UNIVERSIDADE DE LISBOA
Faculdade de Medicina de Lisboa

Is depression a risk factor for dementia?
A translational research

Frederico Simões do Couto de Oliveira Fernandes

Orientadores:
Professora Doutora Maria Luísa Caruana Canessa Figueira da Cruz Filipe
Prof. Doutor Joaquim Alexandre Ribeiro

Tese especialmente elaborada para a obtenção do grau de Doutor em Medicina
Psiquiatria e Saúde Mental

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“Ego Petro Yspanus, considerans diuersas egritudinum passiones per negligentiam in corporibus homini generati, quedem utilia et experta ad salutem humana uite sanitatis conseruandam inueni et probaui ratione ueridica que in artis medicine gremio non inueniuntur. Vnde cum sit melis custodire sanitatem quam morbum repugnare, tractandum est de sanitate. (...). Vtilius est enim infirmatem preuenire quam ipsa concepta forte irrecuperabile auxilium postulare (…).

De nocentibus cerebro

(...) hebrietas crebra multum nocent capiti et toti corpori (...) sollicitudo, tristitia, iracundia et cubare capite inclinato (…).”

Pedro Hispano, Liber de Conservanda Sanitate, XIII century

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FSdC participated in the design of the study, in the experiments, in the data analysis and in the writing of the paper.

Frederico Simões do Couto, Nuno Lunet, Sandra Ginó, Catarina Chester, Vanda Freitas, Carolina Maruta, Maria Luísa Figueira, Alexandre de Mendonça. Depression with melancholic features is associated with higher long-term risk for dementia (in press Journal of Affective Disorders doi:10.1016/j.jad.2016.05.026)

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Contents

A. Abstract

Introduction

Depression and dementia are very common and disabling conditions. Most dementia conditions are irreversible, whereby the identification and correction of the risk factors seems to be of paramount importance. Among the several risk factors identified so far, depression emerges as an important target. Several case-control and cohort studies yielded heterogeneous results, but the meta-analyzes performed found approximately a two fold increase in the risk for dementia in depressed patients.

However, the quality of the studies varies widely, and the accuracy of the diagnosis of depression is frequently not the ideal. Furthermore, the nature of the risk is not clear and the following issues have been repeatedly raised: (a) depressive symptoms are quite common in dementia and could be a symptom of this disorder, and not a true, and early, risk factor, (b) depression and dementia are heterogenous disorders, with distinct biologies, and eventually the risk is different, depending on the specific disorder involved, and (c) what is the role of antidepressants? That is, does the risk diminish if the depression is cured? Antidepressants have neuroprotective properties at the molecular level, but the evidence is much less consistent *in vivo*.

To answer these questions, two studies were performed: a clinical longitudinal controlled study and a preclinical behavioral study.

Methods

A cohort of 322 depressed patients (exposed cohort), recruited for a taxonomic study of depression between 1977-84, was built. Subjects without depression, admitted for surgery at the same time as the exposed subjects, were the group not exposed to depression. Subjects were contacted again between 2009 and 2013, to assess their dementia status. The risk for dementia in the depressed cohort was compared to the risk in the surgical cohort using binary regression, and the *odds ratio* were computed (OR). The same analysis was performed in subjects younger than 45 years old (considered to have early onset depression). To quantify the association between different depression subtypes

(namely melancholic, anxious, and psychotic) and dementia, crude and adjusted hazard ratios (HR) were obtained with 95% confidence intervals (95% CI) using Cox proportional hazards regression.

The preclinical behavioral study assessed the cognitive effect, using the Morris Water Maze test (MWM), of escitalopram in rats submitted to a maternal separation protocol (MS; MS is a protocol that induces depressive-like behaviors). A two-way ANOVA was carried out for analyzing probe trial time (using MS and and escitalopram as treatment factors), computing main effects and interactions. Two-way ANOVA repeated measures was used for the learning curve of MWM.

Results

In 133 (41.3%) depressed subjects, followed-up for a mean (standard deviation) of 25.7 (7.2) years, the diagnosis of dementia could be established or excluded. Among these, 44 (33.1%) developed dementia versus 20 (15.0%) among the subjects with no depression at baseline, and this result is significant [OR 2.50 (1.14-5.49; 95% CI); p=0.022]. Subjects with early onset depression had an increased risk for dementia when compared to the surgical cohort patients [OR 6.85 (95% C.I. 1.38-34.00); p=0,019]. Patients suffering from depression with melancholic features had an increased risk of developing dementia compared to those depressed without melancholic features [HR 3.64 (1.78-11.26; 95% CI); p=0.025].

In the preclinical study, all groups of animals showed a significant learning effect in the MWM over time, but no differences have been found upon treatment. However, escitalopram treatment significantly increased the time spent on the platform quadrant in the probe trial in the MS group [F(1.23)=10.764; p=0.004], thus seeming to have improved the memory.

Discussion

The main results of current study are that depression is a risk factor for dementia, with a risk magnitude in line with the longest longitudinal studies with an accurate diagnosis of depression. Two limitations were considered: the lack of formal cognitive assessment at the baseline, and the number of subjects lost to follow. However, the depressed cohort had a low age at baseline, what altogether

with the long follow up, makes unlikely the depressed patients were demented at baseline. The erosion in this study is high, but in line with previous studies with a similar design and follow up time, and the differences between those with and those without a known outcome were minimal and were taken into account.

When exploring the nature of this risk, (a) these results support the hypothesis that depression is an early risk for dementia, again in line with the studies with a stronger design, (b) depression with melancholic features was found as the only depression feature or subtype that was associated with an increased risk for dementia. Melancholia can have a permanent deleterious effect on cognition, but this was the first study showing a higher risk for dementia. Melancholia is associated with hypercortisolism, and it is known that high cortisol damages the hippocampus, providing a biological rational for these findings. However, no biological assessment of HPA activity were made, and this is a limitation of this study. The inclusion of biological markers would support a biological explanation, but would not interfere with the conclusions of the study, and (3) chronic treatment with escitalopram improved hippocampal dependent memory, in a model that induces depressive-like behaviors (MS). Our results are line with the neuroprotective action of antidepressants, but take a step further by showing that escitalopram also improves cognition *in vivo*. Transposition of results from animal studies to humans has limitations, but animal studies allow the use of models that are not easily amenable or ethically allowed to humans, and permitting a reliable evaluation of a number of internal and external factors, such as pharmacological interventions.

Conclusions

The results presented seem to support a role of depression as a risk factor for dementia, and add novel information regarding the nature of this risk. According to these results, depression is not merely a prodrome of dementia, but an early risk factor, and melancholia is the only subtype associated with an increased risk. Also, they point to a neuroprotective action of escitalopram in depression.

Keywords: depression, dementia, risk factor, melancholia, and antidepressants

Sumário

Introdução

A depressão e a demência são condições patológicas causadoras de grande sofrimento. A maior parte das doenças que provocam demência é irreversível, e as terapêuticas existentes têm uma eficácia limitada e não têm efeito demonstrado na progressão da doença. Assim sendo, a identificação dos factores de risco tem uma importância crucial. De entre os vários factores de risco identificados, a depressão reveste-se de uma importância particular.

Vários estudos caso-controlo e de cohort têm demonstrado que a depressão pode ser um factor de risco para demência. Apesar de não ser um achado constante em todos os estudos, as meta-análises realizadas revelam que os sujeitos com depressão têm aproximadamente o dobro do risco de desenvolver demência, quando comparado com não deprimidos. Todavia, os estudos são heterogéneos no que toca à qualidade e, frequentemente, o rigor no diagnóstico de depressão não é o ideal. Para além disso, a própria natureza do risco é pouco clara e algumas questões têm sido frequentemente levantadas.

(a) Não é claro se a depressão é um factor de risco precoce ou um pródromo/manifestação inicial de demência. A depressão é muito comum em demências, especialmente nas fases iniciais, e até tem sido sugerido que surge das mesmas estruturas anatómicas. Assim, estudos caso-controlo que não tenham em conta o tempo entre os dois diagnósticos, ou estudos longitudinais com tempo de seguimento curto, não seriam capazes de distinguir entre estes dois aspectos.

(b) Qual o papel dos sub-tipos de depressão. A depressão é uma doença heterogénea, com diferentes gravidade e sub-tipos, que parecem estar associados a diferentes aspectos biológicos. A abordagem ideal para responder a esta questão seria um estudo idêntico ao anterior, mas com rigor aumentado no diagnóstico de depressão, por forma a permitir distinguir os vários subtipos.

(c) Qual o papel das diferentes demências. Existindo várias causas de demoncia, não é claro a qual das demências a depressão poderá estar associada.

(d) Qual o papel dos antidepressivos, i.e., se a depressão for curada, o risco de demência diminui? Existem evidências pré-clínicas, especialmente *in*

vitro, para um papel neuroprotector dos antidepressivos, pelo que é tentador pensar que o tratamento a longo prazo com antidepressivos pode atenuar o risco de demência. O estudo ideal seria um ensaio clínico longo, em que doentes deprimidos seriam tratados com antidepressivos, tendo como *endpoint* a evolução para demência. Resultam óbvias as impossibilidades materiais e éticas de um trabalho deste tipo. Assim sendo, a evidência terá de ser obtida de outra forma, com as devidas limitações, por exemplo mimetizando o ensaio em modelos animais.

Com base nestas questões, foi desenvolvido o presente estudo. As hipóteses do presente trabalho são (1) A depressão é um factor de risco precoce para demência?, (2) Os diferentes sub-tipos de depressão estão associados a riscos para demência de intensidade diferente?, (3) A depressão é um factor de risco para alguma demência em particular? (4) Se tratarmos com antidepressivos, durante um longo período de tempo, ratos submetidos a um protocolo que induz comportamentos depressivos, a deterioração cognitiva é diferente dos ratos não tratados?

Métodos

Foi realizado um estudo longitudinal, com duas coortes. Um grupo de doentes admitidos no Serviço de Psiquiatria do Hospital de Santa Maria, entre 1977 e 1984, com o diagnóstico de depressão, para um estudo taxonómico de depressão, foi considerada a coorte dos expostos à depressão. Os não expostos foram doentes admitidos no mesmo hospital, na mesma altura, para apendicectomia ou colecistectomia de rotina, sem depressão na altura do internamento.

À entrada, o coorte de deprimidos tinha sido submetido a uma extensa avaliação demográfica, clínica e psiquiátrica, e ainda preenchidos vários questionários, com destaque para a escala de depressões da Associação para a Documentação e Metodologia em Psiquiatra (AMDP - Arbeitsgemeinschaft für Methodik und Dokumentation in der Psychiatrie). Esta escala avalia a presença e a gravidade de todos os sintomas depressivos e foi usada para confirmar o diagnóstico e identificar os sub-tipos de depressão, de acordo com o Diagnostic and Statistic Manual 5 (DSM-5).

Entre 2009 e 2013 os sujeitos de ambos os coortes foram contactados, e os procedimentos do *follow up* tiveram por objectivo determinar se os sujeitos estavam dementes e a data de início da demência. Incluíram uma avaliação demográfica, clínica e psiquiátrica, uma avaliação neuropsicológica extensa, outros instrumentos validados para a população portuguesa (aos próprios e aos familiares), foram contactados os médicos assistentes, revistos os processos hospitalares e eventuais processos em instituições e, caso aplicável, os certificados de óbito. O diagnóstico e a idade de início de demência foram determinados numa conferência de caso, com psiquiatra, neurologista e neuropsicóloga, usando a melhor evidência disponível. Foram usados os critérios do DSM-5 para Perturbação Neurocognitiva Major (demência), implicando sempre pelo menos duas fontes concordantes de informação. Para a determinação do tipo de demência foram usados os critérios habituais em investigação.

O risco de demência no grupo deprimido foi comparado com o risco no grupo cirúrgico utilizando regressão binária, e os respectivos *odds ratios* (OR) foram calculados. A mesma análise foi realizada em indivíduos com idade inferior a 45 anos de idade (considerados como tendo depressão de início precoce). Para quantificar a associação entre os diferentes subtipos de depressão (ou seja, melancólico, ansioso e psicótico) e demência foram obtidos os *hazard ratios* (HR) brutos e ajustados, com intervalos de confiança de 95% (IC 95%), utilizando uma análise de regressão de Cox. Foi ainda estimada a incidência cumulativa de demência tendo em conta o risco competitivo de morte, usando um modelo de regressão de riscos competitivos. O estudo foi desenvolvido de acordo com a Declaração de Helsínquia e foi aprovado pela Comissão de Ética da Faculdade de Medicina de Lisboa e do Centro Hospitalar Lisboa Norte, pela Comissão Nacional de Protecção de Dados e pelo Instituto de Registos e Notariados.

O estudo pré-clínico consistiu na submissão de ratos a um protocolo que se sabe induzir sintomas depressivos - a separação maternal (MS - Maternal Separation). Foi criado um grupo controlo, que não foi submetido a qualquer separação. A metade dos animais de cada grupo foi administrado escitalopram na comida durante um mês. Todos os animais foram submetidos a um teste de comportamento do tipo desespero aprendido (o Forced Swimming Test) e, posteriormente, um teste de memória e aprendizagem (MWM - Morris Water

Maze). Foi avaliada a curva de aprendizagem no MWM e o tempo passado no quadrante onde estava a plataforma (*probe trial*, indicativo de memória de longo termo). O tempo no *probe trial* do MWM foi calculado através de uma *two-way* ANOVA, usando MS e escitalopram como fatores de tratamento), e foram calculados os efeitos principais e as interações. A ANOVA *two-way repeated measures* foi utilizada para a curva de aprendizagem do MWM.

Resultados

Em 133 (41,3%) dos sujeitos deprimidos, que foram acompanhados em média (desvio padrão) durante 25,7 (7,2) anos, o diagnóstico de demência pode ser estabelecido ou excluído. Destes 133, 44 (33,1%) desenvolveram demência, contra 20 (15,0%) nos indivíduos sem depressão no início do estudo, e este resultado é significativo [OR 2.50 (1.14-5.49; 95% CI); p=0.022]. Os indivíduos com depressão de início precoce tiveram um risco aumentado de demência [OR 6.85 (95% C.I. 1.38-34.00); p=0,019], quando comparado com os sujeitos da coorte cirúrgica. Os sujeitos com depressão melancólica tiveram um risco aumentado de desenvolver demência em comparação com aqueles com depressão sem características melancólicas [HR 3,64 (1,78-11,26; I.C. 95%); p = 0,025]. Estes resultados foram confirmados por várias análises de sensibilidade.

No estudo pré-clínico, todos os grupos de animais apresentaram um efeito significativo na aprendizagem no MWM ao longo do tempo, mas não foram encontradas diferenças entre os grupos. No entanto, o tratamento com escitalopram aumentou significativamente o tempo gasto no quadrante onde estava a plataforma (*probe trial*) no grupo MS [F (1,23) = 10,764; p = 0,004], parecendo assim que melhorou a memória.

Discussão

(1) O risco dos sujeitos com depressão desenvolveram demência foi, aproximadamente, o dobro dos sujeitos sem depressão, o que está de acordo com os estudos com desenho mais forte - longitudinais e com mais rigor no diagnóstico de doença depressiva;

(2) os sujeitos com depressão com início antes dos 45 anos têm um risco de demência aumentado, quando comparados com sujeitos não deprimidos, com

a mesma idade e sexo. Este achado aponta no sentido da depressão ser um factor de risco precoce e não apenas um pródromo/manifestação inicial da demência. Este achado está em linha com os estudos longitudinais com tempos de seguimento mais longos, mas não com estudos caso-controlo, em que o tempo que medeia os dois diagnósticos não é tido em conta, ou é determinado de forma pouco rigorosa. Eventualmente o desenho destes estudos não permite avaliar de forma correcta a existência de depressão, incluindo formas de menor gravidade;

(3) os sujeitos com depressão melancólica têm um risco aumentado de demência comparados com não deprimidos, o que não parece acontecer nos sujeitos deprimidos sem sintomas melancólicos. Este achado, que nunca tinha sido estudado antes desta forma rigorosa, poderá explicar a heterogeneidade dos resultados dos vários estudos. A depressão melancólica tem sido associada de forma mais consistente com achados biológicos relacionados com a hiperactivação do eixo hipófise hipotálamo-suprarrenal. Tem sido demonstrado que o cortisol actua nos receptores dos glicocorticóides (GR) no hipocampo, levando à atrofia deste órgão, o que parece fornecer um racional biológico para a depressão melancólica estar associada a um risco aumentado de demência. No entanto, não foram feitas avaliações biológicas da actividade HPA, e esta é uma limitação do presente estudo. A inclusão de marcadores biológicos apoiaria uma explicação biológica, mas não iria interferir com as conclusões;

(4) o tratamento crónico com escitalopram melhorou a memória episódica, num modelo que induz comportamentos depressivos-like (MS). Os nossos resultados estão linha com a ação neuroprotetora de antidepressivos, mas dão um passo em frente, mostrando que o escitalopram também pode melhorar a cognição *in vivo*. O estudo ideal para esclarecer o papel de antidepressivos na modulação do risco de demência em sujeitos deprimidos seria um ensaio clínico. Todavia, um ensaio deste tipo seria muito caro e difícil de justificar eticamente. A transposição dos resultados de estudos animais para os humanos tem limitações, embora estudos com animais permitam a utilização de modelos que não são eticamente permitidos ou facilmente passíveis de serem realizados em seres humanos, e também permitem uma avaliação fiável de vários factores, como p.ex. intervenções farmacológicas.

Conclusões

Os resultados aqui apresentados parecem apoiar a ideia de que a depressão é um factor de risco para a demência. Parecem também ajudar a esclarecer a natureza desse risco, na medida em que a depressão não parece ser meramente um pródromo de demência, mas antes um factor de risco precoce, e que a melancolia é o único sub-tipo associado a um risco aumentado. Além disso, esses resultados apontam para uma ação neuroprotectora do escitalopram nos défices cognitivos crónicos na depressão.

Palavras-chave: depressão, demência, factor de risco, melancolia, antidepressivos.

B. Introduction

The World Health Organization (WHO) proposition "No health without mental health" reflects both the increased awareness of the importance of mental disorders, and the challenge to prevent and treat these disabling conditions. The WHO reports on the burden of mental disorders have been quite impressive, as neuropsychiatric disorders are considered the first cause of disability-adjusted life-years (DALYs; an integrated measure of disease burden[1]) in high income countries (Prince, Patel et al. 2007). In the high income countries, such as Portugal, neuropsychiatric conditions are responsible for around 30% of all DALYs of non-communicable diseases. The first and second conditions responsible for more DALYs are depression (10%) and dementia (2%).

Dementia has a syndromic conceptual definition, and can be caused by several disorders. Most of these disorders are irreversible and the available therapies have limited beneficial effects, highlighting the importance of the identification and control of risk factors. This strategy could assume a major relevance for dementia prevention (Peters, Beckett et al. 2008, Ritchie, Carriere et al. 2010, Fotuhi, Do et al. 2012, Norton, Matthews et al. 2014). Among the several risk factors identified so far, depression emerges as potentially important one (Reitz, Brayne et al. 2011), although controversial (Byers and Yaffe 2011, da Silva, Goncalves-Pereira et al. 2013). Depression is amenable to prevention, has a high prevalence, is diagnosed inexpensively, and the disease can be treated effectively (Kupfer, Frank et al. 2012). If it was possible to delay the onset of dementia by 1 year, the prevalence of dementia would be reduced by 12 million cases in 2050 (Paillard-Borg, Fratiglioni et al. 2009).

However, the way these conditions are related is not clear, and disentangling these possible different relations is a crucial step for the design of preventing strategies. Depression can be a prodrome of dementia, because depressive symptoms are common in dementia, especially at the initial stages. It has even been suggested that depression could arise from the anatomical lesions of dementia (Boland 2000). On the other hand, or perhaps concomitantly, depression can be an early risk factor for dementia.

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[1] The sum of years lived with disability with years of life lost.

Other questions have been raised, as both conditions are heterogenous disorders, and the different subtypes can have different risk magnitudes, or show no relation. We believe that the relative paucity of longitudinal studies, without accurate methods to diagnose both conditions, is limiting a deeper understanding of the nature of this complex relation.

The role of antidepressants is also unclear. That is, if the depression is treated, does the risk wane? The ideal study to answer this question would be a very long clinical trial comparing treated with non treated depressed patients. Obviously, such a trial is ethically unacceptable. Animal models offer an alternative in these circumstances. Depression and other disorders can be induced in animals in a manner that is not easily amenable or ethically allowed to humans, and animal models also permite a reliable evaluation of a number of internal and external factors (e.g. modifications with drug therapy).

In conclusion, depression is probably a risk factor for dementia, but the nature of this relation is complex and unclear. Because dementia disorders are mainly irreversible conditions, a deeper understanding of the nature of this risk will have an important impact in dementia prevention.

1. Dementia

1.1. Historial evolution

Etymologically, dementia derives from the latin word *dementatus*, which means to be out of out of one's mind or to have no mind. It seems that Celsus in the I century A.D. was the first to use the word dementia in a medical context. Throughout history dementia has been indistinguishable from other psychiatric disorders, although at same points the word was used to describe loss of memory and judgment (Mahendra 1987).

Until 19th century, two opposite views on the relation of aging and loss of capacities were kept. One, called discontinuity, reflected the opinion of Cicero that the loss of capacities - *senilis stultitia* - only occur in those at risk. Another point of view, called "continuity" was defended by a contemporary of Cicero, Lucretius - the decline of intelectual (and physical) abilities are inexorable consequences of aging (Huppert, Brayne et al. 1994).

It is not clear whether Pinel or Esquirol used a more contemporary approach for the word dementia. Pinel described cognitive impairment as an irreversible failure in the association of ideias, with several symptoms, thought to occur in the elderly. He also coined the term "senile dementia". Some authors report that Esquirol was the first to classify dementias. As Esquirol changed his point of view throughout his life it is not clear who in fact coined the actual concept. Esquirol defined "acute dementia" (short lived, reversible, following fever or hemorrhage), "chronic dementia" (irreversible, cause by masturbation, melancholia, mania, epilepsy, and others) and "senile dementia" (resulted from aging and consisted in a loss of the faculties of understanding) (Mahendra 1987).

Kraepelin distinguished functional psychosis (*insanities*) from disorders with an obvious brain damage (varieties of *imbecility*). Under the acquired imbecilities (dementia), Kraepelin included apoplectic dementia, old age, and epilepsy (Myron, 2009). Later, putting together the work of Alzheimer, Pick, Kraepelin and others, Roth & Morrissey (1952) divided the mental diseases peculiar to old age in senile, arteriosclerotic, and presenile (including Alzheimer's disease and Pick's disease) psychosis. Psychosis was used in the sense of a severe mental disorder.

Due to the acknowledgement of an organic basis for these conditions, the diseases causing dementia were classified in DSM I and II in the Chapter "Organic

Brain Syndromes" (cited in Kaplan 1st and 2nd Edition), and a closer approach to the present concept was published in DSM III.

The several diseases causing dementia have been elucidated throughout history. On the XIX century, Griesinger[2], who belonged to the school of "somatics", considered that psychic diseases are caused by organic diseases, and was the first to describe senile dementia as a result of arteriosclerois in the cerebral blood vessels (Mahendra 1987). Although the general paralysis of the insane was described in the XVII century, its relation to *Treponema pallidum*, and neurosyphilis were discovered in the first half of the last century (Weiner and Lipton 2009).

The most well known advance in the field, however, was achieved by a german psychiatrist, Alois Alzheimer. Dr. Alzheimer described a case of a 51-years old female patient (August D), who had shown progressive cognitive impairment disease, along with focal symptoms, hallucinations, and delusions (Maurer, Volk et al. 1997). The neuropathological findings at necropsy (senile plaques and neurofibrillary tangles) defined the disease, and are still today considered the hallmarks of the disease (American Psychiatric Association 2013). The eponym was originally referred to presenile dementia, but actually it referes to the largest cause of dementia.

Other causes of dementia has been described, and refined, throughout the years, such as Frontotemporal Dementia (FTD) (Kertesz 2007, Rascovsky, Hodges et al. 2011) or Dementia with Lewy Bodies (DLB) (Goedert, Spillantini et al. 2013). A more detailed description of these subtypes can be found in Section 2.3..

Before progressing to dementia, patients usually exhibit mild, but increasing, cognitive impairments. These deficits reflect the progressive neurodegeneration promoted by the several causing diseases. These stages, their meaning and definition, have been extensively discussed in the literature (reviewed by Gerstenecker and Mast 2014). However, Petersen et al. (1999) defined conceptually the condition, called Mild Cognitive Impairment, and

[2] His quote "*psychical disorders are brain disorders*" are frequently cited, in the context of biological psychiatry

proposed the first operational criteria. In DSM-5 the condition is referred as Minor Neurocognitive Disorder, existing on a spectrum with Major Cognitive Disorder.

1.2. Epidemiology

According to the report from the Alzheimer's Disease International (2009), it is estimated that globally dementia affected 35,6 millions of people worldwide in 2010. These numbers will probably duplicate each 20 years, up to 115,4 millions in 2050.

We are aware of only two studies reporting epidemiological data of dementia in Portugal. The first, performed by Garcia et al (1994), calculated the prevalence of dementia projecting to the Portuguese population the European data gathered by the EURODEM study (Hofman, Rocca et al. 1991, Rocca, Hofman et al. 1991). The estimated prevalences, for 1991 and for individuals over 60 years, were 2.4% for dementia (about 92 000 cases) and 1.3% (about 48 000 cases) for AD.

A more recent epidemiological study, developed in the North of Portugal, yielded an overall prevalence of dementia of 2.7% for individuals between 55 and 79 years-old (Nunes, Silva et al. 2010). The prevalence was higher in rural areas, increased in with age, decreased with education years and was also higher in individuals with cerebrovascular disease.

1.3. Clinical features, diagnosis and neurobiology

Conceptually, dementia is a clinical condition characterized by a cognitive decline. It has been defined operationally by the Diagnostic and Statistical Manual of Mental Disorder 5th Edition (DSM-5; American Psychiatric Association 2013) and by the World Heath Organization Internacional Classification of Diseases 10th Edition (ICD10) (ICD10; World Health Organization 1992). According to DSM-5 criteria (the most used criteria in research) the core symptom of dementia - called "Major Neurocognitive Disorder" (Major NCD) - is a significant decline in one or more cognitive functions. This decline must be significant and have an impact in the independence to perform the activities of daily living, and must not occur exclusively in the course of a *delirium. A*lthough neuropsychological testing can support the diagnosis, the diagnosis of dementia is clinical (Garcia 1984),

according to both ICD10 and DSM-5. DSM criteria are easier to use, are considered accurate (Knopman, DeKosky et al. 2001), and are the most used criteria in research for dementia diagnosis[3].

At least one cognitive domain should be affected, including complex attention, executive function, learning and memory, language, perceptual-motor, and social cognition according to DSM-5. For ICD10 a memory impairment plus several other deficits is mandatory for the diagnosis. Because the core symptoms are the cognitive impairments, it is conceptually distinct from other psychiatric disorders, such as bipolar disorder or schizophrenia; in these disorders, although a cognitive decline can be documented, it is not the core feature.

It is recommended to document the cognitive deficits by neuropsychological tests (NT). NT assess brain functions that, with certain restrictions, can be viewed as separated and that can be correlated with certain brain areas. Attention, executive functions, learning, memory or language are exemples of cognitive domains commonly assessed by NT. A NT yields a quantitative record, expressing the difference from normality. Normality is a key issue for NT, as a normative database for a test is essential to ensure that the deficits seen in a given individual are not related to age, years of education, native language or others.

A very important number of NT have normative data and validated versions for the Portuguese population. It is noteworthy the efforts of the Grupo de Estudos de Envelhecimento Cerebral e Demências, that have already published three editions of Escalas e Testes na Demência (GEECD 2015). The last edition intends to have detailed psychometric (in what concerns to validity - a test's ability to measure what it proposes to measure - and reliability - the consistency of times scores), and normative data for a vast number of tests.

As mentioned above, the cognitive decline should be severe enough to interfere with daily activities. However, this is not easy to define operationally.

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[3] Three important differences exist in the diagnostic criteria for dementia between DSMIV and DSM-5. (1) A single cognitive deficit is required for the diagnosis of dementia, instead of two, and a memory impairment is not mandatory for the diagnosis. (2) Also a new diagnostic category, for less severe forms of cognitive impairment, was created. (3) The etiologies were reformulated and updated with more recent criteria. We believe these changes, although not fully evaluated, reflect the more recent research in the field. Namely the existence of dementia without or with very mild memory impairment, the importance of mild cognitive impairment, and the accumulating knowledge on amyloid detection, the classification of FTD, or the new LBD criteria.

DSM-5 states there must be an interference with the independence in activities of daily living (at the minimum, assistance is required for complex activities) and performance below 2 standard deviations (s.d.) bellow appropriate norms for the diagnosis of Major Neurocognitive Disorder (Major NCD). For Minor NCD (an equivalente of Mild Cognitive Impairment), DSM-5 specifies that these complex activities must be preserved, but a greater effort, compensatory strategies or accommodation are required to be performed. A difference between 1 and 2 s.d. is required in neuropsychological evaluation. These criteria are openly arbitrary, because DSM-5 assumes the continuity between normality, Minor NCD and Major NCD. ICD10 defines a threshold of impairment as an interference in daily activities not enough to loose independence.

The guidelines for the evaluation of dementia in Portugal are still under discussion (DGS 2011). The proposal recommends the use of screening tests for the detection of the cognitive impairment, such as the MMSE (Folstein, Folstein et al. 1975, Guerreiro 1998) or the MOCA (Nasreddine, Phillips et al. 2005, Freitas, Simoes et al. 2011), both validated for the Portuguese population.

If a diagnosis of dementia, or NCD, is made, the next step is to explore the etiology. It is considered good clinical practice the prescription of laboratory analysis (complete blood count, fasting serum glucose, serum creatinine, serum electrolytes (including calcium), liver functions tests and enzymes, thyroid hormones, B12 vitamin, folic acid and syphilis serology) and brain scan (CT or MRI) to exclude reversible conditions and to provide information for the etiological diagnosis (DGS, 2011, Sorbi, Hort et al. 2012). These prescriptions are also valid for Mild Cognitive Impairment (Pereira, Simoes do Couto et al. 2006).

The etiological diagnosis for each dementia is driven by the clinical picture and eventually confirmed by specific exams (Sorbi, Hort et al. 2012). There are several standard criteria that can be used such as the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's disease and Related Disorders Association) criteria (McKhann, Knopman et al. 2011) for AD, the NINDS-AIREN (National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences) criteria (Roman, Tatemichi et al. 1993) for vascular dementia, the criteria proposed by the International Behavioural Variant FTD (fronto-temporal

dementia) Criteria Consortium (Rascovsky, Hodges et al. 2011) for the behavioral variant of fronto-temporal dementia (FTD), and the criteria proposed by McKeith et al (2005) for Lewy Body dementia.

Several conditions can cause dementia (Table 1), although mixed forms are common (Attems, Konig et al. 2005, Santana 2005, Beach, Adler et al. 2015).

Table 1 Causes of dementia

1. Alzheimer's disease
2. Fronto-temporal dementias
3. Lewy body dementia
4. Parkinson's disease dementia
5. Cortiocobasal degeneration
6. Progressive supranuclear palsy (PSP)
7. Vascular dementia
8. Chronic subdural haematoma
9. Hypoxia related to hypoperfusion
10. Mixed dementia (AD and VD)
11. Normal pressure hydrocephalus
12. Wilson´s disease
13. Huntington's disease
14. Vitamins deficitis
    Vitamina B1, vitamina B12, niacine, folate.
15. CNS infections
    Syphilis, borreliosis, tuberculosis, brucellosis, viral encephalitis, VIH infection, prion diseases.
16. Endocrine diseases
    Thyroid, parathyroid, and adrenal.
    Chronic metabolic disturbances (e.g. uremia)
17. Intoxications[a]
    Aluminum, copper, mercury, plumb, zinc, arsenic, alcohol.
    Other (pesticides, flame retardants, solvents, etc).
18. Neoplasia
    Primary and metastatic CNS tumors.
    Paraneoplasic syndroms.
19. Trauma
20. Immune diorders
    Systemic lupus erythematosus, temporal arteritis.
21. Hepatic or renal failures
22. Metabolic conditions
23. Radiation
24. Electric shock
25. Other neurological conditions
    Epilepsy, multiple sclerosis.
26. Other
    Idiopathic cerebral ferrocalcinosis, metachromatic leukodystrophy, neuroacanthocytosis

Adapted from Santana (2005) and Neurgoschl (2004). Notes: [a](Genuis and Kelln 2015).

