but an increase between stress and subsequent recovery. The only difference for the stress by depression interaction can be seen for TPRI on a trend level: there is no difference between the groups in increase of peripheral resistance, but in recovery.

Discussion

Against the background of significant connections between stress-regulation, depression and heart disease we compared cardiovascular and parasympathetic reaction and recovery in a group of high vs. low-depressed subjects. Regarding the significant differences in perceived stress before and after the stressor and cardiovascular activation, the induction of psychological stress was successful. We also found an elevated overall level of subjective tension in the depressed group. Concerning our main research questions, depressed subjects showed an overall reduction of HF-HRV and an impaired cardiovascular reactivity or recovery in HR, MBP, CI and, on a trend level, TPRI. Effect sizes were moderate to large. Descriptively, the highly depressed group had comparable HR during stress but higher HR in the resting phases, lower MBP and CI during stress but nearly the same MBP and CI during relaxation, and higher TPRI during stress but no differences to the non-depressed group during relaxation. When

Table II. Univariate ANOVAs of CVR/autonomic function during stress and relaxation in subjects with high vs. low depression.

			Main effect depression			Main effect stress					Stress×depression				
Variable and Phase	High depression M (SD)	Low depression M (SD)	*F*	*P*	η^2	*F*	*P*	η^2	*P* 1 vs 2	*P* 2 vs 3	*F*	*P*	η^2	*P* 1 vs 2	*P* 2 vs 3
HR (beats/min)															
1 pre	77.71 (10.86)	73.04 (11.65)													
2 stress	83.15 (11.18)	83.55 (11.48)	1.03	0.316	0.02	130.62	0.000	0.73	0.000	0.000	10.36	0.000	0.18	0.003	0.002
3 post	75.82 (9.87)	71.02 (10.14)													
MBP (mmHg)															
1 pre	92.26 (10.81)	92.50 (9.47)													
2 stress	98.84 (11.09)	103.39 (10.70)	0.40	0.532	0.01	150.43	0.000	0.76	0.000	0.000	8.93	0.000	0.16	0.011	0.003
3 post	92.57 (10.06)	93.13 (9.36)													
CI (l/(min*m^2)															
1 pre	2.98 (.45)	3.14 (.51)													
2 stress	3.09 (.44)	3.46 (.54)	3.20	0.080	0.06	37.97	0.000	0.44	0.000	0.000	7.07	0.001	0.13	0.003	0.017
3 post	2.92 (.46)	3.12 (.53)													
TPRI (dyne*s*m^2/cm^5)															
1 pre	2493.22 (559.57)	2371.67 (422.00)													
2 stress	2644.27(554.11)	2429.54 (372.10)	1.30	0.260	0.03	10.35	0.000	0.18	0.001	0.007	2.54	0.084	0.05	0.222	0.067
3 post	2534.68 (539.77)	2407.66 (452.10)													
HF-HRV (ln ms^2)															
1 pre	2.27 (.46)	2.54 (.43)													
2 stress	2.26 (.49)	2.50 (.39)	4.58	0.037	0.09	3.45	0.036	0.07	0.593	0.031	0.19	0.824	0.00	0.655	0.666
3 post	2.32 (.47)	2.59 (.47)													

HR, heart rate; MBP, mean blood pressure; CI, cardiac index; TPRI, total peripheral resistance; HF-HRV, high-frequency heart rate variability.

looking at the contrasts, we only found differences for HF-HRV and TPRI, probably due to heightened stress and appraisal and therefore reduced parasympathetic and increased alpha-adrenergic influence at the beginning of the examination.

Our results support the findings of other studies. Especially the increased HR during rest in the depressed group could be explained by perseverative cognition as proposed by Brosschot et al. (2005) as well as anticipatory processes and reduced vagal influence. Concerning vagal influence we did not find depression-related differences in recovery as Hughes and Stoney (2000), but an overall reduction in the total power of parasympathetic cardiac control, supporting results of Mueck-Weymann et al. (2002) and Nahshoni et al. (2004). Interpreting the results from a perspective of allostatic load (McEwen 1998), prolonged reduction of parasympathetic control can either lead to or be the result of sympathetic predominance with possible deleterious consequences (Brook and Julius 2000; Thayer and Sternberg 2006). This could also explain the impaired cardiovascular efficacy with higher peripheral resistance, lower blood pressure and cardiac output during stress in the depressed subjects.

