Universidade do Minho
Escola de Medicina

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Tese de Doutoramento em Envelhecimento e Doenças Crónicas

Trabalho efetuado sob a orientação do
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e da
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I hereby declare having conducted my thesis with integrity. I confirm that I have not used plagiarism or any form of falsification of results in the process of the thesis elaboration. I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

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"No man is an island, entire of itself; every man is a piece of the continent, a part of the main. If a clod be washed away by the sea, Europe is the less, as well as if a promontory were, as well as if a manor of thy friend's or of thine own were: any man's death diminishes me, because I am involved in mankind, and therefore never send to know for whom the bells tolls; it tolls for thee."

John Donne in *Devotions Upon Emergent Occasions, and severall steps in my Sicknes*

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Structural and functional asymmetries of the brain: associations with behavior and cognition

Abstract

The brain is remarkably asymmetrical at multiple levels of its organization and function, ranging from microstructural differences such as columnar organization of the *planum temporale*, to gross asymmetries including the counterclockwise torque of the frontal and occipital lobes. Functionally the brain presents lateralized activations during task performance, including the well-known leftward bias during speech production. These asymmetries have been shown to present sex-related differences and to plastically change with age, but also to be altered in disorders such as schizophrenia or dyslexia. It thus seems that brain asymmetry is associated with, or at least reflects, proper functioning, although associations with cognitive function and behavior are still not clear.

Aiming to clarify this matter, we have characterized in detail structural asymmetries and their cognitive correlates in human subjects, as well as functional lateralized activation in humans and rats. In summary, we have: (i) characterized structural laterality in a large population of older subjects with very distinct cognitive profiles using Magnetic Resonance Imaging (MRI); (ii) assessed their structural laterality-cognition associations in both transversal and longitudinal forms; (iii) evaluated lateralized activations during a working memory task (N-Back) using functional (f)MRI; and (iv) assessed local field potentials in left and right brain regions during the execution of a rodent impulsive decision-making task – the Variable Delay-to-Signal (VDS).

We observed that structural asymmetries are common in both left and rightward directions. These left-right differences were associated with learning and memory, inhibition/cognitive flexibility, verbal fluency and mood, often in relationships mediated by sex and education. When these subjects were re-analyzed after an 18-month period, on average no differences regarding the direction and magnitude of the asymmetries were found. Nonetheless, subcortical regions showed a significantly higher dispersion of laterality index variation when compared with cortical regions. Subjects whose thalamic and caudate structural asymmetry suffered higher variations also presented increased changes in general cognition and Stroop interference between the two assessments. Importantly, results regarding thalamic laterality in association with Stroop score were comparable in the transversal and longitudinal data, reinforcing the role of this region.

Functionally, performance in a working memory task (N-Back) in humans and impulsivity (VDS) in rats showed left- or right-specific recruitments. The N-Back induced lateralized activations restricted to lobule VI of the cerebellar hemisphere, precentral and angular gyri. Importantly, superior parietal lobule leftward asymmetry was correlated with better task performance. In the rat model, left and right prefrontal and striatal regions showed a time and hemisphere-dependent role in behavioral inhibition. More than 2 seconds before a timed or a premature response, there seems to be a major effect of the left hemisphere-centered network, which is followed by a left-right hemisphere association, determining the outcome. Additionally, left nucleus accumbens theta power before long wait delays was able to predict the number of premature responses in that delay, thus seeming to play a role in delay intolerance.

In conclusion, the majority of brain areas present either a leftward or rightward structural bias without an apparent regional clustering. This result was shown to be stable across different approaches (region of interest and voxel-based morphometry) and globally over time. We also observed higher variance in subcortical areas in the longitudinal assessment that accompanied the progression of general cognition and Stroop performance in the same time window. On the other hand, functional asymmetries during N-Back performance were sparse, which may be associated with age-related compensatory contralateral activation. On-task asymmetrical activity associated with performance was also observed in the rat; such approach has high temporal and spatial resolution and will allow to establish causal relations between laterality and behavior in future studies.

Assimetrias estruturais e funcionais do cérebro: associações comportamentais e cognitivas

Resumo

O cérebro é marcadamente assimétrico em diferentes níveis organizacionais e funcionais que vão desde diferenças micro-estruturais tal como a organização colunar no *planum temporale*, até assimetrias macroscópicas como o torque de sentido anti-horário nos lobos frontais e occipitais. Funcionalmente, o cérebro apresenta ativações lateralizadas durante o desempenho de uma função, incluindo o bem conhecido viés à esquerda durante a produção de linguagem. Foi já demonstrado que estas assimetrias apresentam diferenças associadas com o sexo e que se alteram de forma plástica com a idade, mas também que estão alteradas em perturbações como a esquizofrenia e a dislexia. Os dados indicam, portanto, que a assimetria cerebral está associada a, ou pelo menos reflete, uma boa função, apesar de as associações com a função cognitiva e comportamento não estarem esclarecidas.