There are important variations in the frequency of these different disorders, depending on the sample characteristics (age, dead or alive, country of origin, how the recruitment took place, etc), on the diagnostic criteria, and others. In clinical series this discrepancies are noteworthy. E.g., the frequency of LBD range from 0 to 30 % (reviewed by Zaccai, McCracken et al. 2005).

A notable serie of a community sample, non demented at study entry, was published very recently involving 1173 autopsies (Beach, Adler et al. 2015) . It was found that the most common cause of dementia at the autopsy was AD (57%), followed by PD (14%), VD (9%), LBD (9%), and PSP (6.8%). However, mixed forms were quite common (around 40%). Other causes are rarer and, as whole, account for about 12%. Reversible causes of dementia are extremely rare, such as vitamina B12 deficiency or hypothyroidism.

In Portugal, it is estimated that AD and VD are equivalent, affecting about 40% of the population (Nunes, Silva et al. 2010). However, in this paper the criteria for diagnosing the different dementia disorders were not clearly defined, and probably more accurate data on this subject is mandatory. In a study performed at our center, in MCI subjects, no reversible dementia was found (Pereira, Simoes do Couto et al. 2006).

a) AD

AD is the most common form of dementia, and is considered the most important dementia condition and a paradigm of dementia.

Although described more than a century ago, the histopathological changes described by Dr. Alzheimer are still used today, with some important refinements, for the definite diagnosis of the disease (Heyman, Fillenbaum et al. 1990, Braak and Braak 1991). AD is characterized by the presence of senile plaques (SPs), neurofibrillary tangles (NTs), and neurodegeneration and cell death specially in the hippocampus and in other medial temporal lobe structures. SPs (or neuritic plaques) are extracellular aggregates of a protein (beta-amyloid or Aβ) and NFTs are composed of hyperphosphorylated tau protein. The mechanisms of SPs and NFTs formation have been studied (reviewed by Blennow, de Leon et al. 2006). Aβ is released when a transmembrane protein, amyloid precursor protein (APP), is cleaved by two amyloidogenic enzymes, the β- and γ-secretases. APP can be

cleaved by a different secretase, called α-secretase, to yield two non-amyloid fragments, non pathogenic.

Drachman (1977) reported memory disturbances after the administration of scopolamine[4] to healthy volunteers, and the blockage of this effect with a pretreatment with physostigmine, a cholinesterase inhibitor. A few years later, a significant neuronal loss in the *nucleus basalis of Meynert* - an important cholinergic nucleus in the brain - in AD patients was described (Whitehouse, Struble et al. 1982). Although a myriad of neurochemical deficits have been described in AD, these findings opened the way to the most consistent explanation for its cognitive disturbances - the cholinergic hypothesis. Cholinergic hypothesis implies that the disturbances in central muscarinic cholinergic mechanisms are related to the loss of memory present in AD (Bartus, Dean et al. 1982, de Mendonça and Simões do Couto 2005).

The relation between SPs, NFTs, and the neurodegeneration is certainly controversial. It has been proposed that amyloid is the key pathogenic element of AD and the process of amyloid induced degeneration is called amyloid cascade (Blennow, de Leon et al. 2006). The main support for this theory is driven from genetic mutations. Mutations that affect APP synthesis and proteolysis[5], lead to and excessive production and early deposition of amyloid, and to an early-onset familiar form of AD (Wu, Rosa-Neto et al. 2012).

Although it has been considered the main pathogenic hypothesis for AD, it has been challenged repeatedly. Other researchers believe that NFGs play the most important role in the pathogenesis of the disease. They are called “tauoists", and their criticisms have become more consistent in the last few years (Herrup 2015), especially because therapeutics that remove amyloid seem not to stop cognitive deterioration (Karran and Hardy 2014).

Another biological mechanism involved is glutamate mediated neuronal toxicity. Although essential for several cognitive processes, an excessive activation of glutamate receptors can lead to neuronal lesion, or even neuronal dead (Lipton and Rosenberg 1994).

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[4] Scopolamine is competitive blocker of muscarinic receptors

[5] Such as APP, Presenilin 1 (PSEN1) and Presenilin 2 (PSEN2) gene mutations

The diagnosis of AD is based on the clinical features of the disease plus the exclusion of other causes (McKhann, Knopman et al. 2011). This approach, operationally defined by the first NINCDS-ADRDA criteria, can lead to a sensitivity of 81% and a specificity of 71% (Knopman, DeKosky et al. 2001).

The clinical picture of AD is characterized by an insidious onset of a decline in memory and learning. These deficits are progressive, without worsening periods related to cerebrovascular events. Although other cognitive deficits can occur, they usually are less severe than memory and learning.

Remarkable advances happened in the last few years in the field of the diagnosis of AD (de Mendonca 2012). DSM-5 is actually reflecting in a small extent these changes, and included a biomarker for AD, specifically a mutation known to cause the disease. However, other biomarkers are also currently available in reference centers, and are a very important tool for the diagnosis of the disease (specially when the diagnosis is doubtful or have therapeutic or management implications). They are also quite often required as inclusion criteria for clinical trials.

The principal AD biomarkers that can detect the disease in the earlier stages are the quantification of Aβ in the cerebrospinal fluid (CSF) and the positron emission tomography (PET) scan with an amyloid marker (most commonly PIB (Pittsburgh compound), although others are under development). Lower levels of Aβ in CSF and increased captation of the radiolabeled Aβ ligand in the PET scan correlate with amyloid deposition in autopsy and seem to predict the development of dementia (Sperling, Aisen et al. 2011). Biomarkers of synaptic dysfunction, specially PET scan with radio labeled glucose (e.g. [^{18}F]FDG or fludeoxyglucose), show a decreased metabolism in temporo-parietal cortex (McKhann, Knopman et al. 2011). Elevated CSF tau is not specific for AD and is thought to be a biomarker of neuronal injury. Although it appears later in the course of disease, hippocampal atrophy is a biomarker of AD-related neurodegeneration.

It is expected that the inclusion of these biomarkers in the diagnostic criteria will increase the diagnostic accuracy (McKhann, Knopman et al. 2011).

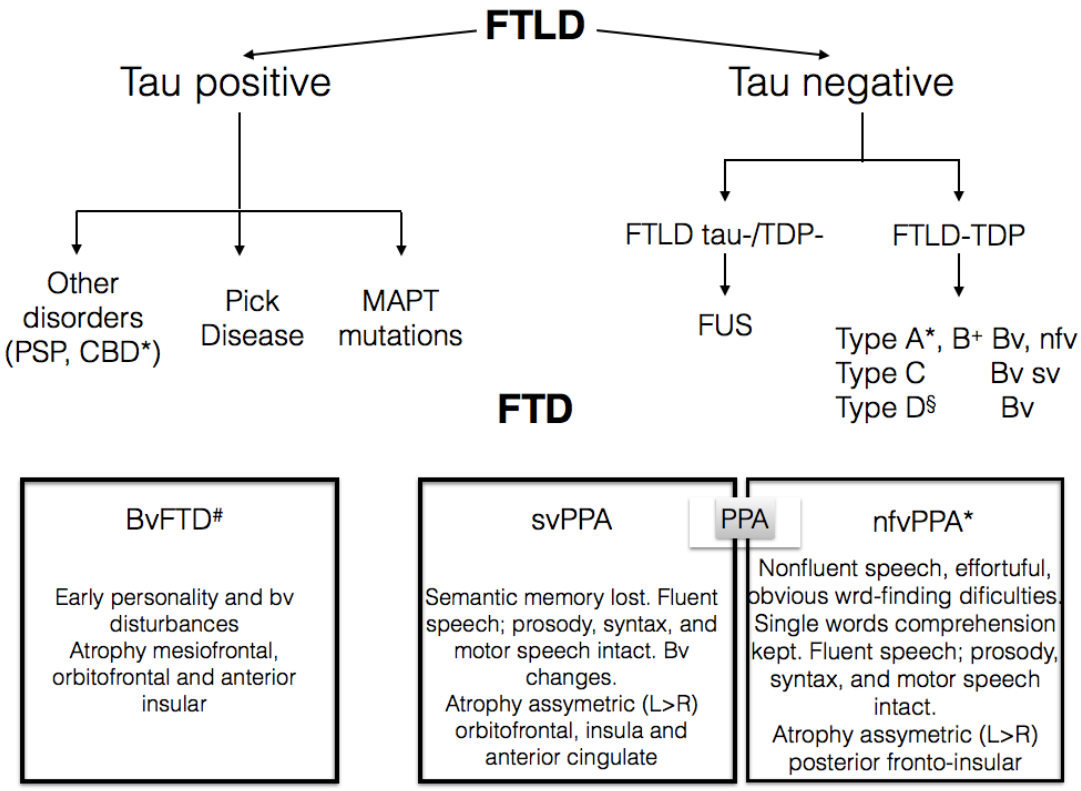
b) Frontotemporal dementia (FTD)

FTD is a heterogeneous group of neurodegenerative disorders affecting frontotemporal areas. The clinical syndrome results from frontotemporal lobar degeneration (FTLD) associated with a range of heterogeneous pathologies (Rascovsky, Hodges et al. 2011). The first case was described in 1892 by Arnold Pick, and Pick's disease was used for a synonymous of FTLD until recently. Today, Pick's disease is used only to described a subgroup of FTLD with specific histopathological features. Based on early and predominant symptoms, DSM-5 distinguishes two different clinical syndromes: the behavioral variant and a language variant. The language variant has often been subdivided in two: semantic dementia and nonfluent primary progressive aphasia (Waldo 2015).

FTLD is caused by an abnormal accumulation of proteins, and its neuropathological subtypes are defined by the different accumulating proteins. Although a considerable overlap existis between these conditions, efforts has been made to classify them (Riedl, Mackenzie et al. 2014). The best studied group represents roughly 40% of all FTLD and is called tau-positive. It is characterized by the accumulation of microtubule-associated protein tau (MAPT) and is called FTLD-tau. It includes the classic Pick's disease, the cases of familial FTLD caused by mutations in MAPT genes, and other neurological disorders (progressive supranuclear palsy and corticobasal degeneration). The tau-negative FTLD includes the accumulation of transactive response DNA-binding protein with molecular weight 43 kDa (TDP-43), and is called FTLD-TDP. This form represents roughly 50% of all forms, is related to mutations on the C9orf72 gene, and includes the FTLD condition and amyotrophic lateral sclerosis. The remaining 5-10% cases are tau and TDP negative, and are characterized by the deposition of another protein, called fused in sarcoma protein (FUS). Additional very rare conditions have been described.

The classification of FTD and FTLD is a controversial area. Each FTLD disease does not necessary presents with a specific FTD syndrome, and even mutated genes can have different clinical presentations. Furthermore, some clinical and histological defined conditions share common features, or can exist simultaneously, and overlap with other disorders, namely atypical Parkinsonian syndromes.

Figure 1 The classification of Frontotemporal Lobar Degeneration and Frontotemporal Dementia



Legend FTLD Frontoremporal Lobar Degeneration, FTD Frontotemporal dementia, PSP Progressive Supranuclear Palsy, MAPT Microtubule-Associated Protein Tau, TDP transactive response DNA-binding protein with molecular weight 43 kDa, FUS Fused with Sarcoma, BvFTD Behavioral variant, PPA Progressive Primary Aphasia, svPPA Semantic Variant, nfv Nonfluent Variant, L Left, R Right, # Associated with MAPT gene mutations, * Associated with Progranuline gene mutations, + Associated with C9orf72 gene mutations (Riedl, Mackenzie et al. 2014)

Both variants - behavioral and language - are characterized by progressive dementia with a relative sparing of learning and memory functions. Clinically, the behavioral variant (bv-FTD) can present itself with disinhibition, loss of empathy, apathy, repetitive behaviors, and hyperorality. The language variants are defined by a prominent decline in language ability. Three forms are usually described: (1) semantic dementia, with a decline in word meaning, but with a fluent speech and preservation of repetition; (2) agramatic/nonfluente, with a decline in speech

fluency and sentence construction, and anomia; (3) logopenic, with slow speech, impaired syntactic comprehension and naming (Harciarek and Kertesz 2011).

The International Behavioural Variant FTD Criteria Consortium (Rascovsky, Hodges et al. 2011) and DSM-5 (American Psychiatric Association 2013) share globally the same criteria. The inclusion of biological markers is also a common feature, and the presence of a known causative mutation and a disproportionate frontal and/or temporal lobe involvement can turn the diagnoses from possible to probable.

Neuroimaging is probably different in these variants (Harciarek and Kertesz 2011, Kirshner 2014). In the bv-FTD the atrophy involves specially the medial frontal lobes and the anterior temporal lobes. In SD, the temporal lobes are atrophic, but asymmetrically (left side usually more affected). Agramatic/nonfluente is predominantly associated with left posterior frontal-insular atrophy, while logopenic variant has left posterior perisylvian or parietal atrophy. These findings were incorporated in the DSM-5, although were not considered diagnostic criteria.

c) Dementia with Lewy Bodies (DLB) and Parkinson's Disease Dementia (PDD)

Parkinson's disease (PD) was described almost 200 years ago and its characteristic neuropathological lesion - Lewy bodies (LB) - almost 100 years after (reviewed by Goedert, Spillantini et al. 2013). The LB are composed of α-synuclein, ant it is believed that this protein is the key feature in the development of the clinical symptomatology (Beach, Adler et al. 2009).

Although LB were found in the brain stem nuclei of PD patients, establishing the neuropathological basis of PD in 50s, it was believed that they were rarely found in the cerebral cortex. Later, some cases suffering from atypical AD with parkinsonism were found to have numerous intracytoplasmic eosinophilic inclusions at the deeper cortical layers and typical LB in the brain stem (Kosaka 1978). The term Lewy Body Disease was proposed to these cases, and 4 types have proposed, with different degrees of brain stem and cortical involvement (Kosaka, Yoshimura et al. 1984, reviewed Kosaka 2014)[6]. Diffuse Lewy Body Disease was applied to the cases with predominant cortical involvement, and a

[6] Type A (brain stem type, consistent with PD), type B (transitional type), type C (diffuse type), and later a cerebral type of LBD was added (with LB in the cortex, but rare in the brain stem; no Parkinsonian symptoms).

relative sparing of the brain stem. When diffuse Lewy body disease was found associated with dementia, it was not clear whether there was a “senile dementia of the Lewy body type” or a “Lewy body variant of Alzheimer’s disease”. Several international conferences and workshops took place and the term Dementia with Lewy Bodies was proposed and the operacional criteria set (McKeith, Galasko et al. 1996).

DLB criteria have refined more recently (McKeith, Dickson et al. 2005), and largely incorporated into DSM-5 (American Psychiatric Association 2013). LBD has been considered the second most frequent cause of neurodegenerative dementia in some series (Donaghy and McKeith 2014).

DLB is characterized by dementia, spontaneous parkinsonism (starting after the onset of the cognitive decline, or at a maximum of one year before), recurrent well formed visual hallucinations, fluctuating cognition, rapid eye movement sleep behavior disorder (RSBD), and severe sensitivity to antipsychotic medications (Beach, Adler et al. 2009). DLB consortium guidelines are considered the gold standard for diagnostic purposes (McKeith, Dickson et al. 2005). They include a central feature (dementia), 3 core features (parkinsonism, visual hallucinations and fluctuating cognition), and 3 suggestive features (RSBD, neuroleptic sensitivity and a biomarker - the low dopamine transporter (DAT) uptake in basal ganglia, demonstrated by PET our SPECT). Several supportive features are listed, considered to be commonly present, but of probably without an impact in the the diagnostic accuracy. DSM-5 (American Psychiatric Association 2013) follows in general the DLB consortium guidelines, although biomarkers were not included in the criteria.

The cognitive deficits seen in DLB include visuospatial, attention and executive functions (Metzler-Baddeley 2007), rather than learning and memory.

d) Vascular dementia (VD)

As mentioned before, dementia caused by cerebrovascular disease was first suggested by Griesinger in the 19th century (Huppert, Brayne et al. 1994). Since then, several terms have been used to described this condition, such as arteriosclerotic dementia, multi-infarct dementia, post-stroke dementia, vascular dementia, vascular cognitive impairment (VCI), and vascular cognitive disorder

(Jellinger 2013). Vascular cognitive disorder encompasses all different types of cerebrovascular lesions that led to cognitive impairment and eventually to dementia.

Brain infarcts are the most important pathology that contributes to cognitive impairment. An increased number of infarcts, an increased infarct size, and certain locations (specially the thalamus, angular gyrus, and basal ganglia) have been associated with an increased risk for dementia, but determining the number, the exact location, or the volume needed to have dementia has not yet been established (Gorelick, Scuteri et al. 2011).

The brain is critically dependent on blood supply, and sophisticated local mechanism regulate the amount of blood supply needed to match the brain energy requirements. These mechanisms are profoundly disrupted in VCI and in AD, leading to chronic hypoxia and inflammation. Besides these microvascular changes, also macrovascular changes are biological mechanisms associated with VCI (Gorelick, Scuteri et al. 2011).

The Religious Order Study ("Nun study") found that only people with AD pathology and subcortical infarcts had dementia (Snowdon, Greiner et al. 1997), suggesting that cerebrovascular lesions increased the risk of developing dementia, or clinical AD, in patients with AD (Gorelick, Scuteri et al. 2011).

Although NINDS-AIREN criteria (Roman, Tatemichi et al. 1993) are still the gold-standard research criteria for VD, the concept of a spectrum of vascular cognitive impairment has been recently incorporated into DSM-5 (American Psychiatric Association 2013) and into other important guidelines (Gorelick, Scuteri et al. 2011).

The diagnosis is made when dementia is present, and the cerebrovascular lesions, shown by neuroimaging, can be related to the cognitive dysfunction. The clinical picture is classically of an acute onset of the cognitive deficit, a stepwise progression, and the presence of focal neurological signs suggesting a sufficient cerebrovascular burden to produce the cognitive deficits. Personality and mood changes are also common, such abulia, depression, and emocional lability. Complex attention and executive functions are usually affected.

Obviously, neuroimaging is a powerful diagnostic tool, especially if silent infarcts exist. The diagnostic accuracy is increased if a genetic disease associated

with cerebrovascular disorder is present, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).

The term *mixed dementia* is used to describe a condition in which AD pathology and cerebrovascular lesions co-occur.

1.4. Treatment

The treatment of secondary dementias is the treatment, if possible, of the condition actually causing the disease. E.g., if the cause of dementia is B12 vitamine deficiency or hypothyroidism, the treatment of these conditions would cure dementia, or at least stop the cognitive deterioration.

The treatment of neurodegenerative dementias is merely symptomatic. As the majority of these conditions is due to AD it is not surprising that the research on the treatment of this disorder is much more extensive.

Primary prevention trials have been done for dementia. A number of factors have been studied (stimulating cognitive, physical and social activities, vascular risk factors - obesity, diabetes, dyslipidemia, etc), but this trials have globally failed, and no therapy is recommended for the primary prevention, or for delaying the onset of dementia (Sorbi, Hort et al. 2012).

a) Alzheimer's disease

There are several targets and approaches for the treatment of AD. The targets are (1) cognition, (2) activities of daily living (ADL), (3) behavioral and psychiatric symptoms of AD (Peter V. Rabins 2014). The approaches for each target can be either pharmacological or non-pharmacological or both.

Currently approved drug treatments for cognition in AD have only shown symptomatic improvement, meaning that an effect in disease progression has never been shown. They can be divided according to their mechanism of action in two classes, cholinesterase inhibitors and NMDA receptor modulators.

Based on the cholinergic deficit of AD, several attempts have been made to increase cholinergic transmission (de Mendonça and Simões do Couto 2005), such as the administration of choline and other acetylcholine precursors, muscarinic receptor agonists, and neutrophic factors. However, the most successful strategy until now has been acetylcholinesterase inhibition, thereby

increasing acetylcholine in the synaptic cleft. There are three cholinesterase inhibitors (CholI) approved for the treatment of AD in Portugal - donepezil, rivastigmine, and galantamine. Although some differences can be found between these compounds, their efficacy does not differ significantly and, more important, an impact on the progression of the disease has not been shown (Rafii and Aisen 2015). Their side effects are mainly an extension of their pharmacological properties, and so cholinergic effects emerge as the most common.

The other target for the treatment of AD has been the the NMDA receptor. As discussed above, glutamate and NMDA receptors involvement in the apoptosis and neurodegeneration of AD has been documented (Lipton and Rosenberg 1994, Cacabelos, Takeda et al. 1999). Therefore, their blockage seems a logical target. However, NMDA receptor has a primordial role in the processes of learning and memory, meaning that its antagonism would have significant cognitive effects. The use of NMDA antagonists for AD has been refined through the use of memantine, a moderate affinity inhibitor of this receptor. Memantine is also an uncompetitive antagonist, with a fast voltage dependent kinetics. These properties seem to explain its different actions with mM and μM glutamate concentrations. Memantine rapidly leave the NMDA channel during physiological activation by mM concentrations of glutamate. However, when sustained activation by μM concentrations of glutamate under moderate pathological conditions occur, memantine acts as antagonist, blocking the receptor (Rammes, Danysz et al. 2008). Its mechanism of action can be understood as restoring the equilibrium of NMDA receptors (Rogawski and Wenk 2003, Zadori, Veres et al. 2014).

Other targets for cognitive improvement are currently being in development in clinical trials, such as BACE inhibition and monoclonal antibodies anti amyloid beta (Rafii and Aisen 2015).

Non-pharmacological approaches for AD have been developed since the 60s and 70s. However, in recent years an increase in the interest in this area led to a significant increase in the number of publications, but also an increase in the heterogeneity of the approaches and their evaluation (Guerreiro 2005, Bahar-Fuchs, Clare et al. 2013, Peter V. Rabins 2014). Nonspecific interventions targeting cognitive functioning, such as reality orientation therapy, have shown benefits in demented or AD patients. More recently, more specific approaches,

such as cognitive rehabilitation and cognitive training, designed to help maintaining or increasing cognition in demented and AD patients have been designed[7]. The Cochrane review in this topic did not find evidence of a positive effect on the cognition of cognitive training. Only one study addressing cognitive rehabilitation was included, and albeit the results seemed promising, no differences in cognition were found (Bahar-Fuchs, Clare et al. 2013).

Globally, non-pharmacological therapies for dementia and AD seem promising, but the heterogeneity and the low quality of the studies, inherent to the nature of the therapy[8], somehow precludes a definitive conclusion on their efficacy (Guerreiro 2005).

b) Vascular dementia

Drug treatments approved for AD have also been tried on VD, but none has received regulatory approval. However, donepezil for VD and galantamine for mixed dementia have shown a symptomatic benefit in these conditions, and they are recommend by several guidelines (Sorbi, Hort et al. 2012), including the Portuguese guidelines (DGS 2011).

Few studies addressed the efficacy of non-pharmacological interventions in VD. Cognitive rehabilitation, cognitive stimulation and acupuncture studies in human VD were inconclusive or showed no efficacy (Sorbi, Hort et al. 2012).

Besides cognitive symptoms, management of vascular risk factors and symptomatic treatment are the recommend treatments for VD.

c) Other neurodegenerative dementias

Rivastigmine is approved for the treatment of PDD in Portugal and has shown efficacy for the symptomatic treatment of DLB (Sorbi, Hort et al. 2012).

Although inconsistent benefits, in several domains, have been shown to occur with several drugs in FTD, currently no drug treatments have been approved for the treatment of this condition (Sorbi, Hort et al. 2012).

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[7] Cognitive rehabilitation is built to address individual needs and cognitive training focusing on the improvement of cognitive functions, such as memory or problem solving.

[8] Other issues mentioned in the these studies are the heterogeneity in the outcome, in the techniques used, in the sample and in the setting.

2. Depression

2.1. Historical evolution

Depression is considered a mood disorder - a group of disorders in which the core symptom is a pathological mood, accompanied by related vegetative and psychomotor disturbances (American Psychiatric Association 2013).

Depression has been conceptualized in different ways, but in general it describes a condition characterized by low, sad or empty mood, less pleasure, changes in sleep and appetite, diminished libido, retardation, guilt, suicidal thoughts, pessimism, feelings and thoughts of hopelessness, helplessness and worthlessness, and eventually psychotic symptoms (Bastos, Gonçalves-Ferreira et al. 2014).

The word *depressão* has been related to the latin etymon *deprimere*, to press down (Machado 2003). The first descriptions of a disease probably similar to what is today considered today depression have been attributed to the ancient Greek physician Hippocrates, and was called melancholia. He described melancholia as a distinct condition characterized by "aversion to food, despondency sleeplessness, irritability and restlessness" (Marneros and Angst 2000). He provided a biochemical cause for this state - an excess of black bile - and called the disorder melancholia (meaning black bile).

Another mood state is called mania, and its etymology is not clear, as several hypothesis have been raised (Marneros and Angst 2000). Anyway, the disease fitting the actual concept of mania was also described by Hippocrates. It is characterized by expansive mood, talkativeness, increased energy, less need to sleep, and eventually psychotic symptoms (American Psychiatric Association 2013, Figueira and Madeira 2014).

The historical concepts of depression, melancholia, mania and bipolar disorder do not correspond to the actual concepts. In fact, many classifications, and conceptualizations took placed throughout the years (reviewed by Marneros and Angst 2000). Mood disorders do not have a clear etiology, and their diagnosis and classification has relied mainly on symptoms. Other classic medical nosographic criteria, such as the evolution, prognosis, response to therapy and etiology (the latter largely unknown) played a secondary role (Sousa 2003).

However, a milestone has been achieved with Kraeplin. Kraeplin cautiously followed his patients for many years, and used the above referred key medical criteria for the classification of mental diseases. The classification of mental disorders he provided is still the basis of the modern classifications. Kraeplin distinguished *dementia praecox* (the actual representative is schizophrenia) from manic depressive illness. He used the term manic-depressive insanity to include, on the one hand, what was called melancholia and related states, and on the other, what was called circular insanity (and other forms of mania). He stressed out that, from a clinical point of view, it was impossible to distinguish depressive episodes occurring in a patient with a previous episode of mania from those without (Marneros and Angst 2000).

Kraeplin's unified conceptualization was soon challenged. Wernick and Kleist proposed the terms and developed the concepts of unipolar (only mania or only depression) and bipolar disorder (merely a co-occurrence of the two disorders), still considering they were the same disorder (Marneros and Angst 2000). Karl Leonhard in 1957 followed a cohort of depressed patients for a longtime and distinguished unipolar (only manic episodes or only depressive episodes) from bipolar (depressive and manic episodes concurring in the same patient). After Leonhard definition, in 1966 the independent seminal studies of Jules Angst (Angst 1966, reviewed by Marneros and Angst 2000, and by Bastos, Gonçalves-Ferreira et al. 2014) and Carl Perris (1966) provided consistency to the separation. Family history and evolution were different in these disorders and the data supported Leonhard's distinction. These studies then were translated into the Research Diagnostic Criteria and into DSMIII (Lara 1984).

Both DSM-5 (American Psychiatric Association 2013) and ICD10 (World Health Organization 1992) separate unipolar from bipolar affective disorders. DSM-5 even treats these disorders in separate chapters, although pure mania is considered a bipolar disorder.

With the rise of psychodynamic views, etiology started to play a determinant role for the classification of the disorders. The DSMI and DSMII largely reflect psychoanalytic views, classifying the disorders as reactions and did not provide diagnostic criteria. Probably, the disappointment with psychodynamic concepts, the use of psychopathological standard measures, and the development of

mathematical applications to clinical psychopathology (Sousa 2003) led a radically different approach to psychiatric disorders, called neokraeplinian (Compton and Guze 1995). These methods were used to establish profiles of symptoms, analyze the symptoms structure, and get the original nosologies, creating empirical classifications - dimensional or typological (Sousa 2003).

These new methods led to a new definition of psychiatric diseases, translated into the Research Diagnostic Criteria (RDC) (or Feighner criteria), created at the Washington University School of Medicine in St. Louis (Spitzer, Endicott et al. 1975, Spitzer, Endicott et al. 1978). These criteria were largely the basis for the next DSM and ICD editions.

In Portugal, Manoel Paes de Sousa and António Fernandes da Fonseca were important researchers of this current. António Fernandes da Fonseca proposed the concept “affective equivalents” to describe certain monosymptomatic conditions of psychic and somatic nature (not obviously mood phenomena) witch gave evidence of being connected to a latent mood state, and that should be considered forms of manic depressive illness (Da Fonseca 1963). He studied a group of patients, all having a twin sibling, and their relatives. He found an increased incidence of somatic disorders, of cyclic nature, with a tendency to resist to medical treatment, and to improve “after the first straightforward attack of periodic depression”. The long term careful observation of the symptoms, the rigorous registries, and the search for common features (personality, evolution, response to treatment, etc) were common features in the works of this current.

Manoel Paes de Sousa studied psychiatry with José Henrique Barahona-Fernandes. Barahona-Fernandes was an eminent Portuguese psychiatrist, who worked with Karl Kleist in Frankfurt, and developed an extremely important work on the field of psychopathology. At 1982, in recognition of his work, Barahona-Fernandes received the Julius Wagner von Jauregg medal, in the World Psychiatry Congress. Manoel Paes de Sousa was also en eminent psychopathologist, and performed a serie of psychopathological studies in a cohort of depressed patients, aiming a classification of depression based on psychopathology. He and his team (Maria Luísa Figueira, Carlos Roldão Vieira, among others) performed an extensive psycopathological evaluation, using among others, an AMDP derived depression scale. AMDP is an abbreviation of

Arbeitsgemeinschaft für Methodik und Dokumentation in der Psychiatrie (Documentation and Methodology in Psychiatry Association), an international working group of German origin, that focus on standardizing data collection and psychopathological concepts (Sousa 1985).

Using K-centroids method and a factorial analysis, three distinct depressive disorders were found - “neurotic”, “psychotic” and an intermediate mixed group (Sousa 1976). Several other studies were published, on the same cohort and with a similar methodology, generally providing a rational for the separation in two classes “neurotic” and “endogenous” (Sousa, Lopes et al. 1980). Unfortunately, the overall results were never published in peer reviewed journals (Sousa 2003) and, after his untimely death, in an extraordinary gesture of generosity, his widow donated Dr. Paes de Sousa files to be developed further research.

With this background of controversy in the classification of depression, we decided to follow as reference the most used research classification manual in psychiatry, the DSM-5. DSM-5 and ICD10 share the most important sub-types, although some differences exist.

2.2. Epidemiology

According to the Estudo Epidemiológico Nacional de Saúde Mental in Portugal (Almeida 2013), the anual prevalence for any mood disorder is 7,9% (1% dysthymia, 6,8% MDD and 1,1% BD). When compared to other European countries these figures are amongst the higher ones, only surpassed by France and Northern Ireland.

Lifetime prevalence of any mood disorder is 19,3% (1,4% dysthymia, 16,7% MDD and 2,6% BD). The most significant risk factors found were being a woman and having lower education. Again, in Portugal these numbers are amongst the highest, comparable to the USA and Lebanon (15% and 19%) (Weissman, Bland et al. 1996).

Depression is associated with an increased risk of dead, by suicide and cardiovascular diseases (Wulsin, Vaillant et al. 1999, Wulsin, Evans et al. 2005).

2.3. Clinical features, diagnosis and neurobiology

a) Bipolar depression

This condition referes to a depressive episode that occurs in a patient with bipolar disorder. DSM-5 and ICD10 criteria does not distinguish the symptoms of a depressive unipolar episode from a depressive episode in a bipolar disorder.

b) Major depressive disorder

Major depressive disorder (MDD), called recurrent depressive disorder in ICD10, is the classic unipolar depression.