Some limitations of our study have to be mentioned. Although matching of the two groups according to sex and age was successful, we had to accept certain limitations concerning internal validity. There were differences in medication, comorbidity, smoking behavior, BMI and occupation between the two groups. The difference in medication is due to treatment conditions and severity of the depressive syndromes. However, bearing in mind their potential cardio-protective effects of reducing HR and increasing HRV (McFarlane et al. 2001; Roose and Miyazaki 2005), the high amount of SSRIs in the depressed group provided rather an opportunity of a stricter testing of our hypothesis concerning CVR and ANS function. Controlling for medication as described above did not change the pattern of our results. Also the use of oral contraceptives might influence cardiovascular parameters (Shufelt and Bairey Merz 2009), but our results are generally in line with other reports that control for contraceptives and menstrual cycle (Mueck-Weymann et al. 2002; Udupa et al. 2007). Although we found a significantly higher body mass index in the depressed group, there was no influence of BMI on our main results. Trying to capture physical activity by screening questions did not provide results that were reliable and comparable between the groups, so we did not include the data in our analyses. This was mostly due to difficulties in ranking of regular physical activities of depressed patients as part of their treatment, but also general problems of measuring fitness by self-report (Shephard 2003). Differences in psychiatric diagnoses and comorbidity were as expected (Kessler 2002). Because we did not use SCID-interviews but clinical ratings, the assessment of DSM-IV diagnoses might not have been as strict as desirable. Nevertheless, because of an intense diagnostic phase and a supervised team focus conference for every patient at the beginning of treatment, we feel confident to have sufficiently captured the main aspects of the particular psychopathology. The BDI mean value of 4.60 in the control group would be classified as no or very mild depressive symptoms (Beck et al. 1988). Also we were particularly interested in a large difference in depressive state between the two groups. Because of the substantial comorbidity in our naturalistic sample, especially concerning anxiety, we cannot rule out effects of other psychiatric conditions on the cardiovascular data. Also our stressors were not targeted at specific symptoms or constructs related to any specific disorder. However, there is evidence that depression and anxiety disorders have more nosological similarities than expected (Hemetta 2008) and autonomic functioning might rather be related to underlying traits than specific diagnosis (Bleil et al. 2008). Heterogeneity concerning age and sex is equally distributed in both groups and should therefore not influence the results, but might even add to the external validity of the study. In the future it would be advisable to recruit larger samples with different levels of depressive symptoms with the possibility of comparing more than two groups without losing statistical power. Smoking seems to be related to depression (MacCaffery et al. 2008), although the exact relationship remains somewhat unclear (Freedland et al. 2005). It is therefore not very representative to recruit a severely depressed, non-smoking sample. The only way to balance this effect out would be to also use a heavily smoking control group, which we did not do. However, even when controlling for smoking, our results stay mostly the same. The control group contained more students and reported less people in a full-time work. Although there are reports on the influence of social status on heart disease, the interaction seems to be highly complex and probably more relevant for longterm adverse conditions, e.g. unemployment (Adler et al. 1993). Unfortunately our sample size does not allow differential testing for social class.

In conclusion, we could add to other studies on cardiovascular and autonomic function in severely depressed subjects without heart disease, for which we were able to show further evidence for reduced cardiovascular adaptability and impaired parasympathetic cardiac control. This is also of special interest for acknowledging and incorporating biolo- 6 J.C. Ehrenthal et al. gical aspects of depression for possible subtypes of the disease in the DSM-V and

ICD-11 (Gupta 2009). Future studies should incorporate stressors either related to specific aspects of psychiatric disease or underlying personality dimensions. This would have the potential of expanding the existing literature, dealing mostly with risk factors, towards a more intervention-related toolkit for assessing and evaluating target areas for psychotherapeutic or pharmacological interventions.

Acknowledgements

None.

Statement of Interest

None to declare.

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The World Journal of Biological Psychiatry, 2010; 11: 594–602

ORIGINAL INVESTIGATION

Is the increase of hypomanic stages during adolescence related to gender and developmental tasks?

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Abstract
Objectives. To detach themselves from their family of origin, adolescents need to develop proactive behaviour which includes increased risk-taking and novelty seeking. These behaviours may be attributable both to developmental issues and to hypomanic-like stages. Since there is a lack of research focusing on hypomania in adolescents the aim of the study was to compare hypomania scores of adolescents with those of adult outpatients suffering from bipolar II disorders, and to investigate possible gender-related differences. *Methods.* One hundred and seven adolescents (mean age: 18 years) took part in the study; 60 of them indicated that they experienced intense romantic love; 47 were controls. Participants completed the Hypomania Check List, and data were compared with those of adult outpatients suffering from bipolar II disorders. *Results.* Scores of adolescents in early-stage intense romantic love differed from those of adolescent controls, but not from those of outpatients suffering from a bipolar II disorder. Factor analyses revealed that both groups of adolescents displayed higher scores for the factor "irritable/risk-taking" hypomania. A gender-related pattern was found, with increased scores for female adolescents. *Conclusion.* Adolescents' developmental tasks surrounding experiences in social, psychosexual and substance use-related engagement may lead to temporary and gender-related hypomanic-like stages.