The core symptoms are low mood and loss of pleasure, accompanied by several other symptoms. Depressive symptoms have been divided in affective/ emotional (sadness, anxiety, anhedonia, etc), somatic (sleep and appetite changes or pain), motor/behavioral (inhibition or agitation) and cognitive (Bastos, Gonçalves-Ferreira et al. 2014). Cognitive symptoms include thought disorder (delusions, pessimism) and classical cognition disturbances, such as memory, attention, and executive functions (Reppermund, Ising et al. 2009, Marazziti, Consoli et al. 2010). Sometimes, cognitive symptoms are so severe, that the acute depressive disorder resembles a dementia disease - it is labelled pseudo dementia (Kiloh 1961, Berrios 1985). A recovery of cognition is expected (Keefe, McClintock et al. 2014), although this a controversial issue as discussed in the next section.

According to DSM-5, the diagnosis can be made with a single episode, although the disease tends to recur, with patients experiencing more than one episode. ICD10 distinguishes between a single depressive episode and a recurrent disorder.

c) Persistent depressive disorder or dysthymia

This disorder includes chronic depressions of a lesser severity than the MDD. It is a large and heterogenous group, that includes the previously called neurotic depressions, depressive neurosis, or depressive personality (Bastos, Gonçalves-Ferreira et al. 2014). The term was coined by Kahlbaum, to distinguish from cyclothymia (Marneros and Angst 2000), but throughout the years it has been found difficult to distinguish the depressive disorder from personality (Freeman

1994). Akiskal conceptualized the disorder as a mood disorder, and this is the actual point of view (Akiskal 1981).

ICD10 states that dysthymia is a very protracted depressive disorder (decades), but its severity is seldom sufficient to fulfill the recurrent depressive disorder criteria. DSM-5 has a pragmatical approach, including in this condition the chronic MDD (more than two years).

Probably due to its intrinsic heterogeneity, several conditions have been associated with this disorder (Akiskal, Lemmi et al. 1984, Arriaga, Cavaglia et al. 1998).

d) Melancholic depression

Both DSM-5 and ICD10 consider melancholic depression (or “somatic syndrome“ as it is called in ICD10) a specifier of a depressive episode. It is defined clinically by a distinct quality of the depressed mood, virtually irresponsive to all kind of pleasurable stimuli, along with a near complete loss of pleasure. Other classic melancholia features can be also present, such the morning mood worsening, terminal insomnia, significant psychomotor retardation or agitation, significant anorexia and weight lost, excessive guilt (self-blaming), and, for ICD10, diminished libido. Melancholic features can also be present in the depressive episodes of bipolar disorders.

Melancholia emerges among depressive disorders as a more quintessentially biological disorder, especially because it is characterized by a more favorable response to biological treatments than to psychotherapy, by a low placebo response, by a lower relevance of psychosocial determinants, and by the existence of abnormalities of the hypothalamic-adrenal-axis (HPA axis) (Fink and Taylor 2007, Parker, McCraw et al. 2013).

HPA axis activity is controlled by the secretion of adrenocorticotrophic hormone-releasing factor (CRF) and anti-diuretic hormone (ADH) from the hypothalamus. These hormones stimulate the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, which in turn stimulates the adrenal cortex to secrete glucocorticoids. HPA is activated in stress situations. Glucocorticoids have several functions, at multiple targets, but in the brain their most significant actions

are at glucocorticoid receptors (GR)[9]. They are responsible for the feedback inhibition of CRF and ADH on the hypothalamus and of ACTH on the pituitary. When activated, HPA has profound effects in the acquisition of new memories and the emotional appraisal of events, in the regulation of neuronal survival, in neurogenesis, and affects the sizes of complex anatomical structures such as the hippocampus (Herbert, Goodyer et al. 2006, Pariante and Lightman 2008).

The association of glucocorticoids with mental disorders dates back to the late 40s of the XX century. Increased urinary excretion of glucocorticoid metabolites was found in mentally ill patients (Reiss, Hemphill et al. 1949). Many subsequent studies found evidences of an increased HPA axis drive in depression: elevated total and free cortisol concentrations in plasma, cerebrospinal fluid and urine, ACTH concentration reported as normal or increased (Carroll, Cassidy et al. 2007), disruption of the orderliness of cortisol secretion (Posener, Charles et al. 2004), non suppression of cortisol with use of dexamethasone (Carroll, Curtis et al. 1976), and more recently the cortisol awakening response (Dedovic and Ngiam 2015). It seems that the glucocorticoid-mediated feedback inhibition is, at least partially, the mechanism responsible for this HPA overdrive in depression (Pariante and Lightman 2008).

HPA axis dysfunction also seems to reflect a susceptibility that can be induced in early life. Rodents pups separated from their mothers - an eventual equivalent of the human child abuse - showed hyperactivity of HPA axis that persists through adulthood (Sanchez, Ladd et al. 2001, Sousa, Vital et al. 2014). Also, patients that suffered from childhood trauma or abuse have an increased activation of HPA axis (Heim and Nemeroff 2001).

The consequences of this HPA dysfunction have been studied in rodents and in humans. Chronically elevated cortisol has been shown to induce decreased neurogenesis and monoamine dysfunction, regional brain changes, and impaired synaptic plasticity (Herbert, Goodyer et al. 2006).

Treatment with antidepressants has been shown to increase GR expression and function and GR-mediated HPA axis feedback inhibition in laboratory animals

[9] Glucocorticoids also act on the brain mineralocorticoid receptor (MR), but their role is less well understood

as well as in humans (Pariante and Lightman 2008). It also seems that antidepressants correct HPA dysfunction (Schule 2007).

However, there is a marked heterogeneity in depressive patients with respect to HPA axis changes. Several causes have been appointed, such as frequency of sampling, analytic methods and heterogeneity of patients (Posener, DeBattista et al. 2000). As mentioned, homogenous samples of melancholic patients consistently show abnormalities related to HPA axis dysfunction (Rothermundt, Arolt et al. 2001, Kaestner, Hettich et al. 2005, Carroll, Cassidy et al. 2007, Fink and Taylor 2007, Parker, Fink et al. 2010, Lamers, Vogelzangs et al. 2013, Parker, McCraw et al. 2013), when compared to non-melancholic patients.

The inclusion of melancholic depression as a distinct depressive disorder, and not as a mere specifier, has been repeatedly proposed (Parker, McClure et al. 2015).

e) Atypical depression

Atypical depression is a form of depression, presenting reverted vegetative symptoms - hypersomnia and increased appetite or weight (Bastos, Gonçalves-Ferreira et al. 2014). Additional symptoms are leaden paralysis, mood reactivity, and increased sensitivity to rejection in personal relations (American Psychiatric Association 2013). These are recognized as atypical features in DSM-5, although the concept is not present in ICD10.

Several studies found evidences for a distinct disorder, including brain laterality, psychological profile, psychiatric co-morbidity, (Pae, Tharwani et al. 2009), differential treatment responses (Hyman Rapaport 2007), and different biological features, specially an excessive inhibition of the stress responses (O'Keane, Frodl et al. 2012, Gold 2015). However, the symptom profile has been frequently challenged (Parker, Parker et al. 2005, Thase 2009).

f) Psychotic depression

Psychotic depression is a depression with psychotic symptoms (delusions and/or hallucinations) according to DSM-5 and ICD10 (Bastos, Gonçalves-Ferreira et al. 2014). However, ICD10 also classifies depressive stupor under the psychotic depression subtype.

Although the classic psychotic symptoms of depression are mood congruente delusions (guilt, hypochondria, deserved punishment, nihilism/Cotard, bankrupt), their significance is controversial. DSM-5 also allows of both congruent- and incongruent-mood psychotic features for the diagnosis.

Psychotic depression has been longtime (Lara 1984) considered a special type of depression, but more recente studies provided a scientific validation of psychotic depression (Johnson, Horwath et al. 1991, Schatzberg and Rothschild 1992).

Psychotic depression has also significant biological abnormalities, although controversial. The most consistent findings describe changes similar to melancholia (Belanoff, Kalehzan et al. 2001), however others point to similarities with primary psychotic disorders (Domschke 2013).

g) Depression with mixed features

This specifier was added to latest DSM-5 edition. The DSM-5 definition of mixed features of major depressive episodes captures sub-syndromal manic/ hypomanic symptoms that can occur in MDD (Perugi, Quaranta et al. 2014). Mild mixed features are a risk factor for the development of bipolar disorder. To take this risk in account, it is clinically useful to use this specifier (American Psychiatric Association 2013).

h) Depression with anxious distress

The relation of anxiety and depression is a longstanding discussion in psychiatry (Lara 1984). Anxiety is a common symptom in depressive disorders, but for ICD10 and for DSM IV only pure depressive and pure anxiety disorders were considered. Whether they are (1) the same disorder with a different clinical picture, (2) totally independent disorders, or (3) independent disorders with significant overlapping areas is a matter of controversy (Arriaga, Cavaglia et al. 1998).

Although ICD10 and DSMIV TR generally consider depression and anxiety disorders as different conditions, DSM-5 includes a specifier for depressive disorders with important anxiety symptoms. This specifier, named “With anxious distress” includes several anxiety symptoms, both mental and somatic. A

Pubmed® search with the quoted frase “With anxious distress” performed in August 2015, yielded no results, probably reflecting the low level of research and knowledge on this new feature.

i) Catatonic depression

Catatonia, originally described by the German psychiatrist Karl Kahlbaum, in 1874 (cited by Fink 2013), is etymologically derived from the greek *cata*, meaning down, and *tone*, meaning tone.

Historically, catatonia is related to a set of motor symptoms, such as mutism, a rigid posture, fixed staring, stereotypic movements and stupor, mainly associated with schizophrenia. However, it seems that, at least in inpatients, the vast majority of catatonias are mood disorders (Parker, McClure et al. 2015).

DSM-5 does not treat catatonia as an independent disorder, but it can be assigned to another psychiatric disorder (e.g. depression with catatonic features), to a medical disorder (e.g. malignant neuroleptic syndrome), or unspecified. DSM-5 diagnosis includes motor immobility (e.g. stupor, or way flexibility), decreased engagement during interview (e.g. mutism), and excessive or peculiar behaviors (e.g. stereotypy, echolalia). ICD10 does not recognize a catatonic depression, assigning a depression with stupor - a nearly complete loss in “reactivity to the environment and reduction of spontaneous movements and activity” - to a depression with psychotic symptoms.

Catatonia can be treated with benzodiazepines and electroconvulsive therapy, irrespectively of the underlying disorder. Though, it has been argued that, although its biological basis is not known, catatonia should be considered a disorder by its own (Fink 2013, Parker, McClure et al. 2015).

j) Other subtypes or specifiers

Other specifiers are recognized in DSM-5, including depression with a peripartum onset and depression with a seasonal pattern.

Both DSM-5 and ICD10 have criteria for the severity of the current episode (mild, moderate and severe).

2.4. Treatment

The treatment of depressive disorder is specially dependent on the severity of the disorder and, with few exemptions, is quite different in unipolar and bipolar depressions (Yatham, Kennedy et al. 2013, Bastos, Gonçalves-Ferreira et al. 2014, Figueira and Madeira 2014, reviewed by Jeong, Lee et al. 2015).

General measures (such as sleep hygiene, physical exercise, or meditation) are recommended for every stage of disease severity (NICE 2009, National Collaborating Centre for Mental Health 2010). Psychotherapy has also an important role in the treatment of depression, either unipolar or bipolar (National Collaborating Centre for Mental Health 2010, Bastos, Gonçalves-Ferreira et al. 2014, Swartz and Swanson 2014, Driessen, Hollon et al. 2015, McQueen and Smith 2015).

However, drug therapy, especially in the moderate and severe stages of unipolar depressions and in every stage of BD, is the cornerstone of the treatment. The main drugs that can be used to treat depression are some mood stabilizers (MS) and, eventually, antidepressants for BD, and antidepressants for MDD, and eventually, for other unipolar depressions. Many other drugs can be used in the treatment of these conditions, such as benzodiazepines, other anxiolytics, thyroid hormones, or pindolol, e.g., but mainly as ancillary or potentiation therapies.

Antidepressants

The first drug that can be called antidepressant, and not merely a mood enhancer, was an agent to kill Koch Bacillus - iproniazid, a molecule similar to isoniazid. In the early 1950's, doctors noted a unexpected happiness namely in severely ill patients, dying form tuberculosis, when they were treated with iproniazid. It was later found that iproniazid acts as a monoamine oxide inhibitor (MAOI) and several clinical studies were performed (Deverteuil and Lehmann 1958). It was marketed in 1958, and discontinued 3 years after in almost all countries because of its hepatotoxicity, and replaced by other MAOI.

Almost simultaneously, a modified molecule of the tricyclic compound chlorpromazine - called imipramine - showed unexpected mood improvement in chronic psychotic patients in Switzerland. A German language paper was published initially (Kuhn 1957), followed by an English publication (Kuhn 1958), revealing the efficacy of this new compound in depressed patients. Imipramine

was shown to inhibit nor-epinephrine (NE), and later, also 5-hydroxytryptamine (5-HT) (or serotonin) re-uptake (Allikmets, Vahing et al. 1969, Lapin and Oxenkrug 1969). This re-uptake inhibition occurs through the inhibition of NE transporter (NET) and 5-HT transporter (SERT). This class of AD is known as TCAs - tricyclic antidepressants. Besides transporter inhibition, TCAs also interfere with an array of other receptors (specially adrenergenic alpha-1, muscarinic M1, and histaminergic H1), responsible for the majority of the severe adverse events associated with this class of drugs (Stahl 2008, Câmara-Pestana and Carmo 2014).

The history of selective serotonin re-uptake inhibitors (SSRIs) is quite different. There was a need for safer antidepressants, and the evidences for a role of serotonine in depression were coming out (Coppen 1967). Researchers believed that a biochemical test would distinguish between the depressions that could improve with 5-HT or those with NE enhancers (Claassen, Davies et al. 1977). In the search for such compounds, fluvoxamine was successfully tried (Saletu, Schjerve et al. 1977), followed by the other SSRIs (fluoxetine, sertraline, paroxetine, citalopram, and escitalopram).

More recently a fourth antidepressant class was introduced, the 5-HT/NE re-uptake inhibitors (SNRI). These antidepressants selectively block these transporters, without significantly interfering with other receptors (Stahl 2008, Câmara-Pestana and Carmo 2014).

Other classes of antidepressant have been discovered throughout the years, such has the selective 5-NE re-uptake inhibitors, the selective dopamine/NE re-uptake inhibitors, among others.

The main criteria for prescribing a specific antidepressant is the adverse events profile, and the antidepressant with a worst adverse event profile tend to be used in refractory depressions (Bastos, Gonçalves-Ferreira et al. 2014).

Although 5-HT and NE re-uptake inhibition have been throughout the years the most important mechanism associated with antidepressant efficacy, an important issue arose soon after antidepressant's discovery. The action of antidepressant on monoamine transporters, and on the monoamines boost, is very rapid, but the clinical effects of antidepressants take certainly a longer time to occur. TCAs induce a selective increase of the inhibitory response of 5-HT, but this

increase of serotonin response took 1 to 2 weeks to develop (de Montigny and Aghajanian 1978). A few years later, it was shown that if the SSRI zimelidine is applied to rat dorsal mesencephalic raphe nucleus, a total suppression of the firing rate[10] of these neurons is observed (Blier and De Montigny 1983). However, two weeks later, under a continued zimelidine treatment, the 5-HT neurons firing rate returned to normal. In the same study, the use of LSD (an 5HT autoreceptor[11] agonist with high efficacy) completely blocked the firing rate, but had no effect on the longtime pretreated neurons. This indicated that a down regulation of the autoreceptor was the cause for the delay of the clinical antidepressants effect. Other antidepressants and ECT have been also been associated with the down regulation of several receptors (Invernizzi and Garattini 2004, Blier and El Mansari 2013).

Further studies showed that these down regulations are not merely an acute desensitization of the receptors, but imply changes in gene expression and editing (Albert and Francois 2010). Chronic treatment with fluoxetine, and other AD, edits and/or increases the expression of several genes related to the neurotransmission of 5-HT, GABA, glutamate, BDNF, and others (Nestler, Barrot et al. 2002, Hertz, Rothman et al. 2015).

Mood stabilizers

MS are the first choice in bipolar depression. The concept of MS is not universal, but in *stricto sensu* are drugs that treat and prevent affective episodes of bipolar patients. A broader definition encompasses those drugs that treat episodes of one pole, without inducing the other. The classic MS is lithium carbonate, although several anti epileptics (sodium valproate, carbamazepine and lamotrigine), and some second generation antipsychotics (aripiprazol, olanzapine and quetiapine) can be also considered MS.

The treatment of bipolar depression is complex, but most guidelines indicate a combination of lithium and/or sodium valproate with lamotrigine, or quetiapine, as first line. It is noteworthy that AD should not be used as first or second line therapy in most guidelines (Jeong, Lee et al. 2015).

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[10] Firing rate is crucial for 5-HT release

[11] These autoreceptors decrease 5-HT release (actually most of them are known as 5-HT1A)

3. Depression and dementia

3.1. How are they related?

As noted before, depressive symptoms are common in dementia - especially in the initial stages, reflecting an eventual reaction to the loss of capacities. Cognitive symptoms can be so severe in patients with depression that they can mimic dementia (so-called pseudomentia). Besides acute deficits, accumulating evidence reveals that depression, especially chronic, can be associated with persistent cognitive deficits. Finally, depression can be an an early risk factor, or a prodrome of, dementia (Byers and Yaffe 2011, Kessing 2012).

3.2. Is depression a risk factor for dementia?

It has been known for a longtime that cognitive deficits are present during acute mood episodes, so much they are a diagnostic criteria for MDD (American Psychiatric Association 2013). The presence of permanent (i.e. in periods without mood symptoms) cognitive deficits in depressed patients was recognized more recently (Robinson, Thompson et al. 2006, Rock, Roiser et al. 2014). Eventually because, according to the Kraepelinian nosology - that continues to provide a touchstone for modern classification systems of mood disorders - depression and bipolar disorder are non-degenerative disorders. However, even Kraepelin wrote about an autopsy report of bipolar patient “(…) widespread arterio-sclerosis of the blood vessels of the brain. This termination appears to be not altogether unusual is maniacal depressive insanity (…)” (Kraepelin, 1913; cit. by da Silva, Goncalves-Pereira et al. 2013). But further than inducing permanent cognitive deficits, in recent years depression emerged as possible risk factor for dementia and AD, initially in case-control studies and later in cohort-studies.

Case-control studies are simpler, easier, and cheaper then cohort studies. In case-control studies demented patients are compared to non-demented patients in what concerns to a prior diagnosis of depression (Fronteira 2013). These studies yield an odds-ratio (OR) value, that must have a 95% confidence interval superior (or inferior) to one, to presume a positive (or a negative) risk association. However, the relative risks cannot be calculated (Grimes and Schulz 2008), and are subject to several biases, specially recall biases (Schulz and Grimes 2002, Fronteira 2013).

Cohort-studies are very expensive and hard-working studies that generate very powerful information regarding causality (Fronteira 2013). In these studies, a cohort of individuals, with and without depression (exposed and non-exposed), are followed for a period of time. They are then examined and compared to their dementia status. The absolute risks can be calculated, so the power of the statistic is stronger.

Many studies have been published using both designs, and some of the most important are summarized in Table 2 and Table 3.

Table 2 Case-control studies

Study	Sample	Depression diagnosis/criteria	Dementia diagnosis /criteria	Conclusions
1	40 AD 80 controls	Question to subject or family	"Rigorous clinical criteria"	NS
2	78 AD 78 controls	Psychiatric disorder, questioning	Histopathology Author's criteria	NS
3	188 AD 80 control	Chart review	ICD9 DSM III R	2.90 (0.64-13.15)*
4	98 AD 162 controls	Question to informant	NINCDS-ADRDA	3.3 (1.1-10.1)
5	170 AD 170 controls	Question to subject or family	NINCDS-ADRDA	3.30 (1.20-8.70)
6	415 AD 415 controls	Episodic depression	Author's criteria	1.71 (1.03-2.83)
7	294 AD 300 controls	Question to subject or family	DSM III R NINCDS-ADRDA	NS
8	81 AD 51 controls	Question to subject or family, DSMIIIR	NINCDS-ADRDA Neurological + NP	2.08 (1.22–3.52)
9	69 AD 69 controls	Question to subject or family	DSM IV NINCDS-ADRDA	2.60 (1.65-4.09)**
10	599 dementia 599 controls	History Medical Files ICD8-10	CAMDEX ICD8-10	2.31 (1.16-4.61) UP* 7.46 (1.50-7.07) BD**
11	65 twin pairs discordant for AD	Registries Informant	DSM III R NINCDS-ADRDA	4.00 (0.64-25.16)**
12	105 VD 802 controls	Question to subject or family	DSM III NINDS-AIREN	2.41 (1.22-4.52)
13	1953 AD 2093 controls (relatives)	Question to subject or family (yes/no)	NINCDS-ADRDA	2.08 (1.70-2.55) General 4.57 (2.87-7.31) UP onset <1y 1.38 (1.03-1.85) UP onset 1-25y 1.71 (1.03-2.82) UP onset >25y
16	76 AD 109 controls	CIDI, DSMIIR, ICD10	DSMIIIR	8.99 (3.67-22.00)*
15	547 dementia 12133 controls	Medical records and clinical history ICD7-10	DSM IV NINCDS/ADRDA NINDS-AIREN	Dementia 1.72 (1.07- 2.76) AD NS Early onset depression NS Late onset depression 3.87 (2.10, 7.14) Age of depression onset NS
16	13568 dementia 13568 controls	Registries ICD8-10	Registries ICD8-10	1.99 (1.56-2.53)

Legend (1) Heyman,1984; (2) French, 1985; (3) Agbayewa, 1986; (4) Shalat, 1987; (5) Broe, 1990; (6) Kokmen, 1991, (7) Speck, 1995; (8) Steffens, 1997; (9) Tsolaki, 1997); (10) Cooper, 1998; (11) Wetherell, 1999; (12) Hebert, 2000; (13) Green, 2003; (14) Heun, 2003; (15) Brommelhoff, 2009; (16) (17) (16) Zilkens, 2014. Notes: * Computed based on crude data published, **retrieved from a meta-analyzes calculation [Silva, 2013]; NS nonsignificant, UP Unipolar depression, BD Bipolar disorder

Table 3 Cohort studies

Study	Sample	Duration	Depression diagnosis	Dementia diagnosis	Conclusions
1	3180 non-demented	3 years	Self-report	DSM-III-R	20.64 (2.84-150.20) for dementia
2	478 old age without dementia (>60 years old)	1-5 years	Informant report “depressed mood” HDRS	DSM-III-R NINCDS-ADRDA	2.94 (1.76-4.91) for dementia, but NS in the multivariate model
3	1600 non demented	3 years	CES-D	MMSE	NS for dementia
4	954 old age (>60 years old)	8 years	CES-Dm	DSM-III-R NINCDS-ADRDA	NS for Dementia NS for AD
5	3363 UP 518 BD 8946 neurotic 5.5 M controls	21 years	ICD 8 and ICD 10 (admission diagnosis)	ICD 8 and ICD 10 (admission diagnosis)	Affective disorders (UP +BP) 13.7 (12.1-15.4) for dementia Neurosis 11.2 (9.6-12.9) for dementia
6	227 non demented non depressed 62 non demented depressed	3 years	DSM-III-R	No reference to diagnostic criteria	1.50 (0.80–2.90) for dementia
7	1911 old age highly educated	3.2 years	Geriatric Mental State Schedule CES-D	DSM-III-R DSM IV	2.21 (1.09-4.48) for dementia NS for dementia in the multivariate analysis > 8 years of education 5.31 (1.88-15.00) for dementia
8	821 religious non-demented > 65 years	7 years	CES-D	NINCDS-ADRDA Histopathology	1.19 (1.07-1.32) for each CES-D score at baseline
9	71 depressed 50 controls	25 years	ICD 8 (endogenous depressive vs other types)	DSM-III-R or evidence from at least other 4 sources	10 cases of dementia in depressed cohort and zero in control cohort (Chi-Square =5.93; p<=0.005) not computed
10	51 AD 79 controls	7 years	CES-D	NINCDS-ADRDA Histopathology	AD 1.33 (1.01-1.76) for each CES-D point increase

Table 3 Cohort studies

Study	Sample	Duration	Depression diagnosis	Dementia diagnosis	Conclusions
11	11741 UP 2007 BD Controls 81 380 osteoarthritis and 69149 diabetes	Time to dementia (>20 years)	ICD8 ICD10 Register-based	ICD8 ICD10 Register-based	UP vs OE 2.13 (2.00-2.26) UP vs DM 1.77 (1.66-1.89) BD vs OE 1.92 (1.62-2.27) BD vs DM 1.56 (1.31-1.84) Both vs OE 2.11 (1.31-1.84) Both vs DM 1.75 (1.65-1.87)
12	18726 UP 4248 BD	Time to dementia (>20 years)	ICD8 ICD10 Register-based	ICD8 ICD10 Register-based	For dementia UP vs BD 1.46 (1.01-2.13) BD n. of episodes NS MDD disorder >4 episodes higher risk when compared to 1 episode; every episode increase the risk by 13%
13	3346 non demented	2 and 5 years	Self-report	NINCDS-ADRDA	NS at baseline for AD NS at 2 years for AD NS at 5 years for AD
14	1357 non demented	14 years	CES-D	DSM-III-R NINCDS-ADRDA	2.41 (1.50-3.87) for dementia* 2.92 (1.70-5.02) for AD* Woman NS (both dementia and AD) Man NS (dementia) and AD 2.63 (1.28-5.40)
15	766 >65 years old (mean age 74.5)	5 years	CES-D	DSM-III-R	2.75 (1.04-3.79) for AD 2.37 (1.32-5.54) for dementia Duration of depression NS
16	1932 men, mean age 76.3	6 years	CES-D	DSM-III-R NINCDS-ADRDA	UP 2.5 (1.1-3.4) for dementia UP 3.0 (1.5-5.9) for AD UP. NS for VD

Table 3 Cohort studies

Study	Sample	Duration	Depression diagnosis	Dementia diagnosis	Conclusions
17	486 (60-90 years old), without dementia	6 years	Self reporting CES-D	NCDS-ADRDA	UP 2.44 (1.26-4.21) for dementia EODep 3.23 (1.36-7.70) for dementia LODep NS for dementia UP 2.46 (1.15-5.26) for AD EODep 3.76 (1.41-10.06) for AD LODep NS for AD
18	949 community (mean age 71 years)	17 years	CES-D	DSM-III-R NINCDS-ADRDA	Dementia 1.72 (1.04-2.84) AD 1.76 (1.03-3.01)
19	1239 community subjects, dementia free, mean age 55.5 years	25 years	CES-D	DSM IIIR NINCDS-ADRDA	Dementia One episode of EDS: HR 1.87 (1.21–2.88) Dementia >1 episodes of EDS: 2.08 (1.23–3.52)
20	7989 dementia free >65 years old	5 years	MINI, CES-D	DSM IIIR IV NINCDS-ADRDA NINCD-AIREN	Dementia 1.5 (1.2-2.2) AD NS VD 4.8 (2.20-10.70)
21	280540 veterans, non demented	Retrospective	ICD9 CM	ICD9 CM	Dementia 2.18 (2.08-2.28) AD significant (not shown) VD significant (not shown)
22	13535 subjects 3727 depressed/ depressive symptoms 9808 non-depressed (mean age 81years)	30 years	Question “Ever been sad or depressed?” Admission for depressive disorder ICD 9	ICD 9	Dementia Midlife only 1.19 (1.07-1.32) Late life only 1.72 (1.54-1.92) Midlife + late life 1.77 (1.52-2.06) AD Midlife only NS Late life only 1.9 (1.6-2.4) Midlife + late life 2.0 (1.5-2.7) VD Midlife only NS Late life only NS Midlife + latelife 3.5 (2.4-5.1)

Table 3 Cohort studies

Study	Sample	Duration	Depression diagnosis	Dementia diagnosis	Conclusions
23	4568 man (70-89)	5 years	GDS for actual Electronic records, self-reported, use of AD for EOD	TICS, records	Dementia UP (study entry) 2.59 (1.57-4.27) EOD 1.09 (0.78–1.52)
24	1911 > 65 years	3 years	Geriatric Mental State	Geriatric Mental State	1.48 (1.14-1.92) for dementia

Legend (1) Minani, 1995; (2) Devanand, 1996; (3) Doufuil, 1996; (4) Chen, 1999; (5) Kessing, 1999; (6) Pálsson, 1999; (7) Geerlings, 2000; (8) Wilson, 2002; (9) Brodaty, 2003; (10) Wilson, 2002; (11) Kessing, 2003; (12) Kessing, 2004; (13) Andersen, 2005; (14) Dal Forno, 2005; (15) Gatz, 2005; (16) Irie, 2008; (17) Geerlings, 2008; (18) Saczynski, 2010; (19) Dotson, 2010; (20) Lenoir, 2011; (21) Byers, 2011; (22) Barnes, 2012; (23) Almeida, 2015; (24) Lugtenburg, 2015. Notes: * retrieved from a meta-analysis [Silva, 2013]; NS nonsignificant, UP Unipolar depression, BD Bipolar disorder; AD Alzheimer's disease, HDRS Hamilton Depression Rating Scale, MADRS Montgomery-Åsberg Depression RatingS cale, EODep early onset depression, LODep late onset depression, GDS Geriatric Depression Scale, OE osteoarthritis, DM Diabetes mellitus, MDD Major depressive disorder.

Four important reviews and meta-analyzes have been performed on this topic. The first (Jorm, van Duijn et al. 1991) and second meta-analysis were performed by the same author (Jorm 2001). The second was a more embracing paper, and found an increased risk both in case-control studies (2.01; 95% CI 1.16-3.50) and in prospective studies (1.87; 95% CI 1.09-3.20). A more recent meta-analysis (Ownby, Crocco et al. 2006), including very important studies (Green, Cupples et al. 2003, Kessing and Nilsson 2003) led to the same conclusion, generating OR of 2.03 (1.73-2.38; 95% CI) for case-control studies and 2.02 (1.80-2.26; 95% CI) for cohort studies. Interestingly, the last meta-analysis published (Gao, Huang et al. 2013) was retracted due to multiple errors of citation, study description and data extraction. A well conducted review was published more recently (da Silva, Goncalves-Pereira et al. 2013), and reported that 11 out of 16 case-controls studies found depression as a risk factor for dementia/AD/VD. For cohort studies 18 in 46 did not find an association.

Despite the very discrepant results, the several meta-analyzes (Jorm 2001, Ownby, Crocco et al. 2006) and the most important reviews (da Silva, Goncalves-Pereira et al. 2013) that have been performed confirmed, in general, an association. The risk (usually OR) for dementia in depressive disorders computed in the meta-analysis is relatively small - between 1.5 and 2.0.

The obvious methodological differences among these studies could account for some of these discrepancies. Methodological differences include the study design, variations in the length of observation periods, different rates of attrition in the cohort studies, different ways to assess depressive disorders and dementia, and the use of different potentially confound variables as covariates (Wilson, Barnes et al. 2002).

The accuracy of the diagnosis of dementia in most of the studies can be considered good. Most of them use DSMIII or IV for the diagnosis of dementia, and standard research criteria for the different disorders, such as the NINCDS/ADRDA for AD, or NINDS-AIREN for VD. In some studies (Wilson, Barnes et al. 2002) the diagnosis was histopathological, providing the strongest accuracy.

In what concerns depression, diagnostic methods vary widely. Retrospective assessment of prior depressive symptoms and episodes can underestimate prevalences. The prevalence of life time depression (and other

psychiatric disorders) is approximately half if retrospectively collected, when compared to prospectively obtained data (Moffitt, Caspi et al. 2010). Composite International Diagnostic Interview (CIDI) is a valid and frequently used instrument to assess major psychiatric diseases (Wittchen 1994). It has been found to to be valid in elderly populations (Heun, Kockler et al. 2003), but it has not been validated in demented or cognitively impaired subjects. Other standard measures used, such CES-D, although validated, cannot replace a clinical interview for the diagnosis of such a complex condition as depression.

Probably the strongest study would be a controlled cohort study, with most consensually accepted and accurate, diagnostic criteria for depression and dementia.