Key words: *Adolescents, bipolar disorders, developmental tasks, gender differences, HCL-32, hypomania*

Introduction

Adolescence is a time of remarkable physical and behavioural changes and these changes are associated with underlying functional and structural processes of the brain (Giedd 2008; Paus et al. 2008). But besides these structural and functional neuronal changes in an adolescent's life psychological and social demands increase: For instance, peer activities such as attending sports activities, concerts or discos in the evening and on weekends increase, and at the same time parental control (also for setting bedtimes; Wolfson and Carskadon 1998) decreases. Employment after school (cf. Millman et al. 2005), homework requirements, and the availability of television or the internet (Eliason et al. 2002) pose a further challenge. Moreover, there is evidence that emotional reactivity increases concurrently with the importance of social feedback. This seems to be particularly true for female adolescents (cf. Hyde et al. 2008).

To accelerate the independence from the family of origin adolescents demonstrate proactive behaviour. This proactive behaviour is believed to be associated with increased risk taking and novelty- and reward-seeking (cf. Paus et al. 2008). As a consequence, adolescence is also the age of the onset of experimenting with psychoactive drugs and peer-related activities such as falling in love or sexually and socially rewarding activities.

Thus the interplay of social, psychological, and neural processes may explain why adolescence is the age of the onset of many psychiatric disorders (Paus et al. 2008): whereas results from a US National Comorbidity Survey Replication study (cf. Kessler et al. 2005, 2008; Giedd 2008; Paus et al. 2008) implicate that anxiety disorders such as phobias and separation anxiety and impulse-control disorders begin in childhood, the onset of other anxiety disorders such as panic, generalized anxiety disorders and post-traumatic stress disorders, substance-use

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(Received 29 September 2009; accepted 1 December 2009)
ISSN 1562-2975 print/ISSN 1814-1412 online
DOI: 10.3109/15622970903521149

disorders, mood disorders and schizophrenic disorders is observed at mid-adolescence, that is, around the age of 14.

However, as to hypomania and bipolar disorders in childhood and adolescence, only limited data are available.

In adults, bipolar II disorders (BP II) were observed in clinical (cf. Angst et al. 2005) and non-clinical samples (Angst et al. 2003; Angst 2004; Meyer et al. 2007). The prevalence of BP II varies between 0.5 and 8%, depending on cohorts and diagnostic criteria (for review, see Raman et al. 2007). Accordingly, Angst et al. (2003) reported findings from a 20-year prospective community sample of 4547 subjects and showed that about 13% of the cohort (mean age: 40 years) represented a very soft expression of bipolarity between a bipolar disorder and normality. There are, however, limited data available about children and adolescents. Recent publications (Dilsvaver et al. 2005; Kowatch et al. 2005; Pavuluri et al. 2005; Soutullo et al. 2005; Holtmann et al. 2009) suggested that BP-II may also be common among children and adolescents. Furthermore, Raman et al. (2007) stated that among 61 outpatients aged about 13.5 years and suffering from major depressive disorders, 12 of them (20%) were misdiagnosed as having a hypomanic disorder. Regarding adolescents, Soutullo et al. (2005) reported prevalence rates between 1.9 and 11%, with a high international variability and definition-related issues. Consequently, careful assessment is needed to avoid under- or misdiagnosed BP II disorders (Dilsaver and Akiskal 2005). Furthermore, in a previous study we could show that adolescents in early-stage intense romantic love displayed a high degree of hypomanic-like behaviours and feelings (Brand et al. 2007).

With regard to the diagnostic criteria of the DSM-IV and the ICD-10 for BP II disorders, the manuals first select subjects with mood changes (elated, expansive, irritable) and then with additional features such as increased sexual activity, social contacts, substance use, creativity, self-confidence, energy, taking risks while driving, as well as lack of sleep, and psychosocial inhibition. However, for adolescents, responsibility does increase; particularly, to name a few, adolescents have to deal with money, car or motorcycle driving, psychosocial and psychosexual disclosure, and exploration of the effects of varying substances (cf. alcohol, cannabis, nicotine, and other psychoactive substances). To tackle these issues may be considered normal and necessary developmental tasks. This means that adolescence is normally the first period in life in which potentially risky experiences are made (cf. Steinberg 1997; Kaplow et al. 2002; Kuttler and La Greca 2004; Paus et al. 2008). Thus, reward-seeking and risk-taking behaviour (see also Fareri et al. 2008) seems to be a common behaviour among adolescents.