3.3. What is the nature of this risk?

However, these discrepancies can also reflect a poor understanding on the nature of this association. Depression and dementia can be related in several ways, and the role of the several players need to be explored: a) depression is merely a prodrome, or a symptom, of dementia?, b) what is the role of different depression subtypes?, c) what is the role of different dementia subtypes?, and d) what is the role of antidepressants? (Byers and Yaffe 2011, Kessing 2012).

a) Depression is prodrome/initial manifestation of dementia, and not an early risk factor

This is one of the main issues that has been raised. Depression can be a prodrome of dementia or even symptom of the disorder. Depressive disorders are quite common, at least in the most common forms of dementia. In cognitively normal elderly people, the prevalence of depression is probably around 15%, but in AD it has been shown to be approximately 20-30%, in VD between 30-50%, and in MCI around 30% (Li, Meyer et al. 2001, Byers and Yaffe 2011). These numbers can be even higher, as 40% of AD patients have depressive symptoms (Chi, Wang et al. 2015). An important proportion of AD patients meet criteria for Major Depressive Episode, ranging from 3-24% (Heun, Kockler et al. 2003), depending on the population, on depression and dementia criteria, and on the type of the dementia.

Three mechanisms have been proposed to characterize this association, not mutually exclusive. One of the mechanisms invoked is the psychological reaction to a self perceived deterioration on the cognitive abilities, although it has been shown that subjects without a cognitive deficit can become depressed and only then develop AD (Paterniti, Verdier-Taillefer et al. 2002). Another mechanism is the neurodegeneration of crucial areas for emotion, in a mechanism similar to the vascular depression. The last explanation is the misdiagnosis of MDD in demented patients, as these disorders share some symptoms, such as impairments in attention, memory and executive functions, changes in sleep patterns, and reduction in social and occupational functioning (Steffens and Potter 2008).

It seems that, besides unipolar depression, demented patients could be also at increased risk of developing bipolar disorder (Nilsson, Kessing et al. 2002).

Studies with a short follow up could not be able to distinguish between these two conditions. Case control studies in general have not taken into account the time frame between the two diagnoses. We are aware of few exceptions, but showing discrepant results. Green et al (2003) showed an increased risk for AD regardless of the time mediating depression and dementia onset. More recently, Brommelholff (2009) showed the opposite, as no increased risk for dementia was found if the depressive disorder started more than 10 years before the onset of dementia.

Cohort studies with short a follow up also have the same limitation. The majority of longitudinal studies have follow up times of less than 5 years. Very few studies have a follow up of more than 20 years, and they all found an increased risk of dementia in depressed patients. However, most of these studies assessed depression retrospectively, and the quality of the diagnosis of depression raises important concerns, as discussed below.

One of the biggest criticisms to these studies is that depression can be a prodrome of dementia or even be misdiagnosed. Ownby (2006) addressed this criticism performing a sophisticated statistical technique (called random-effects metaregression analysis), studying the interval between the diagnosis of dementia and depression, wich was found to be positively related to this risk of developing AD. This interesting finding does not support the hypothesis of depression as a

mere prodrome of dementia. However, this time frame could only be calculated in a percentage of the studies.

Other strategy was to compute different risks for late onset depression and early onset depression, generally if it occurs after 45 or 60 years old, or before. Although not considered in the reference psychiatric diagnostic manuals, there are evidences - clinical, biological and epidemiological - supporting this distinction. Late onset depression is associated with more brain changes, such as frontal lobe atrophy (Almeida, Burton et al. 2003) and white matter changes (Lesser, Boone et al. 1996). However, the results are again discordant, as some studies found an increased risk only in early onset depression, while others found the opposite. Eventually, the distinction between early onset depression and late onset depression is not sufficiently grounded, or late onset depression is definitely not a dementia prodrome.

The studies with a short follow up (FU) are important, and may enable the study of depression as prodrome of dementia (Byers and Yaffe 2011), resulting in a deeper knowledge of the initial manifestations of dementia, and eventually providing earlier diagnosis and treatment.

However, it seems that a long follow up is the right method to distinguish depression as risk factor from a dementia prodrome. The newest diagnostic methods for AD and other dementias probably will help tremendously in distinguishing between depression and these different forms of dementia.

b) What is the role of different depression subtypes?

As mentioned in the previous chapters, depression is a clinically and biologically heterogenous disorder. These heterogeneity has been found to play an important role as a risk factor for other diseases. E.g., it has been proposed that a depression with a predominance of somatic symptoms increases the risk for coronary heart disease, when compared with a depression with cognitive symptoms (Carney and Freedland 2012).

In what concernes to dementia, several subtypes and characteristics of depression have been studied.

1. Early onset depression and late onset depression

The definition of the age limit of late onset depression varies across the studies, between the age of 45 years (Steffens, Plassman et al. 1997) and the age of 60 years (Byers and Yaffe 2011). This issue has been discussed before in this text, as late onset depression could be a prodrome of dementia, and not a true risk factor.

2. Severity: number of episodes, severity of the episodes and duration of the disorder

Each episode that determines an admission seems to increase the risk of dementia by 13% for unipolar depression and by 6% for BD (Kessing and Andersen 2004), when controlled for age, sex and calendar time, but not for education. For unipolar depression (ICD8 and ICD10 diagnosed), an increased risk of dementia was significant only if a patient had 4 or more episodes (HR 6.16; 95% C.I. 1.39-22.72). In BD, however, when the number of episodes were included as a categorial variable, the difference between the number of episodes was not significant. Although a very high number of subjects was included (virtually all Danish population), the diagnosis of both dementia and affective disorders was only considered in patients with at least one admission. So, all outpatients with these diagnoses were excluded. This probably explains the low incidence of dementia found in that study.

Depression data from the Baltimore Longitudinal Study of Aging were also analyzed in what concerns to the severity of depression (Dotson, Beydoun et al. 2010). A concept called “episodes of elevated depressive symptoms“ (EDS), based on CES-D score, was created to capture less severe depressive disorders. The risk for dementia increased about 80% to those who have one of these - EDS - and to more than 100% to those of have two or more. The clinical meaning of EDS remains largely obscure, but this study has one of the longest FU in the literature, and globally the results pointed to a role of the chronicity of depression on the risk for dementia. Another study, evaluating CES-D as a continuos variable, controlled for other important risk factors (such as age and education), the risk found was only marginal for both AD (1.04; 95% C.I. 0.99-1.08) and dementia (1.04; 95% C.I. 1.00-1.08) (Gatz, Tyas et al. 2005).

This study also evaluated the risk according to depression duration (Gatz, Tyas et al. 2005). Although CES-D score at baseline predicted the risk for AD, informant-reported duration of depression was not a predictor of either AD or dementia. The computation of depression duration was dependent on the informant reports, clearly limiting the findings.

Although the results point to an increased risk associated with a more severe disorder, they seem to have limitations.

3. Clinical subtypes of depression

Bipolar disorder was found to carry a greater risk for dementia in some studies. Patients with a manic episode have a slightly increased risk for dementia 1.46 (95% C.I. 1.01-2.13) (Kessing and Andersen 2004), 1.92 (95% C.I. 1.62-2.27), and 1.56 (95% C.I. 1.31-1.84) (Kessing and Nilsson 2003), when compared to patients with one depressive episode, with patients suffering from osteoarthritis or diabetes, respectively. The Camberwell Dementia case register also found a 2-fold risk increase in BD (Cooper and Holmes 1998), although another register study failed to found a risk association (Kokmen, Beard et al. 1991).

Although most depressive disorders, such MDD, are a clearly defined diagnosis, very few studies used standard clinical criteria in their diagnosis (Kessing, Olsen et al. 1999, Brodaty, Luscombe et al. 2003, Kessing and Nilsson 2003, Kessing and Andersen 2004). In what concerns to other subtypes, or specifiers, such as melancholic, ICD10 defined somatic syndrome, atypical, psychotic, or catatonic, we found no studies specifically addressing these subtypes.

One study evaluated neurotic disorders (ICD8 defined), including neurotic depression, and found a 11.2-fold (95% C.I. 9.6-12.9) increase in risk for dementia (Kessing and Andersen 2004). Two studies used CES-D factors to evaluate their influence in the risk, as an approach to differentiate different depressive disorders. DalForno et al. (2005) performed a risk analysis using a CES-D sub-scale based on a cluster of negative affective symptoms, eventually related to melancholic features, but it did not influence the global risk for dementia. Another study tried to find an association between CES-D clusters (depressed affect, positive affect, somatic/vegetative and interpersonal) and the risk for dementia (Gatz, Tyas et al.

2005). Only the somatic/vegetative cluster showed a marginal increased risk (1.10; 95% C.I. 1.02–1.18), and only for AD.

Neurotic disorders (including neurotic depression and anxiety disorders) were found to carry a higher risk for dementia in least one study (Kessing, Olsen et al. 1999). However, a treatment for anxiety - benzodiazepines - also seems to increase the risk (Billioti de Gage, Pariente et al. 2015).

c) What is the role of different dementia subtypes?

The same is true for dementia. AD, VD, FTD, and LBD have distinct risk factors and most studies, specially cohort studies, do not distinguish between them.

In the majority of the studies published, the outcome was dementia as a whole. However, as referred above, the causes of dementia have very different associated biological mechanisms. It is hard to conceptualize that depression could interfere with so different mechanisms such as amyloid deposition, brain irrigation, alpha-synuclein, ubiquitin, and many others. Some studies have determined AD as an outcome, very few VD, and to our knowledge, none has studied FTD or LBD. The finding of an increased risk for AD is more commonly found in a case-control studies than in cohort studies, probably because of the differences in the methodology. Although recall biases are a main caveat in case-control studies, it is supposed to happen both in cases and controls, but specially in cases. This means that AD patient would not disclose a history of depression, increasing the chances of showing a lack of effect. So if a case-control study shows a difference, exclusively from this point of view, this difference probably exists. However, case-control studies have several other limitations discussed above.

Depression is not expected to be a risk factor for AD in a short time, as amyloid deposition occurs over decades (Braak and Braak 1991). Cohort studies with a short follow up probably would be unable to show an effect. Eventually, an increased risk for a more acute onset dementia, such as some forms of VD, could be found. We are not aware of cohort studies with a longer follow up (or controlled) that have studied different types of dementia. It has been shown that depressed non-demented subjects do not have increased amyloid when compared to non-

depressed non-demented subjects, either detected by [^{11}C]PET-PiB (Madsen, Hasselbalch et al. 2012) or by neuropathology (Wilson, Boyle et al. 2015).

Only one case-control study and three cohort studies with the diagnosis of VD as an outcome were performed. One of each group found an effect (Hebert, Lindsay et al. 2000, Lenoir, Dufouil et al. 2011). Interestingly, two studies searching for both outcomes, and with a similar methodology, found the opposite: depression is a risk factor for AD and not for VD (Irie, Masaki et al. 2008) and vice-versa (Lenoir, Dufouil et al. 2011).

In conclusion, studies addressing depression as a risk factor for dementia subtypes revealed discrepant results. Probably a controlled cohort study with longer follow ups would help to clarify this issue.

d) What is the role of antidepressants?

It is expected that antidepressants with strong anticholinergic properties induce cognitive impairment, specially in older adults (American Geriatrics Society Beers Criteria Update Expert 2012). This impairment should be acute and revert after antidepressant discontinuation, and probably will fulfill the criteria for a delirious disorder (American Psychiatric Association 2013).

On the other hand, a number of preclinical observations suggest that antidepressants may have neuroprotective abilities, in animals submitted to protocols known to induce depressive behaviors.

The study of depression using animal models is important, and has advantages, but reproducing or mimicking mental disorders in rodents has also limitations. The main advantage is to permit experiments that could not easily be carried out, or be ethically unacceptable, on human subjects, in the hope of shedding some light on the nature and mechanisms of the disorder (Willner 1990). Although reliability can be an issue for some models, the main limitation is the validity of the model. The principal validity criteria for animal models are construct and predictive validity, face validity being considered less important (Kalueff and Tuohimaa 2004). An ideal model would fit all these three criteria, but it rarely happens in animal models of depression, as discussed below.

Although conceptually it seems wise to separate the protocol inducing depressive-like behaviors from the evaluation of the behaviors themselves, the

paradigm is quite often the same, e.g learned helplessness. Learned helplessness was first described in dogs exposed to an uncontrollable shock (Seligman 1972). When experimentally naive dogs were given a painful electric shock, they “ran frantically about [the cage], defecating, urinating, and howling, until accidentally scramble the barrier and so escapes the shock”. These dogs learned quickly how to escape the shock. When other dog, who had been exposed to uncontrollable shock before the avoidance experiment, was given an escapable shock, also had an initial running reaction. However, this reaction soon stops and the animal “sits or lies, quietly whining, until shock terminates (…). Rather, it seems to give up and passively accepts the shock”, even in subsequent trials. In this exemple the protocol of inducing learned helplessness is different from the evaluation of the behavior “learned helplessness”.

Animal models of depression can be divided according to the duration of the induced depressive behaviors, into acute and chronic, or according to the inducing event (drugs, gene mutations, brain lesions, stimulation or stressful external factors) (reviewed by Kalueff and Tuohimaa 2004). Acute depression models can be pharmacological (e.g. clonidine or reserpine induced) or stress induced (e.g. tail suspension). Chronic depression models can be stress-evoked (such as the unsigned inescapable shock, or the chronic mild stress), promoted by social disruption (such as the maternal or peer separation), and by sensory deprivation (e.g. olfactory bulbectomy or long-term $ZnSO_4$-induced anosmia), among others.

Reserpine, an alkaloid obtained from *Rauwolfia serpentina*, is an important drug in the history of pharmacology. Reserpine is a classic exemple of the effects of the manipulation of the adrenergic synapse, and, on the other hand, is also one of the rationals for the monamine theory of depression. Reserpine was used as an antihypertensive drug (Winsor 1953). Reserpine lowers the concentration of catecholamines (Holzbauer and Vogt 1956), blocking sympathetic response (Muscholl and Vogt 1958), due to inhibition of the vesicular monoamine transporters (VMAT) (Scherman and Henry 1984)[12]. However, soon “calming” and “sedating” effects were reported (Earl 1954), and even the end of

[12] Prior to secretion, monoamines are taken from the cytoplasm and concentrated into vesicles, by a group of transporters, called vesicular monoamine transporters (VMAT). When these transporters are inhibited, monoamines are not stored, and are destroyed by local enzymes, such as the Catechol-*O*-methyl transferase (COMT).

electroconvulsive therapy was proposed (Fouks, Laine et al. 1954), because reserpine would be efficacious to treat acute agitations. However, significant depressive symptoms were reported (Freis 1954). The mechanism of action and the clinical effects were the basis for the proposal of the reserpine animal model of depression. In this model, the animal is injected with reserpine, and acute depressive-behaviors like are observed.

Clonidine was initially used as a nasal decongestant, but the finding that it induces bradycardia and hypotension, drove its use as antihypertensive drug (MacDougall, Addis et al. 1970). Clonidine acts by the stimulation of the α2 pre-synaptic adrenergic receptors, thereby reducing sympathetic efflux from central nervous system (reviewed by Giovannitti, Thoms et al. 2015). Some basic research and clinical studies found a dysregulation of α2 adrenergic receptores in depression (Siever and Davis 1985). This was probably the basis for the clonidine rodent model of depression. These models have some predictive validity, but also have crucial construct validity issues, mainly because they are based on a single mechanism of depression, and it is hard to considerer an homology with human depressive disorder.

Stress induced-models, specially the chronic stress models, have been considered more valid, as they rely on the relations between stress and depression, and also on some common biological findings of these conditions. Although the inescapable shock model has questionable face and construct validity, the unpredictable chronic mild stress (CMS) is generally considered a valid animal model of depression (Willner 1990), and has been used in recent research involving AD and neuroprotection (see below). In CMS the animals are exposed for several weeks to a variety of mild unpredictable stressors (lights or sounds, e.g.) (Hill, Hellemans et al. 2012). This model have shown to promote a state of impaired reward salience similar to anhedonia. Although this model have been considered to have some face and construct validity (Willner 1997), the depressive like-behaviors wane in a few weeks and its reliability is controversial (Hill, Hellemans et al. 2012).

The olfactory bulbectomy is the surgical removal of the olfactory bulbs. This model of depression is based on the findings that the rats submitted to this procedure showed decreased acquisition of behavioral tasks involving both reward

and avoidance training (van Riezen, Schnieden et al. 1976). These deficits are reverted by amitriptyline, and this model has been used specially to predict antidepressant effects of new compounds. Recently it was used to study neurodegeneration and the associated cognitive decline (Hendriksen, Korte et al. 2015), although the variations between species are significant and their meaning in terms of translational value is not clear.

Maternal separation (MS) is another model of depression. This model has its roots on the experimental work on Rhesus monkeys, showing that when infant monkeys were separated from their mothers, they presented an initial stage of 'protest' (agitation, changes in sleep pattern, and screaming). One or two days after, a "despair" phase occurs, characterized by a decrease in activity, appetite, play and social interaction (Harwlow 1958-62, cited by McKinney and Bunney 1969). These separation phenomena are present to some extent in other species, such as rodents (Willner 1990). This MS model has a significant face validity, as a similar sequence of protest followed by despair was described in institutionalized children, a condition called "anaclitic depression" (Spitz (1946) and Robertson and Bowlby (1952), cited by Willner 1990). And child trauma is a known risk factor for metal disorders in adulthood (Heim and Nemeroff 2001, Dvir, Ford et al. 2014). Additionally, rats submitted to a MS protocol show persistent changes of gene expression, neurochemistry, electrophysiology, and morphology (Bakshi and Kalin 2000, Kaufman, Plotsky et al. 2000), altogether with behavioral, neuroendocrine and cognitive signs of over-activation of the HPA axis (Aisa, Tordera et al. 2007, Sousa, Vital et al. 2014).

In general, depending on the aim of the research, the more simple models tend to be used in simple research (such as the preliminary screening of antidepressant actions), while the more sophisticated models are used in more complex research (such as the manipulation of behavior by multiple drugs, or the assessment of a specific behavior).

A slightly different question is the evaluation of depressive-like behaviors in animal models. Behavioral tests are used to assess and quantify the extent to which the animal displays a depression-like phenotype. The most used tests to evaluate depressive-like behaviors in rodents are the Porsolt swimming test (and the tail suspension test), the sucrose preference test, and the novelty suppressed

feeding test, all aiming to measure different components of depression (Powell, Fernandes et al. 2012).

Briefly, the Porsolt behavior despair test, or forced swimming test (FST), is based on the learned helplessness (actually measuring behavioral despair) (Porsolt, Le Pichon et al. 1977). The animal is placed in a cylinder, full of water in such a way that he cannot escape and is forced to swim (Figure 1). After an initial period of vigorous activity, the animal eventually stops moving, making only movements necessary to keep its head above water. This immobility is considered a behavioral equivalente of the human depressive helplessness, and AD decrease immobility time. Other evaluations have been proposed, such as the latency to immobility, but the standard measure is the immobility time (Castagne, Moser et al. 2011).

Figure 1 The FST
The rat, after an initial phase of actively trying to escape, stops moving. It is considered a behavioral equivalente of helplessness.

The sucrose preference test is based on anhedonia (Willner, Towell et al. 1987). Rats have innate preference for sweet food, and when faced with normal tap water and water with sucrose, the animals prefer to drink the sweet water. The non preference is interpreted as a behavioral equivalente of anhedonia and antidepressants have been shown to reverse this non-preference (Willner 1990).

These models and tests have been used to assess the putative neuroprotective actions of antidepressants.

Neuroprotection is an intervention that reverts or prevents further neuronal damage (Brimble and Levi 2006). This intervention is intended to increase the resistance of neurons to an insult. It can be a procedure, such as human refrigeration - probably the first efficacious neuroprotective intervention (Henderson 1963), or the administration of drug. Pedro Hispano in the XIII century, described several neuroprotective strategies, naturally bounded by the erroneous

knowledge of the eras in which it was written: “Here is what is good for the brain (…) a salubrious place, (…) aloes sucotrinum with fennel juice, (…) thyme, rosemary, (…) soundly sleep, (…). Basil and rue for melancholy. Sage and castoreum for paralysis and memory conservation (…)” (Hispano XIII century).

The evidence for neuroprotection is different, depending on the paradigm being studied (Simões do Couto and Mendonça 2007). In cell cultures, neuronal damage or death (e.g. the number of live neurons, or an evidence of neurogenesis) caused by certain noxious stimuli can be attenuated by specific interventions, like the presence of a particular compound (for instance, adenosine) (Ribeiro, Sebastiao et al. 2003). In brain slices, not only neuronal damage (or death) can be attenuated, but also neuronal function (long-term potentiation, e.g.) can be preserved or recovered by neuroprotective interventions. In *in vivo* studies, a neuroprotective intervention can attenuate structural (such as the number of dendritic spines, the morphology of neurons, or the size of a stroke), behavioral (the performance in a memory test, e.g.), or functional deficits (e.g. a focal sign) (Simões do Couto and Mendonça 2007).

In preclinical studies of depression and antidepressants, neuroprotection has been assessed at the morphological, physiological, and behavioral levels (Pehrson, Leiser et al. 2015). At the morphological level neuroprotection has be evidenced by increased neurogenesis, increased number of dendrites, or increased synaptic contacts. Neuroprotection can be evident in hippocampal slices if an LTP dysfunction is attenuated. At the behavioral level, two cognitive tests have been used to assess an eventual neuroprotective action of AD: the novel object recognition (NOR) and the Morris water maze (MWM).

The NOR has emerged as the most popular test for assessing a rodent's ability to recognize a previously presented stimulus, so it is a test for object, non-spacial, memory (Ennaceur and Delacour 1988, Cohen and Stackman 2015). The choice of the name was not very happy, and can be misleading, because the recognition of a novel object is impossible. Anyway, it involves two phases: in the first phase, or training phase, the animal is placed in a familiar arena with two identical novel objects. The animal is left a period of time to explore the objects (encoding) and then is removed from the arena. Later (consolidation of memory), the second phase, or test session, takes place. The animal is left in the same

arena with an exact replica of the initial object and another one, different. Rodents exhibit a natural trend to explore the novel object, and it is expected to exhibit a preference for exploring the novel object. Memory is computed from the percentages of time spent exploring both objects. However, the brain circuits implicated in the test are not fully understood, and discrepant results are common (Cohen and Stackman 2015).

The MWM, created by Morris et al (1982), is sensitive to hippocampal-dependent spatial learning and memory. Several variations exist, but the classic test is the most used (Diogenes, Costenla et al. 2011, Sousa, Vital et al. 2014). The test takes place in a maze, which is a large circular tank, filled with water made opaque with the addition of a small amount of a non-toxic water-based black paint. An escape platform is submerged, non-visible, 1 cm below the water. Several visual cues for spatial reference are placed on the walls of the testing room. The test has two phases: the first phase, or spatial acquisition training, consistes of four trials/day for four consecutive days, in which the platform is placed at a fixed position in the center of one of the four quadrants of the tank. On each day the animals are randomly placed in four different positions facing the wall, and dropped into the water. The latency to find the platform is measured. At the fifth day a probe test was given - the platform is removed and the animals were allowed to swim freely for 60s. The percentage of time spent in the quadrant where the platform was is quantified.

As mentioned before, it has been shown that antidepressant actions extend beyond monoamines re-uptake to include neuroprotection. In non depressed animals, antidepressants increase gene expression of various neuroprotective agents, such as the cyclic AMP response element binding (CREB) or the brain derived neurotrophic factor (BDNF), among others (reviewed by Pehrson, Leiser et al. 2015). Antidepressants also induce changes in proteins related to neurogenesis, such as the Insuline Growth Factor-1 or Heat Shock Protein 10, among others, in normal animals (Khawaja, Xu et al. 2004, Tiraboschi, Tardito et al. 2004, reviewed by Pehrson, Leiser et al. 2015).

However, a direct evidence of neuroprotection in animal models of depression is less clear. Preclinical studies have shown that short-term treatment with fluoxetine and imipramine induces remodeling of dendrites and synapses in

rats submitted to CMS (Bessa, Ferreira et al. 2009). If a longterm treatment is given in the same conditions, increased neurogenesis is observed, paralleling the reversal of the depressive-like behaviors (Mateus-Pinheiro, Pinto et al. 2013). If neurogenesis is blocked (with the use the cytostatic agent methylazoxymethanol), the depressive-like behaviors do not change. Almost all antidepressants have shown similar neurogenic properties (Pehrson, Leiser et al. 2015).

If given for longer periods (although still short - 2-4 weeks), intraperitoneal fluoxetine was shown to reverse cognitive deficits induced by depression in some studies (Harvey, Naciti et al. 2004, Song, Che et al. 2006, Ibi, Takuma et al. 2008). Another study showed a partial reversion (Valluzzi and Chan 2007) and still another one a total absence of reversion (Bianchi, Fone et al. 2009).

In conclusion, although the evidence for a behavioral effect is less clear, in preclinical studies, antidepressants seem to have neuroprotective actions.

It is an appealing hypothesis that long-term treatment with antidepressants could decrease the risk for dementia in depressed patients, although it is not easy to test (Kessing 2012). In fact, clinical data are heterogenous, and seem not to reflect preclinical findings.

Antidepressants use was associated with an increased risk for dementia in the first studies (Jorm, van Duijn et al. 1991). These results were explored in subsequent studies. In a Danish study, although the risk increased along with an increase in the number of prescriptions, actually, for a high number of prescriptions, the risk decreases (Kessing, Sondergard et al. 2009). These changes were found independently of the antidepressant class. These findings were essentially replicated for TCAs, but not for other antidepressants, in a subsequent study by the same researchers, performed in more severe depressed subjects that had been hospitalized due to depression (Kessing, Forman et al. 2011). The authors proposed that increased prescriptions could be associated with a better depression treatment, and so a diminished risk for dementia. They also speculated that for the most severe cases of depression, non-TCAs antidepressants are not enough effective to counterbalance the risk induced by depression (Kessing 2012).

C. Presenting the hypothesis under study

1. Is depression a risk factor for dementia?

We hypothesized that patients suffering from depression, carefully characterized, have an increased risk for dementia when compared to non-depressed subjects, controlling for important confounders.

2. Is depression an early risk factor for dementia or a prodrome of dementia?

We hypothesized that patients suffering from depression, carefully characterized, occurring many years before dementia onset (at an early age), are at increased risk for dementia, controlling for important confounders.

3. What is the role of different depression subtypes?

We hypothesized that patients suffering from different types of depression, carefully characterized, have a different risk for dementia, controlling for important confounders.

4. What is the role of the different dementia disorders?

We hypothesized that patients suffering from depression, carefully characterized, have a different risk for AD, VD and other dementia conditions.

5. What is the role of antidepressants?

We hypothesized that antidepressants reverse the chronic memory deficits in rats submitted to a protocol known to induce depressive behaviors

D. Original studies

During submission to Journal of Affective Disorders, this chapter was improved in the peer review process. These incorporated important suggestions are shown only in the *facsimile* version of the published paper.

Depression with melancholic features is associated with higher long-term risk for dementia

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Abstract

Background: Depression has been reported to increase the risk of subsequently developing dementia, but the nature of this relation remains to be elucidated. Two important unsolved issues have been raised: (1) depression can be a prodrome/ manifestation of dementia or an early risk factor, and (2) the effect may differ according to depression subtypes. Therefore, we aimed to study the association between early-onset depression, as well as between different depression subtypes and the occurrence of dementia.

Methods: We conducted a cohort study including a series of 322 subjects with depression, consecutively recruited between 1977 and 1984. Age and sex-matched subjects without depression admitted for surgery at the same time were the group not exposed to depression. Subjects were contacted again between 2009 and 2013, to assess their dementia status. The risk for dementia in patients with early onset depression was compared to the corresponding controls using binary logistic regression, and the odds ratio (OR) computed. To quantify the association between different depression subtypes (namely melancholic, anxious, and psychotic) and dementia we obtained adjusted hazard ratios (HR) with 95% confidence intervals (95% CI) using Cox proportional hazards regression.

Results: In 133 (41.3%) depressed subjects, followed-up for a mean (standard deviation) of 25.7 (7.2) years, the diagnosis of dementia could be established or excluded. Among these, 44 (33.1%) developed dementia, mostly Alzheimer's dementia, versus 20 (15.0%) among the subjects with no depression at baseline. Depressed subjects had an increased risk of dementia when compared to the controls [OR 2.50 (1.14-5.49; 95% CI); p=0.022], as well as subjects with early onset depression. Patients suffering from depression with melancholic features

had an increased risk of developing dementia compared to those without melancholic features [HR 3.64 (1.78-11.26; 95% CI); p=0.025].
Limitations: The inclusion of biological biomarkers would provide a biological ground and strengthen the results. No formal cognitive evaluation was performed at baseline. About 60% of the subjects with depression at baseline were lost during follow up.
Conclusions: The present study supports early onset depression and depression with melancholic features as important risk factors for dementia. Melancholic features may play an important role in the relation between these diseases, and should be actively included in further studies.

Keywords: dementia; depression; melancholia; hypothalamus-hypophysis-adrenal axis; risk factor

1. Introduction

As most dementing conditions are irreversible, and the available therapies have limited beneficial effects, primary prevention is of paramount importance for reducing the societal impact of dementia (Ritchie et al., 2010; Norton el al, 2014). Among the several risk factors so far identified, depression emerges as a potentially important target (Reitz et al., 2011), because is amenable to prevention, has a high prevalence, and can be diagnosed inexpensively and treated effectively (Kupfer et al., 2012).

Depression has been found to be a risk factor for dementia in several case-control (Cooper and Holmes 1998, Green, Cupples et al. 2003) and cohort studies (DalForno, 2005; Kessing, 2003; Saczynski, 2010; Irie, 2008; Dotson, 2010; Byers, 2011), but not all (see Chen, 1999; Gatz, 2005; Chen, 2008; Brommelholf, 2009). The meta-analyses and recent reviews performed have confirmed this association in general, finding that depression approximately doubles the risk for dementia (Jorm, 2001; Ownby et al., 2006; Silva, 2013).

However, the nature of this relation remains poorly understood. Two unsolved issues have been repeatedly raised (Byers and Yaffe, 2011, Kessing, 2012). The first is that depression, especially if occurring after 60 (called late onset depression) or next to a diagnosis of dementia, can be a prodrome/manifestation of dementia disorder, instead of an early risk factor. In fact, depressive symptoms are quite common in dementia, and depressive symptoms may arise from the anatomic lesions that are part of the neuropathological course of dementia (Boland, 2000). Case-control studies that do not take into account the time between dementia and depression diagnosis, and cohort studies with a short follow up may not be able to distinguish between these two situations. The few studies that specifically compared late onset depression with early onset depression found discrepant results (Green, 2003; Geerlings, 2008; Brommelhoff, 2009; Lenoir, 2011; Almeida, 2015).

The second issue is the subtype of depression. The heterogeneity of depression has seldom been taken into account. A more severe disorder (expressed by higher frequency, duration, and severity of the depressive episodes) has been inconsistently associated with a higher risk for dementia (Geerlings et al., 2008; Kessing et al., 2012; Silva et al., 2013). Bipolar disorder has also been

associated with a higher risk of dementia, but only in some studies (reviewed by Silva et al., 2013). Brodady et al. (2003) explored the role of comorbid anxiety in depression on the risk for dementia and found no influence. On the other hand, the use of benzodiazepines has been reported to carry a higher risk (Billioti de Gage et al., 2012). Psychotic symptoms have been associated with a higher risk for cognitive deficits only in bipolar patients (Martínez-Arán et al., 2004). Few studies looked at the risk for dementia in DSM-5 or ICD10 defined depression subtypes. DalForno et al. (2005) performed an additional risk analysis and found that a Center for Epidemiologic Study–Depression (CES-D) sub-scale based on a cluster of negative affective symptoms, related to melancholic features, did not influence the global risk for dementia.

Different biological mechanisms underlying these different depressive conditions can carry different risk factors for dementia. Late-onset depression may reflect a prodromal symptom of dementia, and unlikely early-onset depression, has been associated with structural brain abnormalities of vascular origin, and may be characterized by a more severe course and a higher prevalence of psychosis (Kessing et al., 2012). Melancholic features, and to a lesser extent psychotic symptoms, have been associated with more consistent biological abnormalities and response to treatment (Brown, 2007; Parker et al., 2013) when compared to their absence.

These unsolved issues - prodrome versus early risk factor and the heterogeneity of depression - regarding the risk for dementia in depressed patients, encouraged us to perform the current study. The objectives were to quantify the association between early onset depression, as well as between different depression subtypes, and the long-term risk for dementia, controlling for well known risk factors for dementia.