Regarding gender, a growing body of research indicates that women are increasingly at risk for developing affective disorders (cf. Kuehner 2003; Hyde et al. 2008). Women seem to have a higher risk for developing rapid-cycling bipolar disorders and dysphoric or mixed mania (Arnold 2003); furthermore, they are more prone to present an initial depressive episode followed by the onset of mania (Viguera et al. 2001), and to suffer from a greater number of depressive episodes (cf. Angst et al. 2002; Kennedy et al. 2005). Results of gender-related differences in bipolar disorders during adolescence were mixed: Geller et al. (2000) found no gender differences in the rates of the criteria for mania, and repeated adverse life events seemed to favour the emergence of juvenile bipolar disorders (Johnson and McMurrich 2006), with female adolescents more at risk than male adolescents (Alloy et al. 2006). An important finding from Dilsaver et al. (2005) was that adolescent girls suffering from BP I or II were more than twice at risk for having suicidal ideation, and nearly three times at risk for having histories of a suicide attempt. In addition, irrespective of gender, the risk for suicidality seems to be increased in adolescents suffering from bipolar II disorders (Holtmann et al. 2007).

To sum up, during adolescence, important functional and structural neural changes occur, and, to detach themselves from the family of origin, adolescents engage more in peer-related psychosocial and psychosexual activities. These activities normally demand more risk-taking and novelty seeking. However, adolescence is also the age of the onset of many psychiatric disorders. With respect to hypomania, research is scarce. For this reason, we re-examined data from previous studies with adolescents (Brand et al. 2007) and adults (Angst et al. 2005). The aim of our study was to shed some light on hypomania in adolescence, particularly to provide a more detailed analysis of the data, first, with respect to the two factors of the Hypomania Check-List 32 (HCL-32; Angst et al. 2005), and second, with respect to gender. The third goal was to compare these results with those of adult patients suffering from bipolar II disorders.

We set up the following two hypotheses: first, based on evidence from developmental psychology that the emergence of an increased risk-taking behaviour seems a common feature among adolescents (cf. Steinberg 1997; Kaplow et al. 2002; Kuttler and La Greca 2004; Fareri et al. 2008) we expected increased HCL-32 scores in the subscale "irritable/risk-taking"

in adolescents when compared to data of adults suffering from BP II. Second, based on the findings from the literature indicating a higher risk for affective disorders in adolescents (Dilsaver et al. 2005; Alloy et al. 2006) and particularly in female adolescents (Viguera et al. 2001; Arnold 2003; Kuehner 2003; Angst et al. 2005; Kennedy et al. 2005; Hyde et al. 2008), we hypothesized gender-related differences with increased values for female participants.

We hold that these questions are important, since it is very probable that adolescents' concerns about alterations in mood states will be encountered in professional psychological and psychiatric contexts. Moreover, there is evidence that the manifestation of hypomanic symptoms in adolescence is predictive of bipolar disorders (Angst et al. 2005). Additionally, bipolar disorders in adolescence are associated with an increased risk for suicidality (Dilsaver et al. 2005; Holtmann et al. 2007). Thus, recognizing and treating hypomania might be crucial in preventing suicide.

Methods

Sample

The sample was already described elsewhere (Brand et al. 2007). In short, 107 adolescents (age M = 17.94, SD = 1.34; 66 females, age M = 17.85, SD = 1.30, and 41 males, age M = 18.19, SD = 1.21) were recruited by word-of-mouth recommendation in two high schools in Basel (Switzerland). Of these adolescents, 60 (35 females and 25 males) reported having recently fallen in love and experiencing intense romantic love, 11 (seven females and four males) indicated they were in a longer-term partnership, and 36 (24 females and 12 males) reported not having fallen or being in love. Age did not differ with regard to gender or state of love (that is, early-stage, longer-term, or not in love at all; $F(2, 104) = 0.56$, n.s.). Mean duration of relationship was 5.3 months (SD = 6.78) for adolescents in early-stage intense romantic love, and 13.08 months (SD = 16.50) for those in longer-lasting relationships ($t(76) = 2.77$, $P < 0.001$). Eighty-eight adolescents were attending high school or studying, 19 were enrolled in vocational training.

Study design

All potential participants were informed about the purpose of the study and assured of the confidentiality of their responses; afterwards, they signed written consent forms. Then, participants were asked whether they were actually "madly" in love, that is, if they had recently fallen in love and were experiencing intense romantic love, whether they had been in love for a longer time, or whether they were singles and not in love.

Singles were assigned to the control group (CG), as were those participants indicating they had been in love for a longer period of time. Participants reporting that they had recently fallen in love and were experiencing intense romantic love were assigned to the romantic love group (RLG). However, to operationalize and thoroughly assess early-stage intense romantic love, those participants indicating to experience intense romantic love answered to three additional items as described with more details below.

All eligible persons were first screened with a brief psychiatric interview (German version of the Mini International Neuropsychiatric Interview; Ackenheil et al. 1999). Participants also completed two questionnaires providing self-ratings of depressive disorders (Depression Scale; Von Zerssen 1976) and anxiety disorders (State-Trait-Anxiety Inventory; Laux et al. 1981). For the two questionnaires values had to be within the norms for healthy people as indicated by the manuals of the questionnaires. Both the screening interview and the self-rating questionnaires were used to assure that only participants without any psychiatric disorders such as anxiety, depressive and eating disorders or substance abuse were included in the study. Furthermore, since romantic love may be also associated with delusion, depressive symptoms and desperation (Welsh et al. 2003), as an exclusion criterion established beforehand, people experiencing early-stage intense romantic love and negative states such as depressive symptoms were also excluded. Afterwards, participants filled out psychological questionnaires as described below.