2. Methodology

2.1 Study design

This study is based on a cohort of depressed patients followed in average 25 years for development of dementia. The cohort comprised 325 patients from the Hospital de Santa Maria, Lisbon, either inpatients or outpatients, with the clinical

diagnosis of depression, recruited between 1977 and 1984 in a taxonomic cluster analysis study of depression.

A surgical comparison sample was recruited retrospectively, to include eligible subjects who were consecutively admitted to Hospital de Santa Maria, Lisbon, for routine surgery (appendicectomy or cholecystectomy) between 1977 and 1984.

All participants were re-evaluated between 2009 and 2013, to establish the outcome - dementia status.

2.2. Baseline assessment

The depressed cohort was submitted to a comprehensive psychiatric and psychological evaluation was performed, and data on demography, clinical features, and personal and family history were collected (Paes de Sousa et al., 1980). This evaluation notably allowed the classification of depression by virtually any diagnostic system.

Surgical cohort demographic and clinical data were collected from clinical files.

2.2.1. Depressed cohort evaluations

a) Association for Methodology and Documentation in Psychiatry System (AMDP)

The AMDP-System was created in Nuremberg in 1960 and has been widely used in Europe in 1970-1980. The Psychopathology Scale contains 100 psychopathology items, including symptoms and other clinical features, derived from classic psychopathology studies from Jaspers, Bleuler, Schneider, and others. It renders a very detailed and standardized evaluation, including affective, behavioral, cognitive, psychotic, sensory, and social dimensions of psychopathology (Busch et al., 1980; Paes de Sousa et al., 1980).

This system has been used for diagnostic or reclassification purposes with other diagnostic systems, such as the DSM IV (Salvatore et al., 2007; Seemuller et al., 2008).

Each symptom is scored for severity (0–3: absent, mild, moderate, severe).

b) Eysenck Personality Questionnaire (EPQ)

This questionnaire (Eysenck & Eysenck, 1975) includes 83 items (full version), allowing the evaluation of the three basic personality dimensions, according to Eysenck's Personality theory: extroversion, neuroticism and psychoticism. The subject responds yes or no, and a positive answer is scored 1. The final result is the sum of the points in each scale (0-23 for extroversion and 0-23 for neuroticism).

The neuroticism dimension assesses emotional stability versus instability and identifies individuals prone to psychological distress. Low scores indicate a trend to more relaxed, unemotional, and self-satisfied subjects. The extraversion dimension measures interpersonal interaction, activity level, need for stimulation, and capacity for joy. The subjects with a low score tend to be more reserved, sober, task-oriented, and quiet.

A low extroversion (a score lower then median) and high neuroticism group (a score higher than median) of subjects was created, as these subjects were previously found to be at a higher risk for dementia (Wang et al., 2009).

c) Clinical Global Impression (CGI))

Clinical global impression - severity (CGI S; Guy, 1976) is 7-point scale to evaluate the current severity of the patient's illness, according to the clinician's total past experience, ranging from 1 (not at all ill) to 7 (extremely ill).

2.2.2. Diagnosis of depression

Using AMDP symptoms at baseline, DSM 5 diagnostic criteria for Persistent Depressive Disorder (dysthymia), Major Depressive Disorder (MDD), melancholic and psychotic features were applied. Through baseline chart review, subjects were considered to have bipolar disorder if they met DSM 5 criteria for bipolar disorder.

The specifier of anxious distress could not be defined by AMDP as only two anxious symptoms ("psychic anxiety" and "somatic anxiety") are present in the scale. A numerical variable "anxiety symptoms" was created adding both scores. Chronic disease was defined if MDD symptoms were present continuously for more than two years.

Based on the information gathered during the follow-up, the initial diagnosis was reviewed and three patients were excluded from the cohort because the diagnosis of depression was found wrong, namely one had schizophrenia, another schizoaffective disorder and the third a brain tumor. So, the cohort recruited 322 subjects.

2.3. Follow-up

2.3.1. Follow up procedures

The Hospital de Santa Maria, the Institute of Notaries and Records, and the National Health Service databases were used to get the subjects' address and next of kin, phone number, vital status, General Practitioner (GP) and other relevant doctors' names. This search was used to find and contact subjects, or next of kin if the subject was dead.

a) Depressive cohort

It was not possible to ascertain the vital status of 125 subjects (data illegible, no records, or too many records found).

In the remaining 197, additional information was sought to establish a diagnosis of dementia. In the 75 subjects found to be dead, the next of kin was contacted to collect demographic information and to apply the Dementia Questionnaire (DQ). GP records, psychiatrist records, neurologist records, hospital clinical files, death certificates and nursing home records were reviewed to collect information regarding dementia diagnosis. In 43 subjects no contact with next of kin was possible or the clinical information in clinical files was not enough to establish or exclude the diagnosis of dementia.

The 124 alive subjects were contacted first by mail, presenting the study and indicating that a later phone contact would be done. Then, a clinical interview and a neuropsychological assessment (NA) were offered. If a subject was living far away, too ill to be submitted to NA, or not willing to come for the clinical/NA, telephone interviews (TICS and/or DQ) to evaluate the cognitive status were applied. Patient's GPs, or other relevant doctors were contacted, and hospital records reviewed when appropriate. No contact at all, and no clinical records were

found in 7 subjects, and a total of 16 subjects refused to participate in the study. In 133 (41.3%) subjects the outcome could be established (Figure 1).

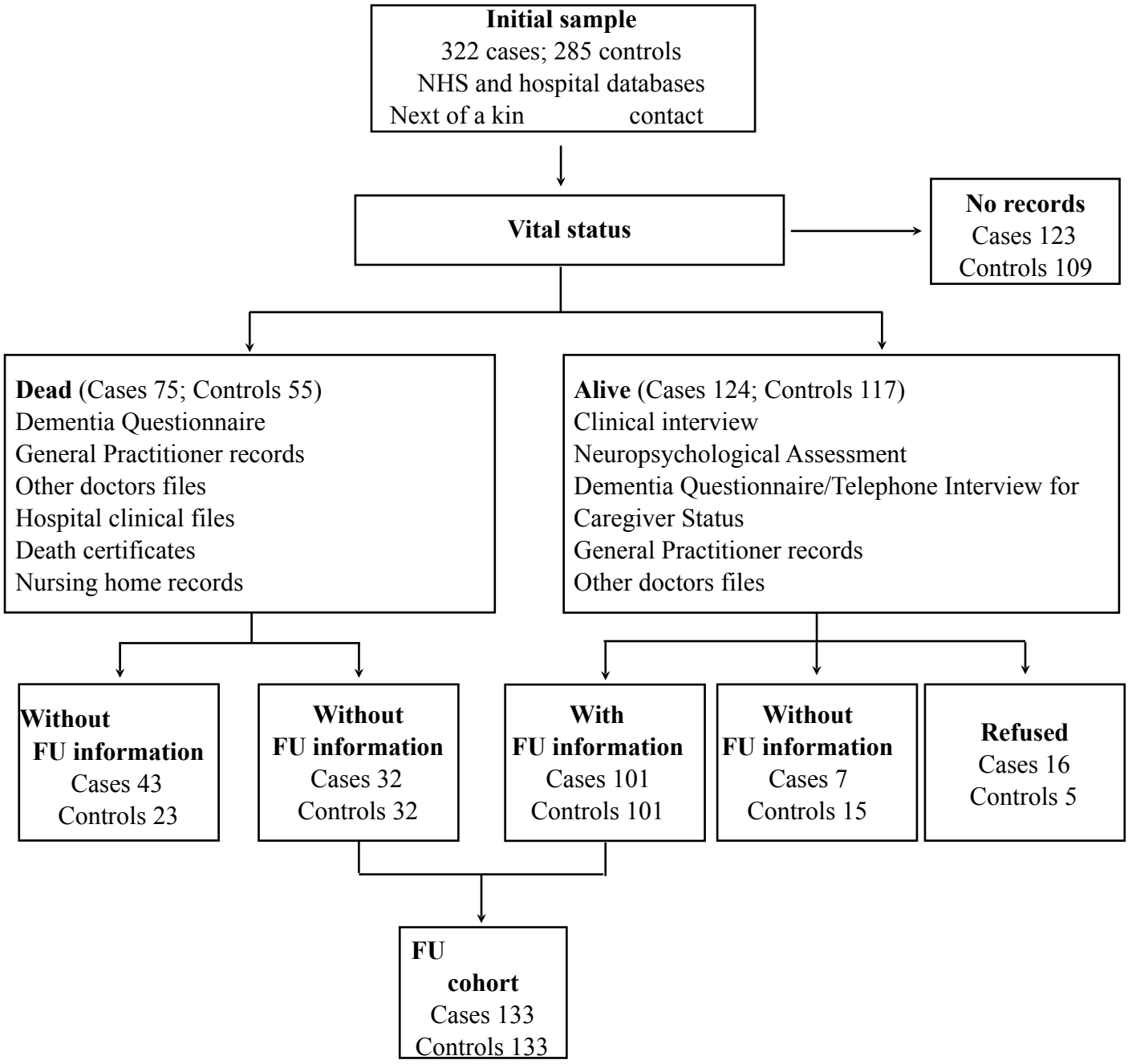

Fig. 2. Diagram displaying the flow of subjects cases in the study. NHS National Health Service, FU Follow up.

b) Surgical cohort

Patients admitted at the Surgery Department of the same hospital, for routine appendectomy or cholecistectomy at the same time as cases, were recruited. The initial sample comprised 287 potentially eligible subjects. A subject was considered eligible if they had not a mood disorder illness prior to or at the time of index surgical hospitalization. This was determined by the interview and by reviewing all the information available from clinical files. Two subjects found to have a mood disorder (one bipolar, one MDD) at time of the index hospitalization were excluded from the cohort. So the cohort included 285 subjects. The same processes described for cases were conducted, reaching a follow up group of 133 subjects.

2.3.2. Follow up assessment (evaluations performed in 2009-2013)

Both cohorts were submitted to the same FU assessments.

a) Neuropsychological assessment (NA)

A comprehensive evaluation was performed, either at patients' home or in the hospital, by experienced neuropsychologists. The evaluation included (1) Battery of Lisbon for the Assessment of Dementia (BLAD; Garcia, 1984), (2) Trail Making Test – part A and part B (TMT; Reitan, 1958, (3) Toulouse-Piéron Test (TP; Toulouse and Piéron, 1986; Mendelsohn, 2000), and (4) California Verbal Learning Test (CVLT; Delis et al., 1987; Ribeiro et al., 2007).

b) Other assessments

1) *Telephone Interview for Cognitive Status* (TICS)

This instrument (Brandt et al., 1988; Madureira et al., 2006) was initially developed for the assessment of AD patients unwilling or unable to return for follow-up. It gathers information on the domains of orientation, concentration, short-term memory, mathematical skills, praxis and language. It was proven to be sensitive and specific, and to have high test–retest reliability. The cutoff used for dementia was less than 26.

2) *Dementia Questionnaire* (DQ)

The Dementia Questionnaire (Silverman et al., 1986) is applied by telephone to caregivers of patients with dementia allowing to quickly diagnose dementia in patients by the DSM IIIR criteria, and in some cases even to suggest the dementia subtype. The DQ can also be applied to caregivers of already dead patients with dementia. A validated version including the age of onset of dementia and dementia subtype was used (Teixeira et al., 2011).

3) *Vascular risk factors assessment*

Subjects with a previous diagnosis of hypertension, diabetes, dyslipidemia, ischemic heart disease or cerebrovascular disease were considered to have vascular risk factors.

2.3.3. Diagnosis of dementia

Dementia was diagnosed at a case conference, including a psychiatrist (F.S.doC.), a neurologist (A.deM.) and a neuropsychologist (C.C.), all experienced in dementia. Cases were determined based on the best available information, using DSM-5 criteria for Major Neurocognitive Disorder (dementia) (APA, 2013).

The clinical and NA information were reviewed if the subject attended the clinical interview and neuropsychological assessment. If a subject was dead or did not attend the formal evaluation, case conference reviewed all the available evidence. In these cases, the diagnosis of dementia was based on at least two of the following: TICS score of 26 or less; DQ yielded a diagnosis of dementia; GPs records with a diagnosis of dementia; a diagnosis of dementia performed by either a neurologist or a psychiatrist; hospital records of dementia; death certificate with a diagnosis of dementia; retrospective case audit to meet DSM-5 dementia criteria; diagnosis of dementia recorded in nursing-home notes. If a diagnosis of dementia was established, reference to a Dementia Clinics was offered to the subject, to undergo the standard of care for evaluation and treatment of dementia.

The type of dementia was determined in the case conference referred above, using all the available information. The criteria for the diagnosis of the most common types of dementia were used: probable Alzheimer's disease according to NINCDS-ADRDA criteria (McKhann et al., 2011), probable vascular dementia

according to NINDS-AIREN criteria (Roman et al., 1993), probable dementia with Lewy bodies (DLB) according to the criteria proposed by McKeith et al. (2005), and behavioral variant of fronto-temporal dementia (FTD) according to the criteria of the International Behavioural Variant FTD Criteria Consortium (Rascovsky et al., 2011). If none of these criteria was met, or no sufficient information could be gathered, the diagnosis made was dementia non-otherwise specified (NOS).

Date of onset of dementia was determined during the clinical interview, by DQ, or chart review.

2.4. Statistical analysis

Statistical Package for Social Sciences® v19 (SPSS) and STATA® version 11.1 (College Station, TX, 2009) were used for the statistical analysis.

Baseline characteristics were compared between subjects in whom the outcome was assessed and in those lost during follow-up, using independent samples *t* Student's test for continuous variables, after verification of homogeneity of variances, and Pearson Chi-square test for categorical variables, with Yates continuity correction for 2X2 tables (CCχ2).

We used a binary logistic regression (with the dependent variable constituted by the conversion to dementia during the follow-up period) to compute the odds ratio (OR) for the association between depression and dementia. Because subjects from the depressed cohort were more tightly followed by the health services, it was possible to have the exact age of dementia onset for the vast majority of them. On the other hand, the data were hard to obtain for surgical cohort subjects, thus preventing the performance of Cox analysis including this group. However, Cox proportional hazards models were used in the depressed cohort to compute crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) to quantify the relation between the different exposures, including depression subtypes, and dementia. For multivariate analyses we considered the variables that were significantly associated ($p<0.05$) with dementia in univariate analysis, as well as variables considered to be potentially relevant confounding factors, according to the literature (Ritchie el al, 2012; Reitz et al, 2011). Age of onset and the presence of vascular risk factors were considered only in sensitivity analyses because data on these variables was available for a subset

of all patients (77 and 123, respectively), and due to the fact that the latter was assessed at follow-up. The proportional hazards assumption was evaluated graphically using “log-log” plots. We estimated the cumulative incidence of dementia, across the follow-up period, taking into account the competing risk of death, using a competing-risks regression model, according to the method of Fine and Gray (Fine & Gray, 1999).

Statistical significance was accepted for $p < 0.05$.

2.5. Ethics

This study was conducted in accordance with the Helsinki Declaration as well as national ethical guidelines. The local Ethics Committee, the National Data Protection Committee, and the national Institute of Notaries and Records approved the protocol.

Subjects who performed follow up evaluation were required to provide informed consent.

If a diagnosis of dementia was established, reference to a Dementia Clinics was offered to the subject, to undergo the standard of care for evaluation and treatment of dementia.

3. Results

3.1. Baseline and descriptive characteristics of both cohorts

In 133 (41.3%) depressed cohort subjects, followed-up for a mean (standard deviation) of 25.7 (7.2) years, the diagnosis of dementia could be established or excluded. Comparing baseline data of the subjects with and without a known outcome, the mean age was lower and the proportion of men was smaller in the subjects with a known outcome, but no statistically significant differences were observed regarding other socio-demographic and clinical characteristics (Table 4).

Table 4. Baseline characteristics of both cohort subjects to known outcome

Characteristics	Depressive cohort				Surgical cohort			
	Lost during follow up (n= 189)	With known outcome (n=133)	Total (n=322)	*p* value[a]	Lost during follow up (n= 152)	With known outcome (n=133)	Total (n=289)	*p* value[a]
Age, mean (SD), y	50.8 (12.7)[b]	41.8 (11.6)	46.9 (13.0)	<0.001	47.7 (14.5)	41.0 (12.1)	44.6 (13.8)	<0.001
Male sex, % (n)	27.0 % (51)	15.0% (20)	22.0% (71)	0.014	20.4% (31)	15.0% (20)	17.9 (51)	0.280
Education, mean (SD), y	6.4 (4.6)	7.1 (4.5)	6.7 (4.6)	0.165	not available	6.63 (4.6)[c]		
Bipolar disorder, % (n)	10.6% (20)	16.5% (22)	13.0% (42)	0.326				
Melancholic features, % (n)	33.3% (63)	35.3% (47)	34.2% (110)	0.722				
Psychotic features, % (n)	24.3% (46)	24.1% (32)	24.2% (78)	0.999				
Anxiety symptoms severity, mean (SD)	1.6 (2.6)	1.9 (2.8)	1.7 (2.7)	0.288				
Clinical Global Impression (CGI), mean (SD)	5.0 (0.8)	5.0 (0.7)	5.1 (0.7)	0.978				

Legend [a]Comparing, within each cohort, those with a known outcome with those without known outcome, based on chi-square test for category variables and independent samples *t* test for continuous variables; [b]baseline age available only for 182 subjects; [c]education years available for 111 subjects. Education years were not written in surgical files.

In both cohorts, baseline age was lower in those whose outcome was determined than in those it was not), but no significant diferences were found in sex proportions.

Almost all subjects were Caucasian, in depressed and surgical cohort (98% and 99%, respectively). For those in whom the outcome was determined, no differences were found in age and years of education between cases and controls.

3.2. Depression as a risk factor for dementia

Forty-four (33.1%), of the 133 depressed subjects included in the analysis, developed dementia. Dementia cause could be assessed in about half of these cases (n=21), and the most prevalent was Alzheimer's disease (AD) (57%), followed by vascular dementia (19%), and Parkinson's disease (14%). Other causes were vitamin B12 deficiency and HIV dementia.

The characteristics of subjects who developed dementia and those that have not are displayed in Table 4. At follow-up 4 patients with unipolar depression were rediagnosed as bipolar, due to a later emergence of a manic episode, and 2 non bipolar patients fulfilled DSM-5 criteria for Persistent Depressive Disorder at baseline, but were re-classified later as MDD.

In the control group, 20 subjects (15.0%) developed dementia, the most common assed cause was AD (n=5). The odds of dementia were increased by 2.50 times (95% C.I. 1.14-5.49; p=0.022) for the depressed cohort when compared to the surgical cohort, controlled for sex and education, computed by logistic binary regression.

Table 5. Cohort characteristics and outcome

	Depressed cohort		Surgical cohort	
Characteristics	**Non demented (n= 89)**	**Demented (n=44)**	**Non demented (n= 113)**	**Demented (n=20)**
Age, mean (SD), y	38.9 (10.4)	47.8 (11.5)	38.6 (11.0)	55.90 (8.6)
<35 years, % (n)	46.1% (41)	11.4% (5)	46.0% (52)	0.0 %(0)
35-45 years, % (n)	23.6% (21)	29.5% (13)	23.9% (27)	10.0% (2)
>45 years, % (n)	30.3% (27)	59.1% (26)	30.1% (34)	90.0% (18)
Male sex	14.6 % (13)	15.9% (7)	14.2 % (16)	20 % (4)
Education, mean (SD), y[a]	7.8 (4.5)	5.9 (4.4)	7.3 (4.7)	2.9 (1.7)
<4 years, % (n)	39.3% (35)	61.4% (27)	56.4 % (53)	100 % (17)
4-9 years, % (n)	31.5% (28)	22.7% (10)	17.0 % (16)	0.0 % (0)
>9 years, % (n)	29.2% (26)	15.9% (7)	26.6 % (25)	0.0 % (0)
Follow up time, mean (SD), y	26.3 (7.2)	24.5 (7.3)	30.4 (5.4)	25.7 (8.0)
Age of onset of the disorder, mean (SD), y[b]	28.4 (10.2)	34.8 (10.2)		
Bipolar disorder, % (n)	14.6% (13)	18.2% (8)		
DSM-5 Diagnosed MDD, % (n)	84.3% (75)	79.5% (35)		
Melancholic features, % (n)	24.7% (22)	56.8% (25)		
Psychotic features, % (n)	20.2% (18)	31.8% (14)		
Anxiety symptoms severity, mean (SD), AMDP score	1.48 (2.59)	2.86 (2.95)		
Clinical Global Impression (CGI), mean (SD)	4.9 (0.7)	5.1 (0.7)		
Chronic Disorder, % (n)	71.3% (57)	92.3% (36)		
Inpatient at baseline, % (n)	55.1% (49)	65.9% (29)		
Ever been admitted for depression, % (n)[c]	82.7% (62)	94.6% (35)		
Suicide attempts, % (n)[d]	43.9% (25)	66.7% (12)		

	Depressed cohort		Surgical cohort	
Characteristics	**Non demented (n= 89)**	**Demented (n=44)**	**Non demented (n= 113)**	**Demented (n=20)**
EPQ (Extroversion), mean (SD)[e]	38.0 (21.2)	35.1 (17.5)		
EPQ (Neuroticism), mean (SD)[e]	81.8 (14.9)	78.8 (14.8)		
High Neuroticism/Low Extroversion Group, % (n)[e]	24.6% (14)	23.3% (7)		
Vascular risk factors, % (n)[f]	59.8% (52)	80.6% (29)	60.6% (57)	75.0% (9)
FU time to dementia (y), mean (SD)		24.5 (7.3)		25.6 (8.0)
Died, % (n)	18.0% (16)	36.4% (16)	20.4% (23)	60.0% (12)

Notes: MDD Major Depressive Disorder, EPQ Eysenck Personality Questionnaire. [a]Data available only in 111 controls; [b]Data available only in 77 subjects; [c]Data on admission status was available in 82 subjects; [d]58 subjects had suicide data; [e]87 subjects had baseline EPQ; [f]Data obtained at follow up, available in 123 depressed subjects and in 101 controls.

3.3. Role of early-onset depression and depression heterogeneity

When the analysis is restricted to subjects younger than 45 years old (to compute the risk for early onset depression) were excluded from both cohorts, the risk for dementia is still significantly increased [OR 6.85 (95% C.I. 1.38-34.00); p=0,019] when compared to the surgical cohort, using a binary logistic regression, controlled for age and years of education.

In the depressed cohort, the probability of dementia increased significantly with age, was not influenced by education and was significantly higher for melancholic features, anxiety symptoms, and severity of the episode assessed with the CGI, in the univariate Cox regression analysis (Table 6). In the multivariate Cox analysis (Table 6), older subjects at baseline and those with depression with melancholic features had an increased risk for developing dementia compared to those without melancholic features (HR=3.64; 95% C.I.

1.78-11.26). The other depression characteristics and education were not associated with a higher risk for dementia.

Table 6 Depressed Cohort: Association between demographic variables, clinical characteristics and other well established risks for dementia, and the risk of dementia

	Univariate analysis		**Multivariate analysis**	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Baseline Age, y				
<35	1 [reference]		1 [reference]	
35-45	7.55 (2.15-26.52)	0.002	7.26 (1.99-26.51)	0.003
>45	15.17 (4.55-50.58)	<0.001	13.18 (3.72-47.50)	<0.001
Baseline Education, y				
0-4	1 [reference]		1 [reference]	
5-9	0.88 (0.42-1.84)	0.730	1.40 (0.60-3.28)	0.437
>9	0.47 (0.19-1.15)	0.097	0.461 (0.16-1.34)	0.156
Bipolar Disorder	1.88 (0.86-4.14)	0.116	0.64 (0.22-1.84)	0.408
Melancholic features	4.48 (2.40-8.39)	<0.001	3.64 (1.78-11.26)	0.025
Psychotic features	1.55 (0.81-2.96)	0.182	1.55 (0.76-3.14)	0.224
Anxiety symptoms	1.18 (1.08-1.30)	0.001	0.97 (0.82-1.22)	0.969
Clinical Global Impression (Severity)	1.84 (1.18-2.87)	0.008	1.19 (0.74-2.36)	0.345
Chronic disorder	1.85 (0.96-3.55)	0.065	1.24 (0.58-2.61)	0.581
Male sex	0.98 (0.39-32.51)	0.974		
Inpatient at baseline	1.01 (0.53-1.92)	0.980		
Ever been admitted for depression	2.20 (0.51-9.52)	0.290		
Suicide attempts	2.03 (0.74-5.63)	0.172		
High Neuroticism/Low Extroversion Group	0.71 (0.33-1.82)	0.568		

Notes: Variables found significant in the preliminary analysis and those known to influence the risk for dementia were included in the Cox proporcional hazards models.

Two sensitivity analyses were performed in this cohort, including age of the affective disorder (determined in few cases) and vascular risk factors (data

obtained prospectively). In a new model, the inclusion of age of affective disorder onset and vascular risk factors did not change the significance of the associations between both melancholic features and age at baseline with dementia risk.

Studies with a very long follow up, especially including geriatric outcomes and depressed subjects, are faced with the difficult problem of how to account for the competing risk of death (Wulsin et al, 1999). Because Cox proportional hazards regression can overestimate the risk of disease, a risk competing analysis was performed. The cumulative incidence of dementia, according to melancholic features of depression, was higher among subjects with melancholic features during most of the follow-up period, reaching approximately 50% at around 30 years of follow-up, according to a competitive risk model (Figure 3).

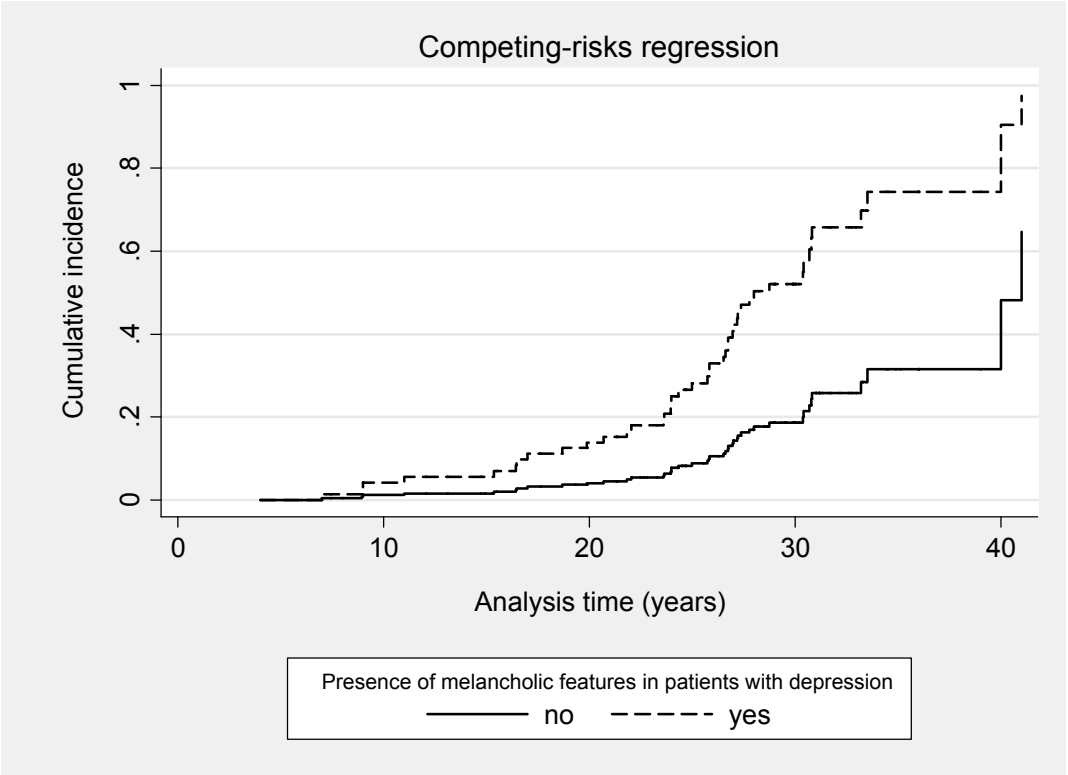

Figure 3 Cumulative incidence of dementia in the depressed cohort, across the follow-up period, taking into account the competing risk of death, using a competing-risks regression model.

When comparing both melancholic and non-melancholic depressed patients with non-depressed subjects, only melancholic patients showed a statistically

significant increased risk for dementia, using age and education were used as covariates in a binary logistic regression (Table 6).

Table 6 Association between depression, melancholia, and risk of dementia

	OR (95% CI)	*p* value
Surgical cohort	1 [reference]	
Depressed non-melancholic	1.60 (0.80-3.22)	0.062
Depressed melancholic	6.42 (3.05-13.52)	<0.001
Baseline Age, y		
<35	1 [reference]	
35-45	4.52 (1.45-14.10)	0.013
>45	11.57 (4.08-32.76)	<0.001
Baseline Education, y	0.87 (0.793-0.948)	0.002

4. Discussion

The main finding of the present study is that melancholic features of depression are an important and independent risk factor for dementia.

Depression has been globally associated with a two-fold increase risk of dementia (Silva et al, 2013), just like we found in this study. But, as far as we know, melancholic features have not been previously specifically assessed as a risk factor for dementia. Melancholia has been associated with persistent cognitive impairment after depression remission (Lin et al, 2014; Roca et al, 2015), but not with dementia. It is possible that the follow up time has been too short in both studies (6 weeks and 6 months, respectively) to detect an increased incidence of dementia. Remarkably, in the present study, melancholic features were independently associated with dementia. As previously mentioned, the study from DalForno et al (2005), failed to find an association of negative affective symptoms with dementia risk. However, the assessment of negative affective symptoms was

based on a CES-D subscale which does not match exactly international standard criteria for melancholic features. Most risk factors that have been studied in previous investigations, such as more severe disorder, greater severity of the episode, diagnosis of bipolar disorder, and the presence of psychotic and anxious symptoms, were associated with an increased risk for dementia in the univariate analysis, but their significance disappeared in the multivariate analysis. Since melancholic features tend to repeat across lifetime episodes (Coryell et al, 1994), are associated with a more severe course, occur in virtually all psychotic episodes, and happen more frequently in bipolar patients (Taylor and Fink, 2008), it is possible that the higher risk associated with those characteristics in previous studies could be at least partially mediated by melancholia. However, the role of bipolar disorder as a risk factor for dementia might have been underestimated because of the small number of bipolar patients in the present cohort. In any case, the present results strongly emphasize that analysis of melancholic features should be included in future studies.

There is a biological rational for the association between melancholic features and dementia, as an important body of evidence pinpoints the biological mechanisms underlying melancholia with cognitive impairment. Melancholia has been associated with hypothalmus-pituitary-adrenal (HPA) axis dysfunction (Brown, 2007; Parker et al, 2013), and hyper- and hypocortisolism characterize different subtypes of depression (Hasler el al, 2004). This HPA axis dysfunction has been widely studied in animal models and in human disorders (Lupien et al., 1998; Finsterwald et al., 2013), and involves down regulation of glucocorticoid receptors and/or increased circulating glucocorticoids triggering a cascade of events that leads to cognitive impairment (McEwen & Margarinos, 1997; Lupien et al, 2008). Melancholia may particularly induce hippocampal damage (Lamers et al., 2012), and has been associated with cognitive decline (Withall et al., 2010; Sachs-Ericsson et al., 2013).

Present results also support a role of depression as an early risk factor for dementia. The depressed cohort included mainly patients with early onset depression, that is, with a young age both at baseline and, when available, at depression onset. The follow up time between the diagnosis of depression and of dementia was very long making it highly improbable that depression was a

manifestation of dementia. To confirm these impressions we performed an additional analysis on depression occurring before 45 years, and found they carry an 11-fold increase in the risk for dementia. Our results are in line with studies with a long follow up (Kessing, 1999; Broadly, 2003; Saczynski, 2010; Dotson, 2010; Barnes, 2012), with a low probability of misdiagnosing the depressive disorder. The few studies that specifically explored the differences between late and early onset depression and found a very small increased risk or no differences for EOD assessed depression retrospectively by a simple questioning (Green, 2003; Almeida, 2015), using CES-D or GDS (Lenoir, 2011; Almeida, 2015), or included subjects mainly with neurotic depression (Brommelhofl, 2009). The accuracy of depression diagnosis is probably lower when compared to the present study, and this could have led to the inclusion of less severe depressive disorders or, according to these results, non-melancholic patients.

The precise diagnosis of dementia was not possible to ascertain in about half the cases in the depressed cohort and in about 2/3 of the controls, a natural consequence of the way clinical information was collected. So it was not possible to identify if depressive subtypes are associated with a specific dementia. It has been suggested that depression is a risk factor for vascular dementia (VD) and for Alzheimer's disease (AD) (Lenoir et al., 2011; Brunnström et al, 2013). The Honolulu-Asia aging study (Irie et al., 2008) found a higher risk for AD, however a higher load of cortical plaques and tangles was not associated with AD and depression (Wilson, 2003). Although in half of the cases the dementia cause could not be identified, the present results suggest that depression could be associated essentially with an increased risk of AD and not VD.

This study has important strengths. It is one of longest longitudinal studies performed to evaluate the risk of dementia in depressed patients. Another strength, is that the diagnosis of depression was done with extreme detail and rigor. The vast majority of previous studies assessed depression with very simple instruments, such as CES-D, that cannot capture the complexity and heterogeneity of the depressive disorder. Still another strength is that important and different risk factors and confounders were assessed (such as personality, severity of the episode, or vascular risk factors), that have been seldom evaluated

together. If different subtypes of depression carry different risks for dementia, it would be elicited by a study with this design.

4.1. Limitations

We should also note the limitations of this study. Melancholia definition is controversial, and DSM-5 definition has been challenged, on the grounds of the limitation of defining melancholia by reliance on symptoms (Parker & Paterson, 2014). These authors propose the inclusion of biological markers related to HPA dysfunction. The inclusion of a biological biomarker would strengthen the findings of this study. Another limitation is the absence of a formal cognitive evaluation at baseline. However, dementia was excluded clinically, the mean age for the onset of the affective disorder was around 35 years old and the mean time to the event was more than 25 years, making it very unlikely that dementia was present at baseline. Losses to follow-up may limit the validity of longitudinal studies, particularly very long studies. Even though, the present study was able to include more than other similar studies (41% versus, for instance, 33% in Brodaty et al., 2003), and, except for sex and age, no significant differences were found between subjects lost to follow up and those with a known outcome.

4.2. Conclusions

Depression is an early risk factor for dementia and a mere prodrome. Depression is a heterogeneous disorder, and it is possible that the frequency of melancholic features could explain the discrepancies found in the risk for depression as a whole in the different studies. Melancholic features of depression should be actively identified in the clinical setting, and DSM-5 criteria seem appropriate for this purpose. Due to a more favorable response to biological therapies, appropriate treatment of melancholia could decrease the risk for dementia.

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Escitalopram improves memory deficits induced by maternal separation in the rat

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Abstract

Maternal separation (MS) induces depressive-like behavior and long-term changes in cognition in rats. Escitalopram is an antidepressant drug shown to reverse the depressive-like features caused by this stress model. However, it is not known if it can ameliorate the affected cognition. We now characterized the effect of escitalopram on hippocampal-dependent memory in rats submitted to the MS protocol. Male Wistar rats were assigned either to control (CTR) or maternal separated (MS) group. MS were separated from their dams between 2–14 postnatal days (PND) for 180 min daily. Escitalopram was given in food pellets (0.34 g/kg/day first 2 weeks and 0.41 g/kg/day the subsequent period, average dose 25 mg/kg) from PND 43 onwards, during 1 month. Depressive behavior was assessed in the forced swimming test (FST), and memory performance in the Morris water maze (MWM). Escitalopram significantly improved the FST's latency to despair in the MS group (n=6), but did not change the immobility time. All groups showed a significant learning effect in the MWM over time, but no differences have been found upon treatment (n=6). However, escitalopram treatment significantly increased the time spent on the platform quadrant in the probe trial in the MS group. We report here that chronic treatment with escitalopram is able to improve hippocampal dependent memory in a chronic stress model, while not changing the learning ability. Moreover, this is accompanied by an amelioration of the depressive like behavior. These results support the use of escitalopram to tackle underlying cognitive deficits caused by stress in early-life.

Keywords

Hippocampus; Stress; Morris water maze; Forced swimming test; Antidepressant

1. Introduction

Depression can induce long-term cognitive deficits or even be a risk factor for dementia (Ownby, Crocco et al. 2006). As there is no cure for most dementia conditions, strategies directed to the correction of risk factors amenable to prevention are extremely important. Depression can be effectively treated in the majority of cases, so it emerges as an important target for the prevention of cognitive deficits and dementia. Treatment of depression is complex and multidisciplinary, but usually implies drug treatment with antidepressants. The long-term effect of antidepressants on human cognition (and eventually on the prevention of dementia) has been poorly studied and clearly remains to be elucidated (Kessing, Sondergard et al. 2009).

Maternal separation (MS) is an animal model of depression that can be induced in rodents in a manner that is not easily amenable or ethically allowed to humans, and permitting a reliable evaluation of a number of internal and external factors (e.g. pharmacological interventions). This model is considered a validated model of depression and anxiety (Ladd, Huot et al. 2000, Kalueff and Tuohimaa 2004). We and others have previously shown that rodents separated from their mothers, according to this protocol, have poorer cognitive performances on memory tasks, probably related to the effect of hypothalamus-pituitary-adrenal (HPA) axis disruption (Aisa, Tordera et al. 2007, Batalha, Pego et al. 2013) on the hippocampus (Huot, Plotsky et al. 2002, Aisa, Elizalde et al. 2009).

Escitalopram is a widely used and highly efficacious antidepressant belonging to the Selective Serotonin Reuptake Inhibitors (SSRI) class. Escitalopram and other antidepressants can reverse depressive-like behaviors in rodents in the Forced swimming test (FST) (El Khoury, Gruber et al. 2006). Although MS impacts in memory and learning and escitalopram is effective in reversing depressive – like behavior, it is not known if this antidepressant can improve cognitive deficits induced by MS.

To study the effect of antidepressant treatment on cognition in a rat model of depression, we have submitted rats to the MS protocol and then to widely used tests for depressive-like behavior – FST – and for memory – the Morris water maze (MWM). We hereby report that the escitalopram diminished FST latency-to-

despair and improved the latency in the probe test of the MWM, in MS treated animals.

2. Material and methods

2.1. Animals

Pregnant Wistar rats were purchased (Harlan, Barcelona) on gestation days 12–15 and were due in our animal facility. All animals were handled according to European Community guidelines and Local Law on animal care (1005/92). The animals were kept on an environment controlled for temperature (22±2 °C), humidity (55±10%) and light (12-h light/dark schedule; lights on at 7:00 a.m. and off at 7:00 p.m.).

Pups were randomly assigned to the MS protocol (n=12) or to animal facility rearing (CTR, n=12). Local Ethics Committee has approved the research protocol.

2.2. Maternal separation protocol

The MS protocol followed has been validated and described before (Lopes, Marvin-Guy et al. 2008, Batalha, Pego et al. 2013). At postnatal day (PND) 2, pups from four different litters, were collected together, gender assessed, and the pups were randomly distributed to foster dams (male/female ratio kept constant). Pups assigned to MS group were removed from their cages and dams at postnatal days (PND) 2–14 for 180 min daily. They were removed as a group from the nest, weighed and placed as a group in an isolation cage in an adjacent room kept at 32.0±0.5 °C. At the end of the separation period pups were returned to their home cage and rolled in the soiled home cage bedding before reuniting them with the mother. CTR animals were only briefly manipulated to change the beddings in their cages twice/weekly. At day 21 the pup's sex was determined and they were weaned and housed in individual cages. Only male rats were in included in the study. After weaning, pups were weighed weekly. No changes were found in weight due to MS protocol as observed before (Lopes, Marvin-Guy et al. 2008).

2.3. Treatment procedures

The majority of hippocampal granule neurons develops and extends their axons between PND 1 and 21 (Amaral and Dent 1981). We have initiated the treatment

in puberty, after the full development of hippocampal circuitry, period when interventions, either drugs or stimulation, are more effective in the hippocampus (El Khoury, Gruber et al. 2006).

Rats were left undisturbed from PND 15–42. On postnatal day 43, half of the rats from each group (MS and CTR) were assigned to dietary treatments with the antidepressant escitalopram (0.34 g/kg/day chow for the first 2 weeks; 0.41 g/kg/day chow during the rest of the experiment) (CTR+AD; MS+AD), or were given placebo, admixed to food pellets (CTR; MS). The escitalopram doses were increased sequentially, according to the method developed by H. Lundbeck A/S (Copenhagen, Denmark) and tested by A. Mørk (El Khoury, Gruber et al. 2006), resembling the clinical situation where a range of escitalopram (or other antidepressants) is used to treat depressed patients. These doses have been shown effective in reverting depressive-like behaviors in the forced swimming test (FST) in the MS model of depression (El Khoury, Gruber et al. 2006). Lactamin AB, Sweden, prepared Denmark dietary escitalopram according to instructions from H. Lundbeck A/S, Denmark. The escitalopram intake was controlled by weighing the animals and monitoring the food intake per animal (weekly intake in Table 7). The pellets made available were weighed prior and post intake and dose estimated according to the weight of each animal. The administered escitalopram dose was approximately 25 mg/kg/day. Animals were kept on their respective diet until the end of the experiment on PND 73.

Table 7

Amount of ingested escitalopram pellets and dose equivalent.

Week	Animal weight (g)	Food ingested/day (g)	Dose equivalent (mg/kg/day)
1	180.42	19.19	24.7
2	230.98	25.84	25.3
3	218.8	21.60	27.6
4	231.2	19.80	23.9
5	209.25	19.87	26.5
6	210.79	19.87	26.3

Average 25.4 mg/kg/day.

All animals have been handled for 5 days prior to behavioral testing.

2.4. Forced swimming test

Forced swimming test (FST) was used as a behavior equivalent of learned helplessness to test AD action (Porsolt, Le Pichon et al. 1977, Castagne, Moser et al. 2011). It was carried out on PND 64 and 65 (note that the escitalopram or vehicle diets were continued until PND 73). On the first of the two test days, all animals were gently placed individually in a vertical Plexiglas cylinder (height: 45 cm, diameter: 19 cm) filled with 26 °C water at a depth that makes it impossible to reach the bottom with hind paws (28–30 cm). The animals were removed from the water after 10 min, and dried before being returned to their home cages. The water was changed after each session. On the second day, the animals were placed in the same cylinders for 5 min. This session was video-recorded and an observer blinded to the animals group subsequently scored the behavior of the animals. According to the criteria of Porsolt et al. (1977), the rat was judged to be immobile when it floated passively, making only small movements to keep its nose above the surface. This test measures the latency to immobility (or to despair, LTD) and the time spent swimming versus the time spent floating, i.e. the percentage of time spent immobile or immobility time (IT).

2.5. Morris water maze

The protocol used was the classical Morris water maze test, which is sensitive to hippocampal-dependent spatial learning and memory (Morris, Garrud et al. 1982). The protocol was used as before (Diogenes, Costenla et al. 2011). The maze consisted of a large circular tank (1.8 m in diameter, 0.6 m in height) of water (temperature, 25±1 °C) made opaque with the addition of a small amount of non-toxic water-based black paint. An escape platform (10 cm in diameter) was submerged, non-visible, 1 cm below the water. Several visual cues for spatial reference were placed on the walls of the testing room. The performances were continuously monitored using an automated tracking system (Smart 2.5, PanLab, Barcelona). From PND 80 to 84, rats were given spatial acquisition training consisting of four trials/day for four consecutive days, in which the platform was placed at a fixed position in the center of one of the four quadrants of the tank

(platform Q, left, right and across). On each day the subjects were randomly placed in four different positions facing the wall, and never in the platform quadrant. The inter-trial interval was of at least 15 min, during which animals were towel-dried and placed under an infrared light to prevent hypothermia. The maximum trial duration was 60 s, after which animals were manually guided to the platform if they failed to locate it, and they were allowed to remain there for 20 s. At the fifth day a probe test was given in which the platform was removed and animals were allowed to swim freely for 60 s while recording the percentage of time spent on each quadrant. The latency to find the platform during acquisition and the percentage of time in the platform quadrant in probe test were used to evaluate hippocampal dependent memory.

2.6. Statistics

Statistical Package for Social Sciences v19 (SPSS) was used for the statistical treatment of data. After establishing homogeneity of variances, a two-way ANOVA was carried out for analyzing FST (LTD and IT) and time spent on quadrant after platform withdrawal (using MS (*) and AD ($^{\#}$) treatment as factors). Simple main effect analyses were performed, following significant interactions. Two-way ANOVA repeated measures was used for the learning curve of MWM. Statistical significance was accepted for $P<0.05$. Results are expressed as mean±standard error of mean (SEM).

3. Results

3.1. Forced swimming test

As observed in Fig. 4, latency-to-despair (LTD) was found to be decreased by MS ($F(1,21)=8.373$; $^{*}P=0.010$). LTD decreased from 71.8±7.71 in CTR to 38.8±6.25 s in MS animals. The treatment with escitalopram significantly improved LTD in MS +AD animals to 65.6±7.35 s ($F(1,20)=5.015$; $^{\#}P=0.038$).

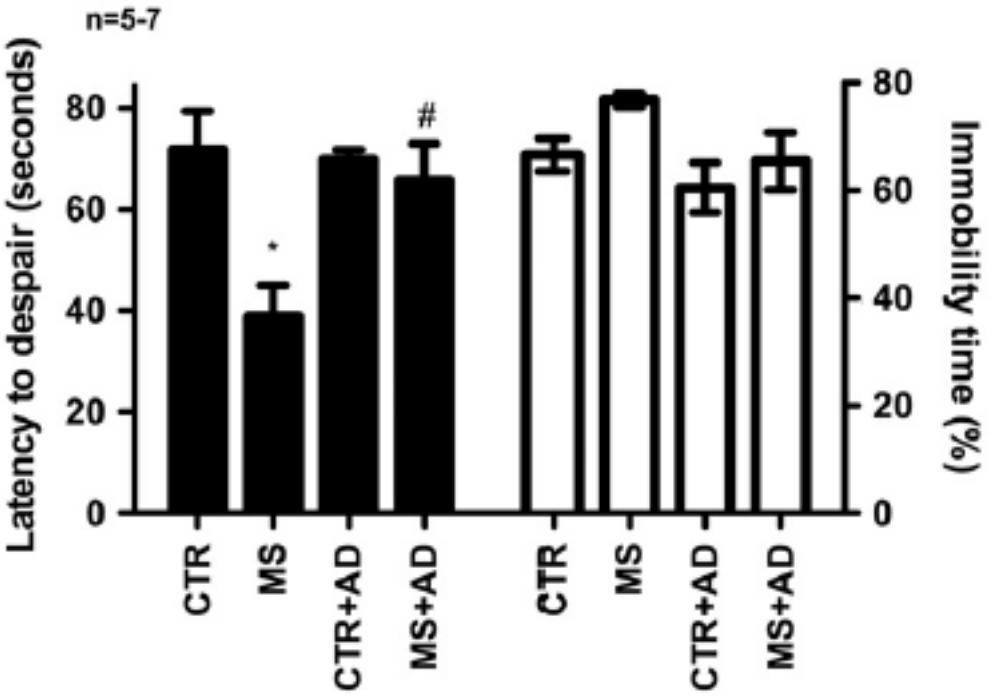

Fig. 4.

Escitalopram reverts MS-induced increase in latency-to-despair. Learned helplessness was assessed by the Porsolt forced swim test, in which latency to despair (LTD) and immobility time (IT) were evaluated. Results are the mean±SEM of 5–7 animals; (*) P=0.010 for MS effect and (#) P=0.038 for AD effect, calculated using two-way ANOVA, followed by simple main affect analysis.

Escitalopram also affected the immobility time (IT) in all groups of rats ($F(1,23)=4.658$; $P=0.043$) but no differences were found when comparing MS and CTR animals ($F(1,23)=0.413$; $P=0.528$).

3.2. Morris water maze

3.2.1. Learning curve

A significant learning effect throughout the 4 days was present, for all groups. The latency to reach the platform significantly improved from 49.4±2.16 s on day 1, to 18.3±2.87 s on day 4 ($F(3,66)=77.542$; $P<0.001$). However, no significant changes between different groups were found ($F(3,22)=0.575$; $P=0.456$; Fig. 5a).

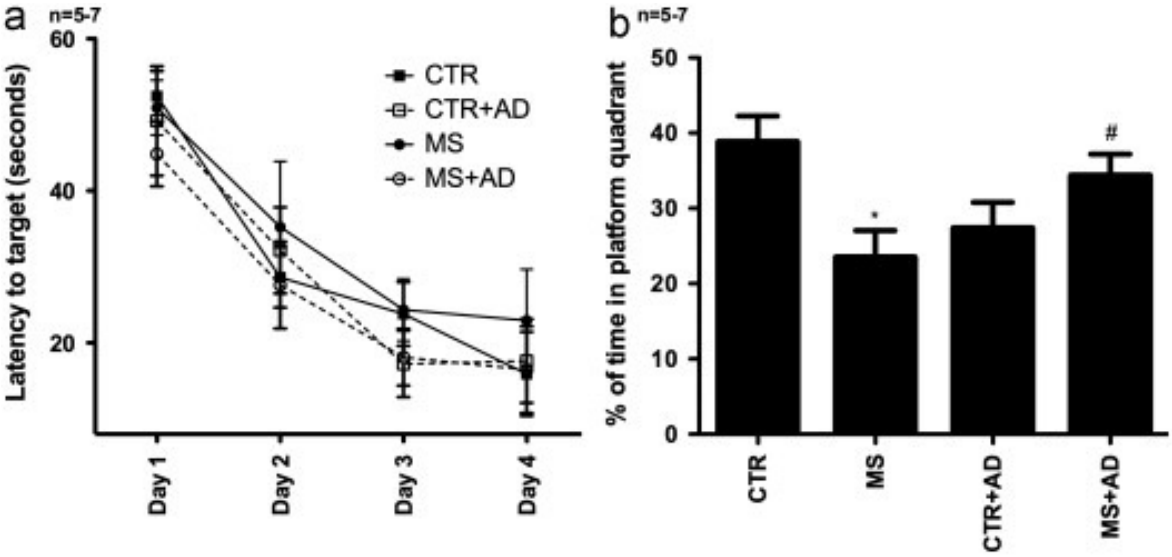

Fig. 5.

Escitalopram reverts MS-induced impairment in the probe trial. Hippocampal dependent memory performance was assessed by the Morris water maze test, in which acquisition (a) and retention (b) were evaluated. Results are the mean±SEM of 5–7 animals; (*) $P<0.01$ for MS effect and (#) $P=0.004$ for AD effect, calculated using two-way ANOVA, followed by simple main affect analysis.

3.2.2. Probe trial

The ability to recall spatial memory is tested following the learning period, by withdrawing the platform. MS animals displayed less time (23.5±7.98%) in the quadrant as compared to the CTR group (38.8±9.18%; Fig. 2b). However, escitalopram reverted this, improving the performance of MS animals ($F(1.23)=10.764$; $^{*}P=0.004$). The time spent on the platform quadrant increased to 34.3±7.04% in MS AD group (Fig. 5b). Escitalopram alone seems to induce a slight decrease in time spent on platform for CTR animals, but this difference is not statistically significant.

The average speed and total swimming distance on probe trial were similar among groups (data not shown).

4. Discussion and conclusions

Maternal separation is known to induce deficits in memory related tasks that persist throughout adulthood. We hypothesized that treatment with an anti-depressant, escitalopram, would be beneficial in reverting these deficits. We now report that chronic treatment with escitalopram for one-month given to adult

animals can improve hippocampal-dependent memory and latency to despair deficits induced by maternal separation (MS).

We found a significant effect of MS for acquisition, but not for learning. Due to the heterogeneity of MS protocols, animal strains (Huot, Plotsky et al. 2002, Hui, Zhang et al. 2011) or both (Anisman, Zaharia et al. 1998, Oitzl, Workel et al. 2000, Frisone, Frye et al. 2002, Zhu, Peng et al. 2011) it is difficult to find a pattern of the cognitive deficits induced by MS. In experimental conditions similar to ours, results are consistent, showing a difference in acquisition and not in learning, as if an effect in learning was more resistant to MS action than the acquisition (Aisa, Tordera et al. 2007, Aisa, Elizalde et al. 2009, Mello, Benetti et al. 2009). The mechanism for this MS induced cognitive deficits has been mostly related to the induction of HPA axis disruption, shown by elevated corticosteroids and corticosterone levels (Aisa, Tordera et al. 2007, Batalha, Pego et al. 2013), similar to what has been observed in depressed patients (Sachar, Hellman et al. 1970, Carroll, Feinberg et al. 1981). This state of hypercortisolism promotes the shrinkage and the degeneration of hippocampal neurons, both in humans (reviewed by Brown, Rush et al. 1999) and animals (Lupien and McEwen 1997, Batalha, Pego et al. 2013).

The main goal of the present study was to evaluate if an antidepressant could decrease these deficits. Antidepressants have several neuroprotective effects in the hippocampus of rats displaying depressive-like behaviors, either neurogenic (Malberg, Eisch et al. 2000) or neuroremodeling (Bessa, Ferreira et al. 2009). However, the impact of these putatively benefic changes is not known, as a direct effect of escitalopram has never been shown before on a memory task in this model of depression.

All groups showed a significant learning effect in the MWM over the days, but no differences have been found upon treatment. However, escitalopram treatment significantly improved the time spent on the platform quadrant in the probe trial in the MS group. The mechanisms underlying the antidepressant-related cognitive effects, reside either on a direct action on hippocampus, by stimulation of the neuronal remodeling (Bessa, Ferreira et al. 2009) or through restoring the normal physiology of the HPA axis (Manthey, Leeds et al. 2011).

MS is an appealing model of depression because it mimics the mother–infant interaction, which is a key factor for developing psychiatric disorders in the future (Freud 1995, Heim and Nemeroff 2001). MS does not induce very strong depressive-like behavior, when compared to some genetically selected strains, such as the “depressed” flinders sensitive line (El Khoury, Gruber et al. 2006). However, it is a more pertinent model when studying susceptibility to early-life events and more related to human conditions. We now report that, in assessing depression, statistical significance was only reached when measuring the effect of escitalopram in latency-to-despair (LTD), but not in immobility time (IT). IT use has been criticized for its low sensitivity in detecting antidepressant actions of SSRI and for having a positive response to psychostimulants (Borsini and Meli 1988, Rupniak 2003). Adding the LTD parameter can improve the sensibility to detect AD actions, especially for tricyclic antidepressants (TCA), and LTD is not affected by psychostimulants (Castagne, Porsolt et al. 2009), excluding changes due to general motor stimulation. Current results reinforce the advantage of assessing LTD to improve sensibility to SSRI antidepressant activity in rats.

Escitalopram alone seemed to induce a slight decrease in the probe test time in the MWM, for CTR rats. This did not reach statistical significance and we do not attribute it to any motor effect since we have used the same dose range as previous studies that found no impact in specific motor parameters (Mello, Benetti et al. 2009). An explanation for the findings is difficult, because its use in healthy subjects is not ethically allowed. However, escitalopram has some, although moderate, anticholinergic properties, and a tendency to cause “cognitive” flattening (Stahl 2009). This could indicate that its beneficial action is limited to animals with underlying deficits. On the other hand, it reinforces the idea that care must be taken and confirms the need to use healthy controls treated with antidepressants in such paradigms. The use of p.o. antidepressant could strengthen the findings, as these methodological approaches can be considered closer to human reality. However, other variables such as genetics and individual susceptibility to dosage must be accounted for. The present data focused on a specific class of antidepressant, the SSRIs. Whether the observed effects are due to a general antidepressant effect or instead to a specific mechanism of action of this class would be interesting to evaluate, testing distinct classes of drugs.

The present results may support a potential effective role of antidepressants, at least of the SSRI class, in the prevention of dementia associated to depression, in patients.

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E. Discussion

1. Is depression a risk factor for dementia?

Present results show that a subject with depression has roughly a 2.5 times higher risk of having dementia when compared to subjects without depression, controlled for important variables known to influence the risk. This is a slightly higher value than most studies, despite a magnitude similar to the three major meta-analyzes carried out earlier.

Most of the case-control studies found a lower risk for dementia in depressed patients than the present study. As previously referred, case-control design relies on a retrospective diagnosis of depression, that tend to be underestimated even in non-demented subjects (Schulz and Grimes 2002). The demented group probably did not accurately report the presence of depression, blurring the differences between groups. Diagnostic criteria for depression are not clear in some studies, and the inclusion of minor forms of depression could have underestimated the risk.

Some longitudinal studies also reported lower risk magnitudes (Chen, Ganguli et al. 1999) for dementia, but the diagnosis of depression in most of these studies was based on CES-D. If the diagnosis of depression was based on more clinical criteria (such as ICD or DSM) (Kessing, Olsen et al. 1999, Brodaty, Luscombe et al. 2003, Kessing and Nilsson 2003), the risk magnitude is higher. Another explanation is the severity of depression. Danish studies relied on inpatients, usually with more severe depressive disorders and, on the other hand, usually with a more accurate diagnosis. In one Danish study (Kessing, Olsen et al. 1999), a much higher risk was found (more than 10-fold), but dementia was only considered in the subset of patients readmitted to a psychiatric ward.

We have reasons to believe that the present study has a strong design. It is a controlled, longitudinal study with a very long follow up - one of the longest the literature. It is hard to imagine a higher accuracy of the depression diagnosis, as the basis of Paes de Sousa cohort was the diagnosis of depression. We tried to use the most recent clinical and research criteria for the diagnosis of dementia and dementia diseases.

In conclusion, present results are in line with studies with a stronger design and a better accuracy of depression diagnosis, yielding similar risk magnitudes.

2. What is the nature of the risk?

a) Is depression a prodrome or an early risk factor?

The subjects with early onset depression are also at an increased risk for dementia [OR 6.42 (95% C.I. 3.05-13.52); p<0.001] when compared to the surgical cohort. Moreover, the cohort included mainly patients with early onset depression, that is, with a young age both at baseline (mean 41.8±11.6 years) and, when available, at depression onset (28.4±10.2 and 34.8±10.2, respectively for demented and non-demented), making it highly improbable that depression was a manifestation of dementia. The time to dementia or to the end of the study was also very long (mean 25.7±7.2). Although no formal cognitive evaluation was performed at baseline, it was highly improbable that dementia was the cause for the depressive disorder. Present study supports the hypothesis of depression as an early risk factor for dementia.

However, several case-control and cohort studies found an increased risk only for late onset depression, eventually supporting the prodrome hypothesis. Two case-control studies found a decrease in the risk as the time between the two diagnosis increases (Green, Cupples et al. 2003, Brommelhoff, Gatz et al. 2009). Green (2003) diagnosed depression by direct questioning, and a recall bias cannot be ruled out. Brommelhoff study (2009) had higher standards for the diagnosis of depression (registry based), but only for some subjects. Self report by direct questioning was also enough to be considered depressed, so the inclusion of milder or doubtful forms of depression could have happened.

Although cohort studies with a short follow up seem to support depression as a prodrome of dementia, they do not exclude depression as an early risk factor (Devanand, Sano et al. 1996, Wilson, Schneider et al. 2003, Geerlings, den Heijer et al. 2008). Geerlings et al (2008) found an increased risk in early onset depression, but this is limited by the accuracy of depression diagnosis. Some cohort studies with a long FU time also support the prodrome explanation (Dal Forno, Palermo et al. 2005, Barnes, Yaffe et al. 2012), but both studies essentially rely on the self report of depression. Again, our study is in line with studies with a longer follow up, with a careful diagnosis of depression (Kessing, Olsen et al.

1999, Brodaty, Luscombe et al. 2003, Kessing and Nilsson 2003). Besides being amongst the studies with the longest FU, the present study was very careful on the accuracy of the diagnosis. We believe it provides strong evidence for considering depression as an early risk factor for dementia, and not merely a prodrome.

b) What is the role of different depression subtypes?

One of the main findings of the present work is that depression with melancholic features is an independent risk factor for dementia. When compared to depression without melancholic features, patients with melancholic features have 3.6 more risk per year of suffering from dementia (HR=3.64; 95% C.I. 1.78-11.26).

All the other depression characteristics evaluated (age of onset of depression, bipolar disorder, psychotic features, anxiety symptoms, chronicity of the disorder, severity of the episode, personality, and hospitalization) seem not to be predictors of dementia in depressed patients. Although some of these characteristics were significant in univariate analysis, their significance disappeared in the multivariate model. This could indicate that the risk of causing dementia is driven through melancholic features.

To our knowledge this is the first study addressing melancholia as a risk factor for dementia. DalForno et al (2005) failed to find an association of negative affective symptoms with dementia risk. However, the assessment of negative affective symptoms was based on a CES-D subscale, which does not match exactly the international standard criteria for melancholic features. Melancholia has also been associated with persistent cognitive impairment in euthymic patients (Lin et al, 2014; Roca et al, 2015), but not with dementia. It is possible that, in both studies, the FU time had been too short (6 weeks and 6 months, respectively) to detect an increased incidence of dementia.

Melancholic features tend to be more frequent in bipolar disorder (Mitchell and Malhi 2004), to confer more severity to the episodes, to occur concomitantly with psychotic symptoms, and to repeat across episodes (Parker, Fink et al. 2010). Eventually, the increased risk found in several studies for an increased number or severity of episodes (Kessing and Andersen 2004, Dotson, Beydoun et al. 2010, Saczynski, Beiser et al. 2010) was due to melancholia. Although the same

explanation could be given for bipolar disorder - the risk for dementia is driven through melancholic features, our study has a relatively low number of bipolar patients, and could be underpowered to show a difference.

Interestingly, a relatively strong biological rational existis for the association. As mentioned previously, melancholia is associated with several biological changes, the most consistent is HPA overactivity (Parker, Fink et al. 2010), with increased cortisol levels. The involvement of cortisol in the stress response (McEwen, Weiss et al. 1968), and its actions in the hippocampus have been fairly studied. Chronic stress, maternal separation, and chronic administration of corticosterone (the equivalent of cortisol in rats) produces hippocampal dendritic retraction and reduces neuropil volume in several hippocampal areas (Sousa, Lukoyanov et al. 2000, Ortiz, McLaughlin et al. 2013, Sousa, Vital et al. 2014). In humans suffering from Cushing syndrome, the hippocampus is smaller (Starkman, Gebarski et al. 1992), and the same is true in a classic stress disorder, the post-traumatic stress disorder patients (Bremner, Randall et al. 1995).However, the relation between childhood trauma, HPA dysfunction, and depression is not totally clear (Suzuki, Poon et al. 2014). As far as we know, there is no direct evidence of hippocampal atrophy specifically in melancholic patients, but the evidences, including the present study, point to the same direction.

c) What is the the role of different dementia disorders

AD was the most prevalent cause of dementia in both cohorts, although it seems to be much more prevalent in the depressed cohort (57% vs 20%). However, in a large number of subjects dementia causes were not determined, a natural consequence of the rigorous criteria applied. A direct comparative analysis could not be performed.

Anyway, the very few Portuguese epidemiological data published (Nunes, Silva et al. 2010) found roughly the same prevalence for AD and VD (around 40%), and thus lower for AD than our depressed cohort. As previously mentioned, this study has several limitations regarding VD diagnosis.

A higher risk for AD in depressed patients has been found in several case-control and cohort studies, but not in others (Table 2. and 3.), and the same is true for VD. The few studies that examined depression as a risk factor for different

dementia causes (comparing different risks) used CES-D or SCL-90 to diagnose depression, with the limitations discussed before. Two cohort studies (Kohler, van Boxtel et al. 2011, Lenoir, Dufouil et al. 2011) followed elderly community individuals free of dementia for 9 and 4 years respectively, and found that a depressed mood at baseline predicted the occurrence of VD, but not AD. Another study, in which around 2000 men with a mean age of 76 years were followed for 5 years, found exactly the opposite (Irie, Masaki et al. 2008). In a recent analysis of the Religious Order study (Wilson, Capuano et al. 2014) it has been found that late life depression is not associated with the neuropathological findings of AD or VD.

The high level of AD patients in Paes de Sousa depressed cohort does not support the hypothesis of a higher risk of VD in depressed patients. However, the limitations referred do not allow the conclusion that the risk is higher for AD.

3. What is the role of antidepressants?

We have shown that rats submitted to a MS protocol have cognitive changes that are reversed by longterm treatment with escitalopram, an antidepressant from the SSRI class.