Materials

To define, operationalize and to more thoroughly assess early-stage intense romantic love, as suggested by Marazziti and Canale (2004), three questions were taken from a self-rating questionnaire to diagnose obsessive compulsive disorders, though modified for our purposes (Y-BOCS; Yale Brown Obsessive Compulsive Scale; Goodman et al. 1989): "How much time do you think of the other person?" (five-point scale ranging from "not at all" to "the whole day"), "While thinking of the other person, do you feel distracted?", and "How well can you resist the need to think of the other person?" (five-point scales: "not at all" to "extremely"). The maximum score

was 12; the cut-off was set at six points.[1] Participants who indicated they were in love, either as a recent occurrence or for a long time, and who scored six points or higher were considered to be at a stage of intense romantic love. Sixty out of 62 (96.78%) of the participants indicating that they had recently fallen in love and experiencing intense romantic love met these criteria and were assigned to the group of adolescents at an early stage of intense romantic love (see Brand et al. 2007 for more details).

To assess hypomanic state, all participants filled out a self-assessment tool for hypomanic symptoms in outpatients, the Hypomania Check List-32 (HCL-32; Angst et al. 2005). Hypomanic state is assessed by summarizing 32 statements concerning behaviour (e.g., "I spend more money/too much money"), mood (e.g., "My mood is significantly higher"), and thoughts (e.g., "I think faster") within the last 4 weeks. Answers were "yes" or "no". The questionnaire has good psychometric properties and it has repeatedly been proven to accurately assess hypomanic states (Carta et al. 2006; Vieta et al. 2007). Furthermore, of the 32 items, two factor-analytically computed subscales were carried out ("active/elated" hypomania and "risk-taking/irritable" hypomania); the validity of the two subscales has proved to be applicable also to non-clinical samples of both adults (Meyer et al. 2007) and adolescents (Holtmann et al. 2009). Most importantly, Holtmann et al. (2009) reported that the internal structure of the HCL-32 for adolescents reflected well juvenile bipolarity with substance use and symptoms of ADHD and conduct disorders, suggesting that the HCL-32 self-rating tool has a high validity. The subscale "active/elated" hypomania is composed of items such as: "I feel more energetic and more active"; "I am more self-confident"; "I enjoy my work more"; or "I am more sociable (make more phone calls, go out more)". The subscale "risk-taking/irritable" hypomania is composed of items such as: "I am less shy or inhibited"; "I want to meet or actually do meet more people"; "I am more interested in sex and/or have increased sexual desire"; "I tend to drive faster or take more risks when driving"; "I spend more/too much money"; "I take more risks in my daily life"; "I drink more alcohol", or "I smoke more cigarettes".

The study was conducted according to the Helsinki Declaration, and it was approved by the local ethical committee.

To compare the results of both groups of adolescents, parts of the data of adult outpatients with BP II (AP BP II) were taken from a study conducted with Italian adult outpatients[2] ($N=186$; 115 female and 71 male patients; age (years): M = 43.20, SD = 13.08).

Statistical analyses

Several ANCOVAs controlling for age were performed to compare the data of the three groups (romantic love group = RLG; controls = CG; adult outpatients = AP BP II). To analyze the influence of gender, ANOVAs with the factor Group (RLG, CG) and Gender (male, female) were performed. Post-hoc analyses using Bonferroni–Holm corrections for *P* values were applied. Test results with an alpha level of below 0.05 (two-sided) were reported as significant. Statistical analyses were performed with SPSS 16.0 for Windows.

Results

The gender distribution was not significant between the adolescents of the romantic love group (RLG) and the control group (CG) ($\chi^2(df=1, N=107)=0.65$, $P=0.42$), and no statistical difference of age regarding gender was found (females; age: M = 17.85, SD = 1.39; males; age: M = 18.19, SD = 1.21: $t(105)=-1.30$, $P=0.20$). Moreover, gender distribution was not significant between the RLG, CG, and AP BP II ($\chi^2(df=2, N=293)=0.65$, $P=0.72$). Significant mean differences between the RLG, CG, and AP BP II were found for age ($F(2, 290)=196.91$, $P=0.000$); post-hoc comparisons revealed that the age of both groups of adolescents (RLG, CG) was significantly lower compared to the age of AP BP II (*P* values < 0.000). Thus, it seemed justified to introduce age as a control variable.