Our results are in line with the neuroprotective action of antidepressants shown by the several studies referred above (Pehrson, Leiser et al. 2015), but we have shown that antidepressants also improve cognition in rats submitted to this MS protocol. If rats submitted to a MS protocol have biological changes similar to those found in melancholic patients, if these changes induce cognitive deficits, and if cognitive improvement is observed with escitalopram, this supports a protective role of escitalopram, and eventually of other antidepressants, in dementia prevention.

After the publication of our work, a few papers replicated these findings with citalopram (Eriksson, Holst et al. 2012), venlafaxine (Martisova, Aisa et al. 2013), and fluoxetine (LeGates, Altimus et al. 2012). Generally, short-term treatment with antidepressants was not shown to reverse cognitive deficits (Pehrson, Leiser et al. 2015). A few papers also reported a role of escitalopram in the pathological mechanisms of AD. Escitalopram decreases tau phosphorylation in a cell line (Ren, Wang et al. 2015) and a similar action of citalopram was found in rats, improving spatial memory (Ren, Gong et al. 2015). A clinical study describes a

decreased new Aβ production in patients treated with citalopram, using a radiolabelled leucine isotope do detect new amyloid production (Sheline, West et al. 2014). Amyloid clearance difference was not statistically significant.

The MS protocol is quite interesting for several reasons. First, it is an empirically appealing protocol, especially for psychiatrists, because it somehow mimics the childhood trauma, a known risk factor for depression later in life (Heim and Nemeroff 2001, Heim, Young et al. 2009). Second, rats submitted to this protocol show permanent over activation of HPA axis, expressed by high corticosterone levels, loss of cortisol circadian rhythm, and blunted dexamethasone suppression (Nishi, Horii-Hayashi et al. 2014). Similar biological changes are found in melancholic patients. Third, rats submitted to this protocol show hippocampal dendritic retraction (Chocyk, Bobula et al. 2013), long term potentiation (LTP) impairment, and longterm cognitive impairment, essentially memory and learning changes (Sousa, Vital et al. 2014).

One limitation is that the cognitive deficits found in these rats could not be considered dementia. However, rats do not spontaneously produce amyloid, and the concept of dementia (impairment of daily life activities) can hardly be applied to rats. Even more importantly, our lab showed that these deficits persist in old animals (Sousa, Vital et al. 2014).

The other limitation is related to the use of animal models in psychiatric disorders. With few exemptions, rats are different from humans, and the direct transposition of animal data to humans must be done cautiously. Psychiatric symptoms such as guilt, shame, delusions or hallucinations are extremely difficult to reproduce in animals. However, the behavioral manifestations of despair or of anhedonia, or more cognitive functions, such memory or attention, were considered reasonably mimicked in rodents (Kalueff and Murphy 2007).

However, we acknowledge that the ideal study to clarify the role of antidepressants in the modulation of dementia risk in depressed patients would be a clinical trial. Such trial should have a long follow up, comparing depressed patients treated and non treated with antidepressants, and having dementia as the outcome. This clearly would be an extremely expensive trial, and ethically hard to justify.

Anyway some papers published recently have addressed this issue. An analysis of the Religious Order Study found that antidepressants did not influence the development of AD, VD or LBD neuropathology (Wilson, 2015). However, this study only included late life depression, and if they are to have an effect, antidepressants should have been taken longer. Very recently, a retrospective study involving 3688 depressed patients (Wang, Gao et al. 2015), found that patients on antidepressants have an increased risk for dementia. This increased risk is found even when compared to patients with severe depression not on antidepressants. The amount, and duration, of antidepressants taken seems to be crucial in the development of their action, and the study had not taken this into account.

We believe that our work provided a rational for a putative effect of antidepressants in preventing dementia in depressed people.

F. Conclusion

The results presented seem to support a role of depression as a risk factor for dementia, and add novel information regarding the nature of this risk. According to these results, depression is not merely a prodrome of dementia, but an early risk factor, and melancholia is the only subtype associated with an increased risk. Also, they point to a neuroprotective action of escitalopram in depression, eventually leading to dementia prevention.

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Appendix (*facsimile* of published papers)

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Research paper

Depression with melancholic features is associated with higher long-term risk for dementia

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ABSTRACT

Background: Depression has been reported to increase the risk of subsequently developing dementia, but the nature of this relation remains to be elucidated. Depression can be a prodrome/manifestation of dementia or an early risk factor, and the effect may differ according to depression subtypes. Our aim was to study the association between early-onset depression and different depression subtypes, and the later occurrence of dementia.

Methods: We conducted a cohort study including 322 subjects with depression, recruited between 1977 and 1984. A comparison cohort (non-exposed) was recruited retrospectively, to include 322 subjects admitted at the same hospital for routine surgery (appendicectomy or cholecystectomy), at the same period as the depressed cohort. Subjects were contacted again between 2009 and 2014, to assess their dementia status. We computed the risk for dementia in subjects with early onset depression and quantified the association between different depression subtypes (namely melancholic, anxious, and psychotic) and dementia.

Results: The odds of dementia were increased by 2.90 times (95% C.I. 1.61–5.21; $p < 0.0001$) for the depressed cohort when compared to the surgical cohort. When the analysis was restricted to patients younger than 45 years old at baseline, the odds for dementia in the depressed cohort were also significantly higher when compared to the surgical cohort (8.53; 95% C.I. 2.40–30.16). In the multivariate Cox analysis, subjects having depression with melancholic features had an increased risk for developing dementia compared to those without melancholic features (HR=3.64; 95% C.I. 1.78–11.26; $p=0.025$).

Limitations: About 59% of the participants with depression and 53% of those non-exposed were lost during follow up. The inclusion of biological biomarkers would strengthen the results. The sample included a low number of bipolar patients.

Conclusions: These results support depression as an early risk factor for dementia. Depression with melancholic features was found as an important risk factor for dementia, playing a main role in the relation between these disorders.

1. Introduction

As most dementing conditions are irreversible, and the available therapies have limited beneficial effects, primary prevention of cognitive decline is of paramount importance (Ritchie et al., 2010; Norton et al., 2014). Among the several risk factors so far identified, depression emerges as a potentially important target (Reitz et al., 2011), because is amenable to prevention, has a high prevalence, and can be diagnosed inexpensively and treated effectively (Kupfer et al., 2012; Malhi et al., 2015).

Depression has been found to be a risk factor for dementia or Alzheimer's dementia (AD) in several case-control (Cooper and Holmes, 1998; Green et al., 2003) and cohort studies (DalForno et al., 2005; Kessing and Nilsson, 2003; Saczynski et al., 2010; Irie et al., 2008; Dotson et al., 2010; Byers and Yaffe, 2011), but not all (Chen et al., 1999, 2008; Gatz et al., 2005; Brommelhoff et al., 2009). The meta-analyses and reviews performed have confirmed this association in general, finding that depression approximately doubles the risk for dementia (Jorm, 2001; Ownby et al., 2006;

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Silva et al., 2013).

However, the nature of this relation remains poorly understood. Two unsolved issues have been repeatedly raised (Byers and Yaffe, 2011; Kessing, 2012). The first is that depression, especially if occurring after 60 years old (called late onset depression) or next to the diagnosis of dementia, can be a prodrome/manifestation of a dementing disorder, instead of an early risk factor. Depressive symptoms are quite common in dementia, and depressive symptoms may arise from the anatomic lesions that are part of the neuropathological changes of dementing disorders (Boland, 2000). Case-control studies that do not take in account the time between dementia and depression diagnosis, and cohort studies with a short follow up, may not be able to distinguish between these two situations. The few studies that specifically compared late-onset depression with early-onset depression found discrepant results (Green et al., 2003; Geerlings et al., 2008; Brommelhoff et al., 2009; Lenoir et al., 2011; Almeida et al., 2016).

The second issue is the subtype of depression. The heterogeneity of depression has seldom been taken into account. A more severe disorder (expressed by higher frequency, duration, and severity of the depressive episodes) has been inconsistently associated with a higher risk for dementia (Kessing and Andersen, 2004; Geerlings et al., 2008; Kessing, 2012; Silva et al., 2013). Bipolar disorder has also been associated with a higher risk of dementia. In the review and meta-analysis by Silva et al. (2013) the majority of studies confirmed the association in accordance with subsequent published studies. Brodaty et al. (2003) explored the role of comorbid anxiety in depression on the risk for dementia and found no influence. On the other hand, the use of benzodiazepines has been reported to carry a higher risk (Billioti de Gage et al., 2012). Psychotic symptoms have been associated with a higher risk for cognitive deficits only in bipolar patients (Martínez-Arán et al., 2004). Few studies looked at the risk for dementia in DSM5 or ICD10 defined depression subtypes. DalForno et al. (2005), in a community based study, performed an additional risk analysis finding that a Center for Epidemiologic Study–Depression (CES-D) sub-scale based on a cluster of negative affective symptoms, related to melancholic features, did not influence the global risk for dementia. Different biological mechanisms underlying these different depressive conditions can carry different risks for dementia. Melancholic features, and to a lesser extent psychotic symptoms, have been associated with more consistent biological abnormalities and response to treatment (Brown, 2007; Parker et al., 2013) when compared to their absence.

These unsolved issues – prodrome versus early risk factor and the heterogeneity of depression – regarding the risk for dementia in depressed patients, encouraged us to perform the current study. The objectives were to assess the association between early-onset depression and the long-term risk for dementia, and to analyze the risk for dementia of different depression subtypes, controlling for well known risk factors for dementia.

2. Methodology

2.1. Study design

This study is based on two cohorts followed in average 25 years for development of dementia. The exposed cohort (depression cohort) comprised 325 patients from the Hospital de Santa Maria, Lisbon, with the clinical diagnosis of depression, recruited between 1977 and 1984 in a taxonomic cluster analysis study of depression (Paes de Sousa et al., 1980).

A surgical comparison cohort (non-exposed) was recruited retrospectively, to include 325 subjects who were consecutively admitted to Hospital de Santa Maria, Lisbon, for routine surgery (appendicectomy or cholecystectomy) at the same period as the depressed cohort.

Participants were re-evaluated between 2009 and 2014, to establish the outcome - dementia status.

2.2. Baseline assessment

Data on demography, clinical history, and personal and family history as part of routine clinical files were collected for both cohorts. For the depressed cohort a comprehensive psychiatric and psychological evaluation was performed.

2.2.1. Evaluations

2.2.1.1. Association for methodology and documentation in psychiatry system (AMDP). The AMDP-System was created in Nuremberg in 1960 and has been widely used in Europe in 1970–1980. The Psychopathology Scale contains 100 psychopathology items, including symptoms and other clinical features, derived from classic psychopathology studies from Jaspers, Bleuler, Schneider, and others. It renders a very detailed and standardized evaluation, including affective, behavioral, cognitive, psychotic, sensory, and social dimensions of psychopathology (Busch et al., 1980; Paes de Sousa et al., 1980).

Each symptom is scored for severity (0–3: absent, mild, moderate, severe).

This evaluation notably allowed the classification of depression by virtually any diagnostic system and has been used for diagnostic or reclassification purposes with other diagnostic systems, such as the DSM IV (Salvatore et al., 2007; Seemüller et al., 2008).

2.2.1.2. Eysenck personality questionnaire (EPQ). This questionnaire (Eysenck and Eysenck, 1975) includes 83 items (full version), allowing the evaluation of the three basic personality dimensions, according to Eysenck's personality theory: extroversion, neuroticism and psychoticism. Only the extroversion and neuroticism dimensions were analyzed in this study. The subject responds yes or no, and a positive answer is scored 1. The final result is the sum of the points in each scale (0–23 for extroversion and 0–23 for neuroticism).

The neuroticism dimension assesses emotional stability versus instability and identifies individuals prone to psychological distress. Low scores indicate a trend to more relaxed, unemotional, and self-satisfied subjects. The extraversion dimension measures interpersonal interaction, activity level, need for stimulation, and capacity for joy. The subjects with a low score tend to be more reserved, sober, task-oriented, and quiet.

A low extroversion (a score lower then median) and high neuroticism group (a score higher than median) of subjects was created, as these subjects were previously found to be at a higher risk for dementia (Wang et al., 2009).

2.2.1.3. Clinical global impression (CGI)). Clinical global impression – severity (CGI S; Guy, 1976) is 7-point scale to evaluate the current severity of the patient's illness, according to the clinician's total past experience, ranging from 1 (not at all ill) to 7 (extremely ill).

2.2.2. Diagnosis of depression

Using AMDP symptoms at baseline, DSM 5 diagnostic criteria for Persistent Depressive Disorder (dysthymia), Major Depressive Disorder (MDD), melancholic and psychotic features were applied. Through baseline chart review, subjects were considered to have bipolar disorder if they met DSM 5 criteria for bipolar disorder.

The specifier of anxious distress could not be defined by AMDP as only two anxious symptoms (“psychic anxiety” and “somatic anxiety”) are present in the scale. A numerical variable “anxiety symptoms” was created adding both scores. Chronic disease was

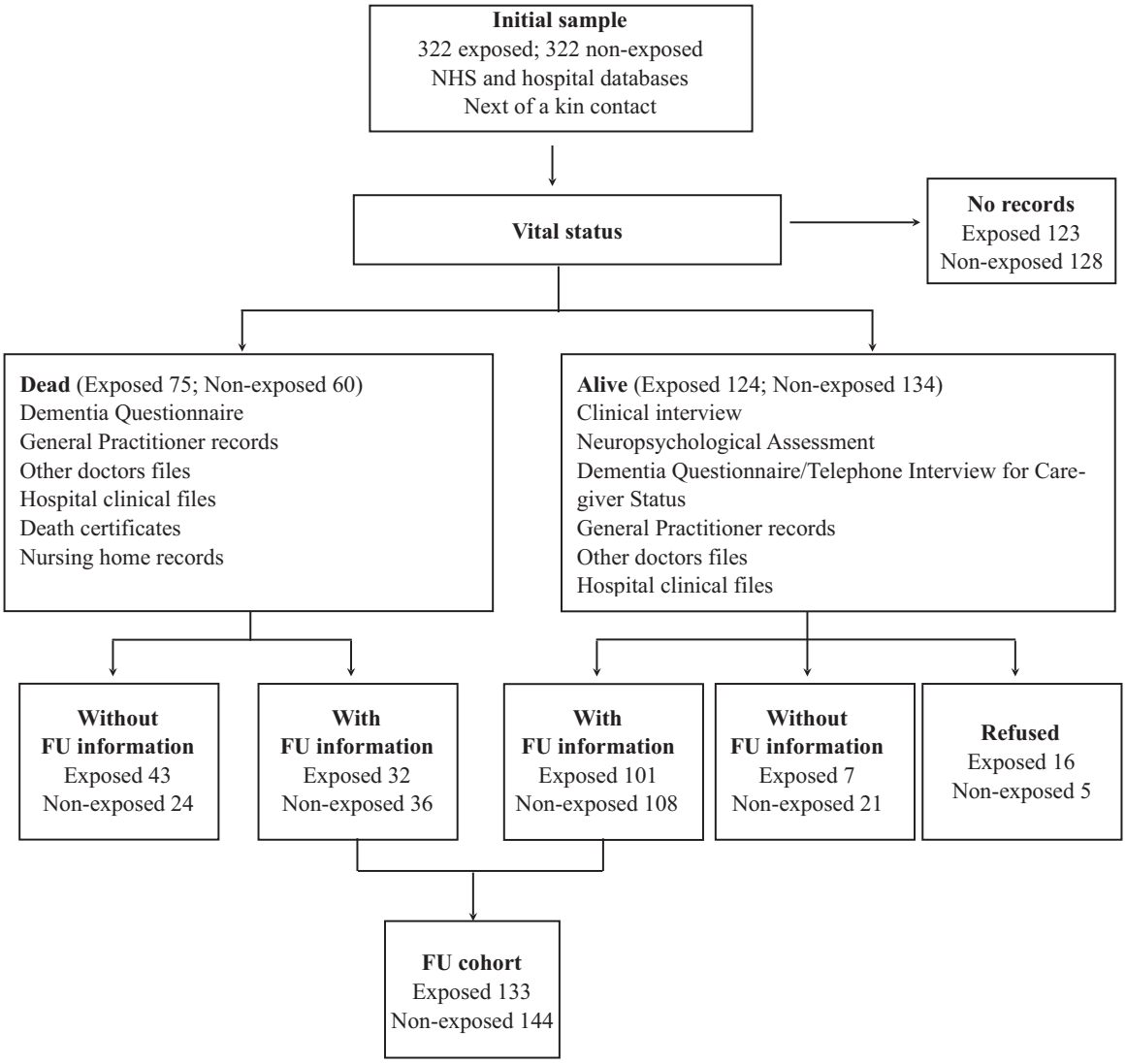

Fig. 1. Diagram displaying the flow of subjects cases in the study. NHS National Health Service, FU Follow up. In 278 (43.2%) cohort subjects the diagnosis of dementia could be established or excluded. The rates of follow up in relation to death are 42.7% in the exposed group and 60.0% in the non-exposed group.

defined if MDD symptoms were present continuously for more than two years.

2.3. Follow-up

2.3.1. Follow up procedures

The Hospital de Santa Maria, the Institute of Notaries and Records, and the National Health Service databases were contacted to get the subjects' address and next of kin, phone number, vital status, General Practitioner (GP) and other relevant doctors' names. This search allowed to find and contact subjects, or next of kin if the subject was dead.

2.3.1.1. Depressed cohort. Based on the information gathered during the follow-up, the initial diagnosis was reviewed and 3 patients were excluded from the depressed cohort because the diagnosis of depression was found wrong, namely one had schizophrenia, another schizoaffective disorder and the third a brain tumor. So, the depressed cohort recruited 322 subjects (Fig. 1).

It was not possible to ascertain the vital status of 123 subjects (no records found, data illegible or too many records found for a given name).

In the remaining 199 (61.2%), additional information was sought to establish a diagnosis of dementia. In the 75 subjects found to be dead, the next of kin was contacted to collect demographic information and to apply the Dementia Questionnaire (DQ). GP records, psychiatrist records, neurologist records, hospital clinical files, death certificates and nursing home records were reviewed to collect information regarding dementia diagnosis. In 43 subjects no contact with next of kin was possible or the clinical information in clinical files was not enough to establish or exclude the diagnosis of dementia.

The 124 alive subjects were contacted first by mail, presenting the study and indicating that a later phone contact would be done. Then, a clinical interview and a neuropsychological assessment were offered. If a subject was living far away, too ill to be submitted to neuropsychological assessment, or not willing to come for the clinical/neuropsychological assessment, telephone interviews (Telephone Interview for Cognitive Status (TICS) and/or DQ) to evaluate the cognitive status were applied. Patient's GPs, or other relevant doctors were contacted, and hospital records reviewed when appropriate. No contact at all was possible and no clinical records were found in 7 subjects, and a total of 16 subjects refused to participate in the study. In 133 (41.3%) subjects the outcome could be established (Fig. 1).

2.3.1.2. Surgical cohort. For the non-exposed cohort, we followed the methodology of a similar study (Brodaty et al., 2003). The first 325 subjects who were admitted for routine appendectomy or cholecystectomy at the same period as cases, and who could be matched to cases for age (± 2 years) and sex were identified from surgical lists (Fig. 1). For each depressed subject, the first identified matched control in the surgical list was assessed for his/her eligibility status. A subject was considered eligible if a mood disorder was not present prior to or at the time of index surgical hospitalization. This assessment was made by the review of the subject clinical files at the index hospitalization, and a diagnosis of a mood disorder and prescription of antidepressants, mood stabilizers or antipsychotics were considered exclusion criteria. A further assessment was performed at follow up by direct questioning. Three subjects were excluded from the surgical cohort, because they were found to be depressed (n=2) or to have a diagnosis of bipolar disorder (n=1) at inclusion time. The surgical (non-exposed) cohort included 322 subjects.

Identical procedures were taken to assess their dementia status as with the depressed participants. It was possible to ascertain the vital status of 128 subjects (60.2%). Among these, 5 refused to participate in the study, and in 45 the diagnosis of dementia could be not confirmed or excluded. The diagnosis of dementia could be established or excluded in 144 subjects (44,3%).

2.3.2. Follow up assessment (evaluations performed in 2009–2014)

2.3.2.1. Neuropsychological assessment. A comprehensive evaluation was performed, either at patients' home or in the hospital, by experienced neuropsychologists. The evaluation included (1) Battery of Lisbon for the Assessment of Dementia (BLAD; Garcia, 1984), (2) Trail Making Test – parts A and B (TMT; Reitan, 1958), (3) Toulouse-Piéron Test (TP; Toulouse and Piéron, 1986; Mendelsohn, 2000), and (4) California Verbal Learning Test (CVLT; Delis et al., 1987; Ribeiro et al., 2007).

2.3.2.2. Other assessments

1) Telephone Interview for Cognitive Status (TICS)
   This instrument (Brandt et al., 1988; Madureira et al., 2006) was initially developed for the assessment of AD patients unwilling or unable to return for follow-up. It gathers information on the domains of orientation, concentration, short-term memory, mathematical skills, praxis and language. It was proven to be sensitive and specific, and to have high test–retest reliability. The cutoff used for dementia was less than 26.
2) Dementia Questionnaire (DQ)
   The Dementia Questionnaire (Silverman et al., 1986) is applied by telephone to caregivers of patients with dementia allowing to quickly diagnose dementia in patients by the DSM IIIR criteria, and in some cases even to suggest the dementia subtype. The DQ can also be applied to care givers of already dead patients with dementia. A validated version including the age of onset of dementia and dementia subtype was used (Teixeira et al., 2011).
3) Vascular risk factors assessment

Subjects with a previous diagnosis of hypertension, diabetes, dyslipidemia, ischemic heart disease or cerebrovascular disease were considered to have vascular risk factors.

2.3.3. Diagnosis of dementia

Dementia was diagnosed at a case conference, including a psychiatrist (F.S.doC.), a neurologist (A.deM.) and a neuropsychologist (C.C.), all experienced in dementia. Cases were determined based on the best available information, using DSM-5 criteria for Major Neurocognitive Disorder (dementia) (American Psychiatric Association, 2013).

Clinical and neuropsychological assessment information were reviewed. If a subject was dead or did not attend the formal evaluation, case conference reviewed all the available evidence. In these cases, the diagnosis of dementia was based on at least two of the following: TICS score of 26 or less; DQ yielded a diagnosis of dementia; GPs records with a diagnosis of dementia; a diagnosis of dementia performed by either a neurologist or a psychiatrist; hospital records of dementia; death certificate with a diagnosis of dementia; retrospective case audit to meet DSM-5 dementia criteria; diagnosis of dementia recorded in nursing-home notes. If a diagnosis of dementia was established, reference to a Dementia Clinics was offered to the subject, to undergo the standard of care for evaluation and treatment of dementia.

The type of dementia was determined in the case conference referred above, using all the available information. The criteria for the diagnosis of the most common types of dementia were used: probable Alzheimer's disease according to NINCDS-ADRDA criteria (McKhann et al., 2011), probable vascular dementia according to NINDS-AIREN criteria (Roman et al., 1993), probable dementia with Lewy bodies (DLB) according to the criteria proposed by McKeith et al. (2005), and behavioral variant of frontotemporal dementia (FTD) according to the criteria of the International Behavioral Variant FTD Criteria Consortium (Rascovsky et al., 2011). If none of these criteria was met, or no sufficient information could be gathered, the diagnosis made was dementia non-otherwise specified (NOS). Date of onset of dementia was determined during the clinical interview, by DQ, or chart review.

2.4. Statistical analysis

IBM SPSS Statistics for Windows, version 19.0 (IBM Corp, Armonk, NY, 2010) and Stata Statistical Software: Release 11 (StataCorp LP, College Station, TX, 2009) were used for the statistical analysis.

Baseline characteristics were compared between subjects in whom the outcome was assessed and in those lost during follow-up, using independent samples t Student's test for continuous variables, after verification of homogeneity of variances, and Pearson Chi-square test for categorical variables, with Yates continuity correction for 2×2 tables.

We used a binary logistic regression analysis (with the dependent variable constituted by the conversion to dementia during the follow-up period) to compute the odds ratio (OR) for the associations between depression and dementia. Because data on age of dementia onset in the non-exposed cohort were available only in a minority of subjects, it was not possible to perform a Cox-regression analysis in this group.

Cox proportional hazards models were used to compute crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) to quantify the relation between the different exposures, including depression subtypes, and dementia. For multivariate analyses we considered the variables that were significantly associated ($p < 0.05$) with dementia in univariate analysis (baseline age, depression with melancholic features, depression with anxiety symptoms, and severity of the index episode (CGI)), as well as variables considered to be potentially relevant confounding factors (sex, years of education, bipolar disorder, depression with psychotic features, and chronic disorder), according to the literature (Kessing and Andersen, 2004; Ritchie et al. 2010; Reitz et al., 2011; Silva et al., 2013). Age of onset and the presence of vascular risk factors were considered only in sensitivity analyses because data on these variables was available for a subset of all patients (77 and 123, respectively), and due to the fact that the latter was assessed at follow-up. For each participant the follow-up started at the date of diagnosis of depression, or corresponding index data in the

surgical cohort, and ended at the estimated data of onset of dementia, date of follow-up assessment or date of death, whichever occurred first, as applicable. The proportional hazards assumption was evaluated graphically using "log-log" plots.

The cumulative incidence of dementia was estimated, across the follow-up period, taking into account the competing risk of death, using a competing-risks regression model, according to the method of Fine and Gray (Fine and Gray, 1999).

Statistical significance was accepted for $p < 0.05$.

2.5. Ethics

This study was conducted in accordance with the Helsinki Declaration as well as national ethical guidelines. The local Ethics Committee, the National Data Protection Committee, and the National Institute of Notaries and Records approved the protocol. Subjects who performed follow up evaluation were required to provide informed consent. If a diagnosis of dementia was established, reference to a Dementia Clinics was offered to the subject, to undergo the standard of care for evaluation and treatment of dementia.

3. Results

Comparing baseline data of the subjects with and without a known outcome, the mean age was lower in both cohorts, and the proportion of men was smaller in the subjects with a known outcome only in the depressed cohort. No statistically significant differences were observed regarding other socio-demographic and clinical characteristics (Table 1). Almost all subjects were Caucasian (98%).

3.1. Depression as a risk factor for dementia

Forty-four (22.3%) of the 133 subjects from the depressed cohort developed dementia, compared to 21 (14.6%) of the 144 subjects from de surgical cohort. Dementia cause could be assessed in about half of the cases (in 21 and 8 subjects, depressed and surgical respectively). The most prevalent cause in both groups was Alzheimer's disease (AD) (57% and 63%), followed by vascular dementia (19% and 25%), and Parkinson's disease (14% only in the depressed cohort). Other causes, found only in one patient each, were vitamin B12 deficiency and HIV dementia in the depressed cohort and neurosyphilis in the surgical cohort.

The characteristics of subjects who developed dementia and those that have not are displayed in Table 2. At follow-up 4 patients were rediagnosed as bipolar, due to a later emergence of a manic episode, and 2 non bipolar patients fulfilled DSM5 criteria for Persistent Depressive Disorder at baseline, but were re-classified later as MDD.

A logistic binary regression analysis showed that the odds of dementia were increased by 2.90 times (95% C.I. 1.61–5.21; $p < 0.0001$) for the depressed cohort when compared to the surgical cohort. The higher risk for dementia in the depressed cohort was still significant after adjusting for sex, age, and education years (OR = 3.36; 95% C.I. 7.76–6.80; $p < 0.0001$).

3.2. Depression as an early risk factor

To address the issue of depression as a prodrome of dementia we repeated the analysis considering the subjects with an early onset of depression and those with a longer time frame between the two diagnoses. The definition of the age limit of late onset depression varies across the studies, between the age of 45 (Steffens et al., 1997) and the age of 60 years (Byers and Yaffe, 2011). When the analysis was restricted to patients younger than 45, or 60 years old at baseline, the odds for dementia in the depressed cohort were still significantly higher when compared to the surgical cohort (8.53; 95% C.I. 2.40–30.16 and 3.30; 95% C.I. 1.75–6.33, for those younger than 45 and 60, respectively). When adjusting for age, sex, and education years, similar results were found (8.69; 95% C.I. 2.21–34.23 and 4.00; 95% C.I. 1.87–8.60, for those younger than 45 and 60, respectively).

Age of depression onset was only available in 77 subjects. In those with depression onset before the age of 60 the odds of developing dementia were not different for those with depression onset after the age of 60 (0.84; 95% C.I. 0.38–1.84), even after controlling for age, sex, and education years (0.72; 95% C.I. 0.30–1.74).

A 10 years difference between depression diagnosis and dementia onset has been used as a criterion to reduce the risk of misdiagnosing depression as prodrome of dementia (Brunnström et al., 2013). When restricting the analysis to the subjects with a follow up time longer than 10 years (94.0% in the exposed group and 97.9% in the non-exposed group), depression still emerged as a risk factor for dementia when compared to the surgical cohort (2.95; 95% C.I. 1.62–5.40 and 4.16; 95% C.I. 1.96–8.83, for crude and adjusted OR, respectively).

Table 1
Baseline characteristics of both cohort subjects to known outcome.

	Depressive cohort				Surgical cohort			
	Lost during follow up (n = 189)	With known outcome (n = 133)	Total (n = 322)	*p* Value[a]	Lost during follow up (n = 178)	With known outcome (n = 144)	Total (n = 322)	*p* Value[a]
Age, mean (SD), y	50.8 (12.7)[b]	41.8 (11.6)	46.9 (13.0)	< 0.001	47.7 (14.5)	41.0 (12.1)	44.6 (13.8)	< 0.001
Male sex, % (n)	27.0% (51)	15.0% (20)	22.0% (71)	0.014	24.2% (43)	19.4% (28)	22.0 (71)	0.212
Education, mean (SD), y	6.4 (4.6)	7.1 (4.5)	6.7 (4.6)	0.165	N.A.	6.63 (4.6)[c]		
Bipolar disorder, % (n)	10.6% (20)	16.5% (22)	13.0% (42)	0.326				
Melancholic features, % (n)	33.3% (63)	35.3% (47)	34.2% (110)	0.722				
Psychotic features, % (n)	24.3% (46)	24.1% (32)	24.2% (78)	0.999				
Anxiety symptoms severity, mean (SD)	1.6 (2.6)	1.9 (2.8)	1.7 (2.7)	0.288				
Clinical Global Impression (CGI), mean (SD)	5.0 (0.8)	5.0 (0.7)	5.1 (0.7)	0.978				

N.A. not available. Education years were not written in most surgical files.

[a] Comparing, within each cohort, those with a known outcome with those without known outcome, based on chi-square test for category variables and independent samples *t* test for continuous variables.

[b] Baseline age available only for 182 subjects.

[c] Education years available for 111 subjects.

Table 2
Cohort characteristics and outcome.