Table I shows the descriptive and inferential statistical overview. For the Total score of the HCL-32, a one-way ANCOVA controlling for age revealed significant mean differences between the three groups (RLG, CG, AP DP II). Post-hoc analyses with Bonferroni–Holm corrections for *P* values showed significantly increased scores for the romantic love group, when compared to the control group, but not when compared to adult BP II. Compared to the

[1]The cut-off point of 6 turned out to best discriminate between people highly in love and people just in love (cf. Marazziti and Canale 2004; Brand et al. 2007).

[2]The sample was already described in Angst et al. (2005); outpatients with diagnosed unipolar depressive or bipolar disorders were recruited in an outpatient clinic in Italy. Following the recommendations established by Brislin (1986), a German version of the HCL-32 was first translated into Italian by a professional translator, and then back-translated into German by another translator. Consensus was reached on a final version that was subjected to the translation–re-translation process.

Table I. Descriptive and inferential statistics of HCL-32 total and subscores between adolescents in early stage intense romantic love (RLG), adolescents of the control group (CG), and a sample of adult outpatients suffering from bipolar II disorders (AP BP II).

	Groups						ANCOVAs		Post-hoc comparisons with Bonferroni-Holm correction for *P* values		
	AP BP II ($N=186$)		RLG ($N=60$)		CG ($N=47$)		ANCOVA controlling for age		AP BP II vs. RLG	AP BP II vs. CG	RLG vs. CG
HCL-32	M	SD	M	SD	M	SD	*F*	*P*	*P*	*P*	*P*
Total score	15.90	(5.49)	16.17	(5.84)	9.77	(6.83)	$F(2, 289) = 26.84$	$P=0.000$	$P=0.725$	$P=0.000$	$P=0.000$
Subscale 1 score 'active/elated' hypomania	12.49	(4.36)	9.83	(4.19)	6.02	(4.65)	$F(2, 289) = 24.10$	$P=0.000$	$P=0.041$	$P=0.000$	$P=0.000$
Subscale 2 score 'risk-taking/irritable' hypomania	2.82	(1.82)	3.82	(1.72)	3.43	(1.98)	$F(2, 289) = 19.56$	$P=0.000$	$P=0.000$	$P=0.003$	$P=0.455$

HCL-32, Hypomania Check List 32 (1); AP BP II, adult patients suffering from bipolar II disorders; RLG, romantic love group; C, controls; M, mean; SD, standard deviation; *N*, number of participants of an entire group.

RLG and the AP BP II, the control group showed the lowest sum scores.

For the subscale "active/elated" hypomania, a one-way ANCOVA controlling for age revealed significant mean differences between the three groups (RLG, CG, AP BP II). Post-hoc analyses with Bonferroni-Holm corrections for p-values showed decreased scores of both groups of adolescents (RLG, CG) compared to adult patients suffering from BP II. Moreover, compared to the CG, adolescents of the romantic love group displayed higher scores.

For the subscale "risk-taking/irritable" hypomania, a one-way ANCOVA controlling for age revealed significant mean differences between the three groups (RLG, CG, AP BP II). Post-hoc analyses with Bonferroni–Holm corrections for *P* values showed increased scores for both adolescent groups (RLG, CG) compared to adult patients suffering from BP II. No significant differences were found between the two adolescent groups (RLG, CG).

Thus, the pattern of results showed that adolescents in early-stage intense romantic love (RLG) had an overall sum score of hypomanic symptoms at a similar level to that of adult patients suffering from BP II. Furthermore, compared to adult BP II, both groups of adolescents (RLG, CG) displayed lower scores for the subscale "active/elated" hypomania, but higher scores for the subscale "risk-taking/irritable".

Table II shows the descriptive and inferential statistical overview. For the Total score, main effects were found for the factor Group, with highly increased scores for adolescents of the romantic love group, for the factor Gender, with increased scores for female adolescents, and for the Group × Gender interaction, with increased scores for female adolescent controls, and with highly decreased scores for male adolescent controls, respectively (see Figure 1).

For the subscale "active/elated" hypomania, main effects were found for the factor Group, with increased scores for adolescents of the romantic love group, and for the Group × Gender interaction, with increased scores for female adolescent controls, and with highly decreased scores for male adolescent controls, respectively. No main effect was found for the factor Gender (see Figure 2).

For the subscale "risk-taking/irritable" hypomania, main effects were found for the factor Group, with increased scores for adolescents of the romantic love group, and for the factor Gender, with increased scores for the female adolescents, and with decreased scores for male adolescents, respectively. The Group × Gender interaction failed to reach statistical significance ($P=0.115$); descriptively, the female adolescent controls had scores which were almost twice as high as those of the male adolescents of the same group (see Figure 3).

Table II. Descriptive and inferential statistics of HCL-32 total and subscores between adolescents in early stage intense romantic love (RLG) and adolescents of the control group (CG), separated by gender.

	Groups					
	Romantic love group			Controls		
	Male ($n=25$)		Female ($n=35$)	Male ($n=16$)		Female ($n=31$)
HCL-32	M	SD	M SD	M	SD	M SD
Total score	14.95	(5.39)	17.88 (6.12)	4.25	(2.91)	12.62 (6.53)
Subscale 1 score 'active/elated' hypomania	8.51	(4.07)	11.68 (3.70)	2.38	(1.86)	7.90 (4.54)
Subscale 2 score 'risk-taking/irritable' hypomania	3.52	(1.92)	4.03 (1.56)	2.13	(1.15)	3.81 (2.08)

	Statistical comparisons			
	Factor group	Factor gender	Interaction group × gender	
HCL-32	F P	F P	F P	
Total score	F (1, 103) = 48.82, P = 0.000	F (1, 103) = 5.64, P = 0.019	F (1, 103) = 24.47, P = 0.000	
Subscale 1 score 'active/elated' hyPomania	F (1, 103) = 39.68, P = 0.000	F (1, 103) = 2.25, P = 0.360	F (1, 103) = 30.50, P = 0.000	
Subscale 2 score 'risk-taking/irritable' hypomania	F (1, 103) = 25.64, P = 0.000	F (1, 103) = 9.40, P = 0.003	F (1, 103) = 2.70, P = 0.115	

HCL-32, Hypomania Check List 32 (1); M, mean; SD, standard deviation; n, number of participants within a subgroup.

Figure 1. Total score of the Hypomania Check-List 32 (HCL-32) differed significantly between adolescents experiencing early-stage intense romantic love (romantic love group = RLG) and adolescents of the control group (CG), and between female and male participants. Indications: means and standard deviations (error bars).

To sum up, compared to the adolescent controls, the adolescents of the romantic love group displayed higher scores, and the female simply adolescents generally showed increased scores irrespective of the group.

Discussion

The key findings of the present study were that adolescents in early-stage intense romantic love (RLG) showed hypomania sum scores as high as those of adult outpatients suffering from bipolar II disorders, as self-rated with the HCL-32 (Angst et al. 2005). This was not the case with respect to the subscales "active/elated" and "irritable/risk-taking": irrespective of the adolescent group (RLG,

Figure 2. Subscale of active/elated hypomania of the Hypomania Check-List 32 (HCL-32) differed significantly between adolescents experiencing early-stage intense romantic love (romantic love group = RLG) and adolescents of the control group (CG), and between female and male participants. Indications: means and standard deviations (error bars).

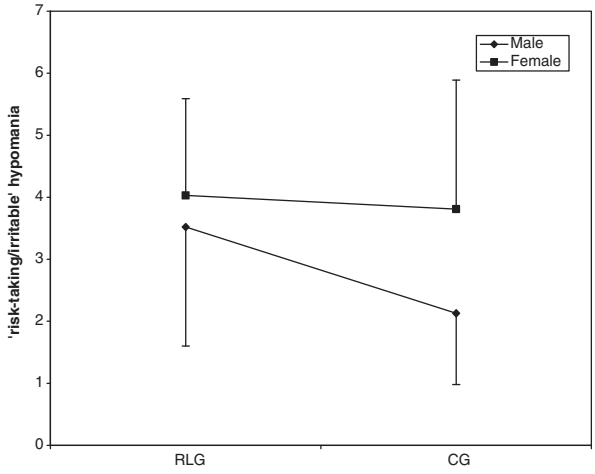

Figure 3. Subscale of risk-taking/irritable hypomania of the Hypomania Check-List 32 (HCL-32) differed significantly between adolescents experiencing early-stage intense romantic love (romantic love group = RLG) and adolescents of the control group (CG), and between female and male participants. Indications: means and standard deviations (error bars).

CG), adolescents showed increased scores for the subscale "irritable/risk-taking" hypomania. Moreover, significant gender differences were observed with increased scores for female adolescents.

The two hypotheses we had formulated are now considered in turn.