	Depressed cohort		Surgical cohort	
	Non-demented (n=89)	Demented (n=44)	Non-demented (n=123)	Demented (n=21)
Age, mean (SD), y	38.9 (10.4)	47.8 (11.5)	37.7 (11.0)	54.2 (9.1)
<35 years, % (n)	46.1% (41)	11.4% (5)	48.8% (60)	0.0%(0)
35–45 years, % (n)	23.6% (21)	29.5% (13)	22.8% (28)	14.3% (3)
>45 years, % (n)	30.3% (27)	59.1% (26)	28.5% (35)	85.7% (18)
Male % (n)	14.6% (13)	15.9% (7)	19.5% (24)	19% (4)
Education, mean (SD), y[a]	7.8 (4.5)	5.9 (4.4)	7.3 (4.7)	2.9 (1.7)
<5 years, % (n)	39.3% (35)	61.4% (27)	48.4% (59)	85.7% (18)
5–9 years, % (n)	31.5% (28)	22.7% (10)	14.8% (18)	0.0% (0)
>9 years, % (n)	29.2% (26)	15.9% (7)	36.9% (45)	14.3% (3)
Follow up time, mean (SD), y	26.3 (7.2)	24.5 (7.3)	29.0 (6.8)	25.1 (7.6)
Age of onset of the disorder, mean (SD), y[b]	28.4 (10.2)	34.8 (10.2)		
Bipolar disorder, % (n)	14.6% (13)	18.2% (8)		
DSM5 Diagnosed MDD, % (n)	84.3% (75)	79.5% (35)		
Melancholic features, % (n)	24.7% (22)	56.8% (25)		
Psychotic features, % (n)	20.2% (18)	31.8% (14)		
Anxiety symptoms severity, mean (SD), AMDP score	1.48 (2.59)	2.86 (2.95)		
Clinical Global Impression (CGI), mean (SD)	4.9 (0.7)	5.1 (0.7)		
Chronic Disorder, % (n)	71.3% (57)	92.3% (36)		
Inpatient at baseline, % (n)	55.1% (49)	65.9% (29)		
Ever been admitted for depression, % (n)[c]	82.7% (62)	94.6% (35)		
Suicide attempts, % (n)[d]	43.9% (25)	66.7% (12)		
EPQ (Extroversion), mean (SD)[e]	38.0 (21.2)	35.1 (17.5)		
EPQ (Neuroticism), mean (SD)[e]	81.8 (14.9)	78.8 (14.8)		
High Neuroticism/Low Extroversion Group, % (n)[e]	24.6% (14)	23.3% (7)		
Vascular risk factors, % (n)[f]	59.8% (52)	80.6% (29)	61.2% (63)	76.9% (10)
Age of dementia onset (y), mean (SD)		72.3 (8.8)		81.5 (8.5)
Died, % (n)	18.0% (16)	36.4% (16)	19.5% (24)	57.1% (12)

MDD Major Depressive Disorder, EPQ Eysenck Personality Questionnaire.

[a] Data available only in 111 controls.
[b] Data available only in 77 subjects.
[c] Data on admission status was available in 82 subjects.
[d] 58 subjects had suicide data.
[e] 87 subjects had baseline EPQ.
[f] Data obtained at follow up, available in 123 depressed and in 116 surgical subjects.

3.3. Role of depression heterogeneity

In the depressed cohort, the probability of dementia increased significantly with age, was not influenced by education and was significantly higher for melancholic features, anxiety symptoms, and severity of the episode assessed with the CGI, in the univariate Cox regression analysis (Table 3). In the multivariate Cox analysis (Table 3), older subjects at baseline and those with depression with melancholic features had an increased risk for developing dementia compared to those without melancholic features (HR=3.64; 95% C.I. 1.78–11.26). The other depression characteristics and education were not associated with a higher risk for

Table 3
Depressed cohort: association between demographic variables, clinical characteristics and other well established risks for dementia, and the risk of dementia.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Baseline age, y				
<35	1 [reference]		1 [reference]	
35–45	7.55 (2.15–26.52)	0.002	7.26 (1.99–26.51)	0.003
>45	15.17 (4.55–50.58)	<0.001	13.18 (3.72–47.50)	<0.001
Baseline education, y				
0-4	1 [reference]		1 [reference]	
5–9	0.88 (0.42–1.84)	0.730	1.40 (0.60–3.28)	0.437
>9	0.47 (0.19–1.15)	0.097	0.461 (0.16–1.34)	0.156
Bipolar disorder	1.88 (0.86–4.14)	0.116	0.64 (0.22–1.84)	0.408
Melancholic features	4.48 (2.40–8.39)	<0.001	3.64 (1.78–11.26)	0.025
Psychotic features	1.55 (0.81–2.96)	0.182	1.55 (0.76–3.14)	0.224
Anxiety symptoms	1.18 (1.08–1.30)	0.001	0.97 (0.82–1.22)	0.969
Clinical Global Impression (Severity)	1.84 (1.18–2.87)	0.008	1.19 (0.74–2.36)	0.345
Chronic disorder	1.85 (0.96–3.55)	0.065	1.24 (0.58–2.61)	0.581
Male sex	0.98 (0.39–32.51)	0.974		
Inpatient at baseline	1.01 (0.53–1.92)	0.980		
Ever been admitted for depression	2.20 (0.51–9.52)	0.290		
Suicide attempts	2.03 (0.74–5.63)	0.172		
High Neuroticism/Low Extroversion Group	0.71 (0.33–1.82)	0.568		

Notes: variables found significant in the preliminary analysis and those known to influence the risk for dementia were included in the Cox proporcional hazards models.

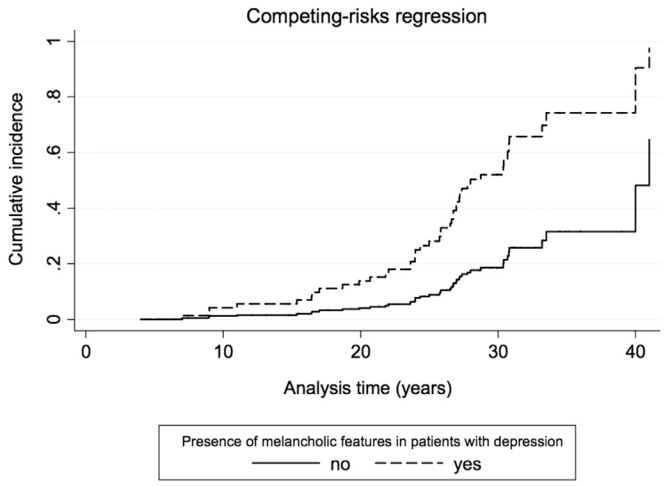

Fig. 2. Cumulative incidence of dementia, across the follow-up period, taking into account the competing risk of death, using a competing-risks regression model.

dementia.

Two sensitivity analyses were performed, including age of the affective disorder (determined in few cases) and vascular risk factors (data obtained at follow up). In a new model, the inclusion of age of affective disorder onset and vascular risk factors did not change the statistical significance of the associations between both melancholic features and age at baseline with dementia risk.

Studies with a very long follow up, especially including geriatric outcomes and depressed patients, are faced with the difficult problem of how to account for the competing risk of death (Wulsin et al., 1999). Because Cox proportional hazards regression can overestimate the risk of disease, a risk competing analysis was performed. The cumulative incidence of dementia, according to melancholic features of depression, was higher among subjects with melancholic features during most of the follow-up period, reaching approximately 50% at around 30 years of follow-up, according to a competitive risk model (Fig. 2).

When comparing melancholic (n=47, 25 events), non-melancholic depressed patients (n=86, 19 events) and surgical cohort subjects (n=144, 21 events), logistic binary regression analysis showed a significantly increased risk for dementia only in melancholic subjects when compared to the surgical cohort subjects (OR=6.66; 95% C.I. 3.19–13.90 and OR=7.72; 95% C.I. 3.18–18.77, for crude and adjusted for baseline age and years of education OR, respectively). Non-melancholic subjects were not at increased risk for dementia, in the same analysis (OR=1.66; 95% C.I. 0.84–3.31 and 2.25; 95% C.I. 0.99–5.10, for crude and adjusted OR, respectively).

Since the precise age of dementia onset was not possible to ascertain in about half the subjects from the surgical cohort, Cox regression analysis was not done in this cohort.

4. Discussion

The main finding of the present study is that melancholic features of depression are an important and independent risk factor for dementia.

Depression has been globally associated with a two-fold increase risk of dementia (Silva et al., 2013), just like we found in this study. But, as far as we know, melancholic features have not been previously specifically assessed as a risk factor for dementia. Melancholia has been associated with persistent cognitive impairment after depression remission (Lin et al., 2014; Roca et al., 2015), but not with dementia. It is possible that the follow up time has been too short in both studies (6 weeks and 6 months, respectively) to detect an increased incidence of dementia. Remarkably, in the present study, melancholic features were independently associated with dementia. As previously mentioned, the study from DalForno et al. (2005) failed to find an association of negative affective symptoms with dementia risk. However, the assessment of negative affective symptoms was based on a CES-D subscale which does not match exactly the standard criteria for melancholic features. Most risk factors that have been studied in previous investigations, such as more severe disorder, greater severity of the episode, diagnosis of bipolar disorder, and the presence of psychotic and anxious symptoms, were associated with an increased risk for dementia in the univariate analysis, but their significance disappeared in the multivariate analysis. Since melancholic features tend to repeat across lifetime episodes (Coryell et al., 1994), are associated with a more severe course, occur in virtually all psychotic episodes, and happen more frequently in bipolar patients (Taylor and Fink, 2008), it is possible that the higher risk associated with those characteristics in previous studies could be at least partially mediated by melancholia. However, the role of bipolar disorder as a risk factor for dementia might have been underestimated because of the small number of bipolar patients in the present cohort. Also, non-melancholic subjects were not found to be at a significantly increased risk for dementia, though results were close to statistical significance, despite the relatively small sample size in this subgroup. In any case, the present results strongly emphasize that analysis of melancholic features should be included in future studies.

There is a biological rational for the association between melancholic features and dementia, as an important body of evidence pinpoints the biological mechanisms underlying melancholia with cognitive impairment. Melancholia has been associated with hypothalamus-pituitary-adrenal (HPA) axis dysfunction (Brown, 2007; Parker et al., 2013), and hyper- and hypocortisolism characterize different subtypes of depression (Hasler et al., 2004). This HPA axis dysfunction has been widely studied in animal models and in human disorders (Lupien et al., 1998; Finsterwald and Alberini, 2013; Suzuki et al., 2014), and involves down regulation of glucocorticoid receptors and/or increased circulating glucocorticoids triggering a cascade of events that leads to cognitive impairment (McEwen and Magariños, 1997; Lupien et al., 2008). Melancholia may particularly induce hippocampal damage (Lamers et al., 2013), and has been associated with cognitive decline (Withall et al., 2010; Sachs-Ericsson et al., 2014).

Present results support a role of depression as an early risk factor for dementia, and not merely a prodrome. Our results are in line with studies with a long follow up (Kessing, 1999; Brodaty et al., 2003; Saczynski et al., 2010; Dotson et al., 2010; Barnes et al., 2012), with a low probability of misdiagnosing dementia as a depressive disorder. The few studies that specifically explored the differences between early- and late-onset depression found a very small increased risk for dementia, or no differences in early onset depression as compared to late-onset depression, however they assessed depression retrospectively by simply questioning (Green et al., 2003), using CES-D or Geriatric Depression Scale (Lenoir et al., 2011; Almeida et al., 2016), or included subjects mainly with neurotic depression (Brommelhoff et al., 2009). The accuracy of depression diagnosis was probably lower when compared to the present study, and this could have led to the inclusion of less severe depressive or non-melancholic patients.

The precise diagnosis of dementia was not possible to ascertain in about half the cases in the depressed cohort and in about 2/3 of the controls, a natural consequence of the way clinical information was collected. So, it was not possible to identify whether

depressive subtypes were associated with specific dementing disorders. It has been suggested that depression is a risk factor both for vascular dementia (VD) and for Alzheimer's disease (AD) (Lenoir et al., 2011; Brunnström et al., 2013). The Honolulu-Asia aging study (Irie et al., 2008) found a higher risk for AD, however a higher load of cortical plaques and tangles was not associated with AD and depression (Wilson et al., 2003). Although in half of the cases the dementia cause could not be identified, the present results suggest that depression would be associated essentially with an increased risk for AD and not VD.

This study has important strengths. It is one of longest longitudinal studies performed to evaluate the risk of dementia in depressed patients. Another strength is that the diagnosis of depression was done very reliably using an extensive and comprehensive psychiatric and psychological evaluation. The vast majority of previous studies assessed depression with rather simple instruments, such as CES-D, that cannot capture the complexity and heterogeneity of the depressive disorder. Still another strength is that important and different risk factors and confounders were assessed (such as personality, severity of the episode, or vascular risk factors), that have been seldom evaluated together. If different subtypes of depression carry different risks for dementia, it would be elicited by a study with this design.

4.1. Limitations

We should also note the limitations of this study. The major limitation is the large proportion of subjects without follow-up information, since only 43% of individuals included in the cohorts had follow-up data. This proportion is high, as compared to case register based studies with a complete follow-up of data on dementia and death (Kessing et al., 1999; Kessing and Nilsson, 2003). Attrition may limit the validity of findings from longitudinal studies, and is more likely to occur in investigations with long follow-up periods; however, the latter are essential to evaluate long-term effects and resemble more closely inception cohorts, which contributes for survival-related biases to be less likely (Saracci, 2006). Shorter studies evaluate short term effects and often include predominantly survivors instead of participants selected closer to the onset of the exposures of interest. Despite the robustness of our findings could be improved with a more complete follow-up, our design allows the evaluation of long-term effects, contributes to minimize survival-related biases and adds to previous research on this topic the assessment of the effects of different depression subtypes. Furthermore, in the present study the completeness of follow-up was greater than in other similar studies (43% versus, for instance, 33% in Brodaty et al. (2003)). Melancholia definition is controversial, and DSM5 definition has been challenged, on the grounds of the limitation of defining melancholia by reliance on symptoms (Parker and Paterson, 2014). These authors propose the inclusion of biological markers related to HPA dysfunction. The inclusion of a biological biomarker would strengthen the findings of this study. Another limitation is the absence of a formal cognitive evaluation at baseline. However, dementia was excluded clinically, the mean age for the onset of the affective disorder was about 35 years old and the mean time to the event was more than 25 years, making it very unlikely that dementia was present at baseline.

4.2. Conclusions

This study supports depression as an early risk factor for dementia, and not only a prodrome. Depression is a heterogeneous disorder, and it is possible that the frequency of melancholic features could explain the discrepancies found in the risk for depression as a whole in the different studies. Melancholic features of depression should be actively identified in the clinical setting, and DSM5 criteria seem appropriate for this purpose. Due to a more favorable response to biological therapies, appropriate treatment of melancholia could decrease the risk for dementia.

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Conflict of interest

None.

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Neuropharmacology and analgesia

Escitalopram improves memory deficits induced by maternal separation in the rat

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ABSTRACT

Maternal separation (MS) induces depressive-like behavior and long-term changes in cognition in rats. Escitalopram is an antidepressant drug shown to reverse the depressive-like features caused by this stress model. However, it is not known if it can ameliorate the affected cognition. We now characterized the effect of escitalopram on hippocampal-dependent memory in rats submitted to the MS protocol. Male Wistar rats were assigned either to control (CTR) or maternal separated (MS) group. MS were separated from their dams between 2–14 postnatal days (PND) for 180 min daily. Escitalopram was given in food pellets (0.34 g/kg/day first 2 weeks and 0.41 g/kg/day the subsequent period, average dose 25 mg/kg) from PND 43 onwards, during 1 month. Depressive behavior was assessed in the forced swimming test (FST), and memory performance in the Morris water maze (MWM). Escitalopram significantly improved the FST's latency to despair in the MS group ($n=6$), but did not change the immobility time. All groups showed a significant learning effect in the MWM over time, but no differences have been found upon treatment ($n=6$). However, escitalopram treatment significantly increased the time spent on the platform quadrant in the probe trial in the MS group. We report here that chronic treatment with escitalopram is able to improve hippocampal dependent memory in a chronic stress model, while not changing the learning ability. Moreover, this is accompanied by an amelioration of the depressive like behavior. These results support the use of escitalopram to tackle underlying cognitive deficits caused by stress in early-life.

1. Introduction

Depression can induce long-term cognitive deficits or even be a risk factor for dementia (Ownby et al., 2006). As there is no cure for most dementia conditions, strategies directed to the correction of risk factors amenable to prevention are extremely important. Depression can be effectively treated in the majority of cases, so it emerges as an important target for the prevention of cognitive deficits and dementia. Treatment of depression is complex and multidisciplinary, but usually implies drug treatment with antidepressants. The long-term effect of antidepressants on human cognition (and eventually on the prevention of dementia) has been poorly studied and clearly remains to be elucidated (Kessing et al., 2009).

Maternal separation (MS) is an animal model of depression that can be induced in rodents in a manner that is not easily amenable or ethically allowed to humans, and permitting a reliable evaluation of a number of internal and external factors (e.g. pharmacological interventions). This model is considered a validated model of depression and anxiety (Ladd et al., 2000; Kalueff and Tuohimaa, 2004). We and others have previously shown that rodents separated from their mothers, according to this protocol, have poorer cognitive performances on memory tasks, probably related to the effect of hypothalamus-pituitary-adrenal (HPA) axis disruption (Aisa et al., 2007; Batalha et al., 2012) on the hippocampus (Huot et al., 2002; Aisa et al., 2009).

Escitalopram is a widely used and highly efficacious antidepressant belonging to the Selective Serotonin Reuptake Inhibitors (SSRI) class. Escitalopram and other antidepressants can reverse depressive-like behaviors in rodents in the Forced swimming test (FST) (El-Khoury et al., 2006). Although MS impacts in memory and learning and escitalopram is effective in reversing depressive – like behavior, it is not known if this antidepressant can improve cognitive deficits induced by MS.

To study the effect of antidepressant treatment on cognition in a rat model of depression, we have submitted rats to the MS protocol and then to widely used tests for depressive-like behavior – FST – and for memory – the Morris water maze (MWM). We hereby report that the escitalopram diminished FST latency-to-despair and improved the latency in the probe test of the MWM, in MS treated animals.

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2. Material and methods

2.1. Animals

Pregnant Wistar rats were purchased (Harlan, Barcelona) on gestation days 12–15 and were due in our animal facility. All animals were handled according to European Community guidelines and Local Law on animal care (1005/92). The animals were kept on an environment controlled for temperature (22 ± 2 °C), humidity ($55 \pm 10\%$) and light (12-h light/dark schedule; lights on at 7:00 a.m. and off at 7:00 p.m.).

Pups were randomly assigned to the MS protocol ($n=12$) or to animal facility rearing (CTR, $n=12$). Local Ethics Committee has approved the research protocol.

2.2. Maternal separation protocol

The MS protocol followed has been validated and described before (Batalha et al., 2012; Lopes et al., 2008). At postnatal day (PND) 2, pups from four different litters, were collected together, gender assessed, and the pups were randomly distributed to foster dams (male/female ratio kept constant). Pups assigned to MS group were removed from their cages and dams at postnatal days (PND) 2–14 for 180 min daily. They were removed as a group from the nest, weighed and placed as a group in an isolation cage in an adjacent room kept at 32.0 ± 0.5 °C. At the end of the separation period pups were returned to their home cage and rolled in the soiled home cage bedding before reuniting them with the mother. CTR animals were only briefly manipulated to change the beddings in their cages twice/weekly. At day 21 the pup's sex was determined and they were weaned and housed in individual cages. Only male rats were in included in the study. After weaning, pups were weighed weekly. No changes were found in weight due to MS protocol as observed before (Lopes et al., 2008).

2.3. Treatment procedures

The majority of hippocampal granule neurons develops and extends their axons between PND 1 and 21 (Amaral and Dent, 1981), We have initiated the treatment in puberty, after the full development of hippocampal circuitry, period when interventions, either drugs or stimulation, are more effective in the hippocampus (El-Khoury et al., 2006)

Rats were left undisturbed from PND 15–42. On postnatal day 43, half of the rats from each group (MS and CTR) were assigned to dietary treatments with the antidepressant escitalopram (0.34 g/kg/day chow for the first 2 weeks; 0.41 g/kg/day chow during the rest of the experiment) (CTR+AD; MS+AD), or were given placebo, admixed to food pellets (CTR; MS). The escitalopram doses were increased sequentially, according to the method developed by H. Lundbeck A/S (Copenhagen, Denmark) and tested by A. Mørk (El-Khoury et al., 2006), resembling the clinical situation where a range of escitalopram (or other antidepressants) is used to treat depressed patients. These doses have been shown effective in reverting depressive-like behaviors in the forced swimming test (FST) in the MS model of depression (El-Khoury et al., 2006). Lactamin AB, Sweden, prepared Denmark dietary escitalopram according to instructions from H. Lundbeck A/S, Denmark. The escitalopram intake was controlled by weighing the animals and monitoring the food intake per animal (weekly intake in Table 1). The pellets made available were weighed prior and post intake and dose estimated according to the weight of each animal. The administered escitalopram dose was approximately 25 mg/kg/day. Animals were kept on their respective diet until the end of the experiment on PND 73.

All animals have been handled for 5 days prior to behavioral testing.

Table 1
Amount of ingested escitalopram pellets and dose equivalent.

Week	Animal weight (g)	Food ingested/day (g)	Dose equivalent (mg/kg/day)
1	180.42	19.19	24.7
2	230.98	25.84	25.3
3	218.8	21.60	27.6
4	231.2	19.80	23.9
5	209.25	19.87	26.5
6	210.79	19.87	26.3

Average 25.4 mg/kg/day.

2.4. Forced swimming test

Forced swimming test (FST) was used as a behavior equivalent of learned helplessness to test AD action (Porsolt et al., 1977; Castagné et al., 2011). It was carried out on PND 64 and 65 (note that the escitalopram or vehicle diets were continued until PND 73). On the first of the two test days, all animals were gently placed individually in a vertical Plexiglas cylinder (height: 45 cm, diameter: 19 cm) filled with 26 °C water at a depth that makes it impossible to reach the bottom with hind paws (28–30 cm). The animals were removed from the water after 10 min, and dried before being returned to their home cages. The water was changed after each session. On the second day, the animals were placed in the same cylinders for 5 min. This session was video-recorded and an observer blinded to the animals group subsequently scored the behavior of the animals. According to the criteria of Porsolt et al. (1977), the rat was judged to be immobile when it floated passively, making only small movements to keep its nose above the surface. This test measures the latency to immobility (or to despair, LTD) and the time spent swimming versus the time spent floating, i.e. the percentage of time spent immobile or immobility time (IT).

2.5. Morris water maze

The protocol used was the classical Morris water maze test, which is sensitive to hippocampal-dependent spatial learning and memory (Morris et al., 1982). The protocol was used as before (Diógenes et al., (2011)). The maze consisted of a large circular tank (1.8 m in diameter, 0.6 m in height) of water (temperature, 25 ± 1 °C) made opaque with the addition of a small amount of non-toxic water-based black paint. An escape platform (10 cm in diameter) was submerged, non-visible, 1 cm below the water. Several visual cues for spatial reference were placed on the walls of the testing room. The performances were continuously monitored using an automated tracking system (Smart 2.5, PanLab, Barcelona). From PND 80 to 84, rats were given spatial acquisition training consisting of four trials/day for four consecutive days, in which the platform was placed at a fixed position in the center of one of the four quadrants of the tank (platform Q, left, right and across). On each day the subjects were randomly placed in four different positions facing the wall, and never in the platform quadrant. The inter-trial interval was of at least 15 min, during which animals were towel-dried and placed under an infrared light to prevent hypothermia. The maximum trial duration was 60 s, after which animals were manually guided to the platform if they failed to locate it, and they were allowed to remain there for 20 s. At the fifth day a probe test was given in which the platform was removed and animals were allowed to swim freely for 60 s while recording the percentage of time spent on each quadrant. The latency to find the platform during acquisition and the percentage of time in the platform quadrant in probe test were used to evaluate hippocampal dependent memory.

2.6. Statistics

Statistical Package for Social Sciences v19 (SPSS) was used for the statistical treatment of data. After establishing homogeneity of variances, a two-way ANOVA was carried out for analyzing FST (LTD and IT) and time spent on quadrant after platform withdrawal (using MS (*) and AD (#) treatment as factors). Simple main effect analyses were performed, following significant interactions. Two-way ANOVA repeated measures was used for the learning curve of MWM. Statistical significance was accepted for $P<0.05$. Results are expressed as mean ± standard error of mean (SEM).

3. Results

3.1. Forced swimming test

As observed in Fig. 1, latency-to-despair (LTD) was found to be decreased by MS ($F(1,21)=8.373$; $^{*}P=0.010$). LTD decreased from 71.8 ± 7.71 in CTR to 38.8 ± 6.25 s in MS animals. The treatment with escitalopram significantly improved LTD in MS+AD animals to 65.6 ± 7.35 s ($F(1,20)=5.015$; $^{\#}P=0.038$).

Escitalopram also affected the immobility time (IT) in all groups of rats ($F(1,23)=4.658$; $P=0.043$) but no differences were found when comparing MS and CTR animals ($F(1,23)=0.413$; $P=0.528$).

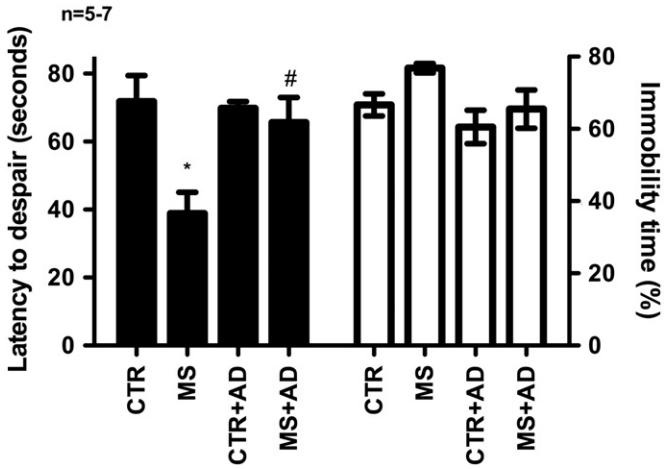

Fig. 1. Escitalopram reverts MS-induced increase in latency-to-despair. Learned helplessness was assessed by the Porsolt forced swim test, in which latency to despair (LTD) and immobility time (IT) were evaluated. Results are the mean ± SEM of 5–7 animals; (*) $P=0.010$ for MS effect and ($^{\#}$) $P=0.038$ for AD effect, calculated using two-way ANOVA, followed by simple main affect analysis.

3.2. Morris water maze

3.2.1. Learning curve

A significant learning effect throughout the 4 days was present, for all groups. The latency to reach the platform significantly improved from 49.4 ± 2.16 s on day 1, to 18.3 ± 2.87 s on day 4 ($F(3,66)=77.542$; $P<0.001$). However, no significant changes between different groups were found ($F(3,22)=0.575$; $P=0.456$; Fig. 2a).

3.2.2. Probe trial

The ability to recall spatial memory is tested following the learning period, by withdrawing the platform. MS animals displayed less time (23.5 ± 7.98%) in the quadrant as compared to the CTR group (38.8 ± 9.18%; Fig. 2b). However, escitalopram reverted this, improving the performance of MS animals ($F(1.23)=10.764$; $^{*}P=0.004$). The time spent on the platform quadrant increased to 34.3 ± 7.04% in MS AD group (Fig. 2b). Escitalopram alone seems to induce a slight decrease in time spent on platform for CTR animals, but this difference is not statistically significant.

The average speed and total swimming distance on probe trial were similar among groups (data not shown).

4. Discussion and conclusions

Maternal separation is known to induce deficits in memory related tasks that persist throughout adulthood. We hypothesized that treatment with an anti-depressant, escitalopram, would be beneficial in reverting these deficits. We now report that chronic treatment with escitalopram for one-month given to adult animals can improve hippocampal-dependent memory and latency to despair deficits induced by maternal separation (MS).

We found a significant effect of MS for acquisition, but not for learning. Due to the heterogeneity of MS protocols, animal strains (Huot et al., 2002; Hui et al., 2011) or both (Anisman et al., 1998; Oitzl et al., 2000; Frisone et al., 2002; Zhu et al., 2011) it is difficult to find a pattern of the cognitive deficits induced by MS. In experimental conditions similar to ours, results are consistent, showing a difference in acquisition and not in learning, as if an

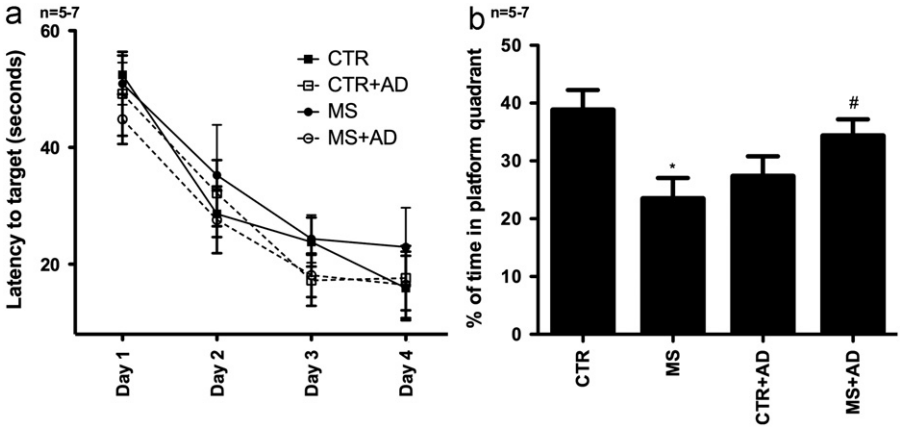

Fig. 2. Escitalopram reverts MS-induced impairment in the probe trial. Hippocampal dependent memory performance was assessed by the Morris water maze test, in which acquisition (a) and retention (b) were evaluated. Results are the mean ± SEM of 5–7 animals; (*) $P<0.01$ for MS effect and ($^{\#}$) $P=0.004$ for AD effect, calculated using two-way ANOVA, followed by simple main affect analysis.

effect in learning was more resistant to MS action than the acquisition (Aisa et al., 2007, 2009; Mello et al., 2009). The mechanism for this MS induced cognitive deficits has been mostly related to the induction of HPA axis disruption, shown by elevated corticosteroids and corticosterone levels (Aisa et al., 2007; Batalha et al., 2012), similar to what has been observed in depressed patients (Sachar, 1971; Carroll, 1981). This state of hypercortisolism promotes the shrinkage and the degeneration of hippocampal neurons, both in humans (reviewed by Brown et al., 1999) and animals (Batalha et al., 2012; Lupien and McEwen, 1997).

The main goal of the present study was to evaluate if an antidepressant could decrease these deficits. Antidepressants have several neuroprotective effects in the hippocampus of rats displaying depressive-like behaviors, either neurogenic (Malberg et al., 2000) or neuroremodeling (Bessa et al., 2009). However, the impact of these putatively benefic changes is not known, as a direct effect of escitalopram has never been shown before on a memory task in this model of depression.

All groups showed a significant learning effect in the MWM over the days, but no differences have been found upon treatment. However, escitalopram treatment significantly improved the time spent on the platform quadrant in the probe trial in the MS group. The mechanisms underlying the antidepressant-related cognitive effects, reside either on a direct action on hippocampus, by stimulation of the neuronal remodeling (Bessa et al., 2009) or through restoring the normal physiology of the HPA axis (Mantheya et al., 2011).

MS is an appealing model of depression because it mimics the mother–infant interaction, which is a key factor for developing psychiatric disorders in the future (Freud, 1895; Heim and Nemeroff, 1999). MS does not induce very strong depressive-like behavior, when compared to some genetically selected strains, such as the "depressed" flinders sensitive line (El-Khoury et al., 2006). However, it is a more pertinent model when studying susceptibility to early-life events and more related to human conditions. We now report that, in assessing depression, statistical significance was only reached when measuring the effect of escitalopram in latency-to-despair (LTD), but not in immobility time (IT). IT use has been criticized for its low sensitivity in detecting antidepressant actions of SSRI and for having a positive response to psychostimulants (Borsini and Meli, 1988; Rupniak, 2003). Adding the LTD parameter can improve the sensibility to detect AD actions, especially for tricyclic antidepressants (TCA), and LTD is not affected by psychostimulants (Castagné et al., 2009), excluding changes due to general motor stimulation. Current results reinforce the advantage of assessing LTD to improve sensibility to SSRI antidepressant activity in rats.

Escitalopram alone seemed to induce a slight decrease in the probe test time in the MWM, for CTR rats. This did not reach statistical significance and we do not attribute it to any motor effect since we have used the same dose range as previous studies that found no impact in specific motor parameters (Xi et al., 2011). An explanation for the findings is difficult, because its use in healthy subjects is not ethically allowed. However, escitalopram has some, although moderate, anticholinergic properties, and a tendency to cause "cognitive" flattening (Stahl, 2009). This could indicate that its beneficial action is limited to animals with underlying deficits. On the other hand, it reinforces the idea that care must be taken and confirms the need to use healthy controls treated with antidepressants in such paradigms. The use of *p.o.* antidepressant could strengthen the findings, as these methodological approaches can be considered closer to human reality. However, other variables such as genetics and individual susceptibility to dosage must be accounted for. The present data focused on a specific class of antidepressant, the SSRIs. Whether the observed effects are due to a general antidepressant effect or instead to a specific mechanism of action of this class would be interesting to evaluate, testing distinct classes of drugs.

The present results may support a potential effective role of antidepressants, atleast of the SSRI class, in the prevention of dementia associated to depression, in patients.

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