First, according to evidence from developmental psychology (cf. Steinberg 1997; Kaplow et al. 2002; Kuttler and La Greca 2004) we expected increased HCL-32 scores in the subscale "irritable/risk-taking" in adolescents when compared to the data of adults suffering from BP II disorders. The results of our study confirmed this assumption, irrespective of the group of adolescents (RLG, CG). Thus, our data fit well in the existing literature which claims that adolescence is a particular period in the life span, that is: To accelerate the independence from the family of origin, but also to establish one's own position and function in the peer group and adult society, the adolescent needs to develop proactive behaviour. In so far as behaviour such as increased sexual activity, social contacts, substance use, creativity, self-confidence, energy, taking risks while driving, as well as decrease of sleep, and psychosocial inhibition is concerned this may be attributed to hypomania as well as to "normal" increased risk-taking at this stage in life. Thus, increased responsibility (for money), car or motorcycle driving, psychosocial and psychosexual disclosure, exploration of the effects of varying substances (cf. alcohol, cannabis, and other psychoactive substances) may be considered normal and necessary developmental tasks. Over the life span, adolescence is normally the first period in which these potentially risky experiences are made (cf. Steinberg 1997; Kaplow et al. 2002; Kuttler and La Greca 2004; Paus et al. 2008). This proactive behaviour is believed to be associated with increased risk taking and novelty- and reward-seeking (cf. Paus et al. 2008). Moreover, our findings suggest that hypomanic stages can be identified by self-assessment (cf. Holtmann et al. 2009). Particularly, being "madly" in love led to increased mood states comparable to those of adult patients with BP II disorders (cf. Brand et al. 2007). However, focusing on the HCL-32 subscales, the pattern of results is more complex, that is, compared to adults with BP II disorders, adolescents showed lower scores in the subscale "active/elated" hypomania and higher scores in the subscale "risk-taking/irritable" hypomania, suggesting that adolescents are living less elatedly but more risky. However, "active/elated" refers to behaviours and cognitive-emotional states such as feeling more energetic and active, enjoying work, being more sociable, or being more self-confident; "risk-taking/irritable" refers to behaviours and cognitive-emotional states such as being less shy, taking more risks when driving, spending more/too much money, taking more psychoactive substances, being irritating and exhausting for others, or being more interested in sexual activities.

Our second hypothesis which postulated gender-related differences, with increased values for female participants, was confirmed by our results. The pattern of results seems to mirror well the literature which demonstrates a higher risk for affective disorders in older adolescents (Dilsaver et al. 2005; Alloy et al. 2006) and moreover particularly in female adolescents (Viguera et al. 2001; Arnold 2003; Angst et al. 2005; Kennedy et al. 2005; Hyde et al. 2008). In our opinion our data present an important contribution to the existing literature because we were able to demonstrate an increased risk for hypomania in female adolescents.

If the subscale "risk-taking/irritable" hypomania is regarded as reflecting instabilities in social contact and rhythms (cf. Meier and Maier 2006), we may assume that female adolescents may be at a higher risk for developing affective disorders and for being more prone to suicide-related behaviour and ideation (cf. Dilsaver et al. 2005; Holtmann et al. 2007). The underlying mechanisms leading to increased scores of hypomania in female adolescent participants compared to male adolescent participants could not be assessed. However, though highly speculative, it could be postulated that female adolescents might be more responsive to and more seeking for social and peer feedback compared to male adolescents (cf. Hyde et al. 2008). A longitudinal study focusing on self-esteem showed that self-esteem

decreased from early to mid-adolescence for female, but not for male participants, suggesting that female adolescents might be more susceptible to psychosocial changes (Heaven and Ciarrochi 2008). In this vein, Kuehner (2003) summarized in her review that intrapsychic and psychosocial gender role related risk factors are identified which may contribute to the higher depression risk in women.

Limitations

Despite the intriguing findings of our study, we want to warn against an overgeneralization of the present findings. First, the low sample size may have led to low statistical power. Second, one might object that it is difficult to compare results obtained from healthy adolescents with results taken from outpatients suffering from BP II disorders and being twice as old as the adolescent participants. Therefore any conclusion drawn from this comparison should be regarded very cautiously. We performed statistical comparisons controlling for age, and there is as yet very little research concerning hypomania during adolescence in general, and with respect to the self-rating questionnaire used in our study (HCL-32; for exception see Holtmann et al. 2009). Therefore, in the absence of findings from adolescent and adult healthy people, we performed the statistical comparison with a sample of adult outpatients suffering from BP II disorders. Third, gender differences might have resulted because male adolescents consider a certain risk taking behaviour as more desirable and "normal" than female adolescents. Fourth, we cannot exclude that some gender differences are unspecific because it is well-known that women give more positive responses to symptom checklists than men. Fifth, self-ratings were not compared to experts' ratings; thus, self-reports could not be compared to clinical diagnoses. Last, self-ratings do not substitute a thorough clinical assessment based on criteria from the DSM-IV or the ICD-10. In this view, the present findings are clearly preliminary data.

To sum up, our data support the dimensional view of a very soft expression of hypomania between normality and subthreshold, with possible confounding developmental task-related biases and gender-related issues. Accordingly, further research focusing on the understudied affective disorders in children and adolescents (cf. Kuttler and La Greca 2004; Kowatch et al. 2005; Pavuhuri et al. 2005; Soutullo et al. 2005), also with respect to gender differences (cf. Geller et al. 2000; Alloy et al. 2006; Johnson and McMurrich 2006), are needed.

Acknowledgments

We thank Alexandre Mueller for data entry. Moreover, we thank Alex Gamma (Zurich, Switzerland) for the data of the Italian sample of outpatients suffering from BP II.

Statement of Interest

The study was conducted without external funding, and all authors declare no conflicts of interest.

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