Com o objetivo de clarificar esta questão, caracterizámos em detalhe as assimetrias estruturais e suas associações cognitivas em humanos, assim como a atividade lateralizada durante o desemprenho de uma tarefa em dois modelos distintos (humano e rato). Sumariamente, (i) caracterizámos a lateralidade estrutural de uma grande população envelhecida com perfis cognitivos bastante distintos utilizando ressonância magnética (MRI); (ii) avaliámos a associação entre estas assimetrias estruturais e a cognição de forma transversal e longitudinal; (iii) avaliámos ativações lateralizadas durante o desempenho de uma tarefa de memória de trabalho (N-Back), utilizando ressonância magnética funcional (fMRI); e (iv) registámos a atividade eletrofisiológica em regiões cerebrais esquerdas e direitas durante a execução de uma tarefa de tomada de decisão impulsiva ("Variable Delay-to-Signal" – VDS) em ratos..

Observámos que as assimetrias estruturais são comuns em ambas as direções. Estas diferenças esquerda-direita demonstraram estar associadas a aprendizagem e memória, inibição/flexibilidade cognitiva, fluência verbal e humor depressivo, geralmente em correlações mediadas por sexo ou educação. Quando estes indivíduos foram reavaliados após um período de 18 meses, não foram encontradas, em média, diferenças em termos de direção ou magnitude das assimetrias. No entanto, as regiões subcorticais mostraram maior dispersão na variação do índice de lateralidade em comparação com as corticais. Os sujeitos cuja assimetria do tálamo e do caudado sofreu

maiores variações apresentaram também maiores diferenças em termos de cognição geral e interferência na tarefa Stroop entre as duas avaliações. De notar, os dados relativos à lateralidade do tálamo em associação com a tarefa Stroop mostraram-se semelhantes nos dados transversais e longitudinais, reforçando o papel desta região.

Funcionalmente, o desempenho de uma tarefa de memória de trabalho (N-Back) em humanos e impulsividade (VDS) em ratos esteve associado a recrutamentos lateralizados. O N-Back induziu ativação lateralizada restrita ao lóbulo VI do hemisfério cerebelar e nos giros pré-central e angular. De notar, uma correlação entre a assimetria à esquerda do lóbulo parietal superior e um melhor desempenho na tarefa foi encontrada. No modelo animal, regiões pré-frontais e estriatais esquerdas e direitas demonstraram ter um papel na inibição comportamental que é dependente de tempo e de hemisfério. Mais de 2 segundos antes de uma resposta atempada ou prematura, os efeitos aparentam ser centrados numa rede esquerda, seguindo-se uma associação hemisfério esquerdo/hemisfério direito que determina a resposta. Adicionalmente, a atividade em teta do núcleo accumbens antes de intervalos de espera grandes foi capaz de prever o número de respostas prematuras nesse intervalo, aparentando desempenhar um papel na intolerância à espera.

Em conclusão, a maioria das áreas cerebrais apresenta um viés estrutural à esquerda ou à direita sem aparente agrupamento em regiões específicas. Estes resultados mostraram-se estáveis na análise através de duas abordagens diferentes (região de interesse e morfometria baseada em vóxeis) e globalmente ao longo do tempo de análise. Observámos ainda uma maior variação do índice de lateralidade em regiões subcorticais na avaliação longitudinal que acompanhou a progressão dos dados de cognição geral e Stroop na mesma janela temporal. Por outro lado, o número de assimetrias funcionais encontradas durante o N-Back foi reduzido, o que poderá estar associado a uma ativação contralateral compensatória devido à idade. Também no rato foi encontrada atividade assimétrica durante o desempenho da tarefa; esta abordagem apresenta alta resolução tanto a nível espacial como temporal e permitirá em estudos futuros o estabelecimento de relações causais entre a lateralidade e o comportamento.

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List of Abbreviations

#

2CA – Clinical Academic Center

2-csrtt – 2-choice serial reaction time task

3-MT – 3-Methoxytyramine

3si – initial 3 second delay

3sf – final 3 second delay

5-HIAA - 5-Hydroxyindoleacetic acid

5-HT – serotonin

6s – 6 second delay

12s – 12 second delay

Δ

Δcog – neuropsychological variation

ΔLI – laterality index variation

A

AAL – Automatic Anatomical Labeling

Acc – accuracy

ACTH – adrenocorticotropic hormone

ADHD – attention deficit and hyperactivity disorder

AI – asymmetry index

AP – anterior-posterior

AR – androgen receptor

B

BIS/BAS – Behavioral Inhibition/Behavioral Activation scale (also recognized as behavioral avoidance/approach)

BMI – body mass index

BOLD – blood oxygen level-dependent

C

C – chance

Caud – caudate

CCKAR – cholecystokininn-A receptor

COMPETE – Competitiveness Factors Operational Programme

CORT – cortisol

CLTR – Consistent long term retrieval

CRUNCH – Compensation Related Utilization of Neural Circuits Hypothesis

D

D – dextral

DA – dopamine

DARTEL – Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra

Dbackward – backwards component of the Digits Span Test

DD – delay discounting

df – degrees of freedom

Dforward – forward component of the Digits Span Test

DOPAC – 3,4-Dihydroxyphenylacetic acid

DR – delayed recall

DREADDS - Designer Receptors Exclusively Activated by Designer Drugs

DSST – Digit-Symbol Substitution Test

DTI – diffusion-tensor imaging

DV – dorso-ventral

E

EPI – Echo Planar Imaging

EPM – elevated plus maze

ERP – event-related potential

F

FA – fractional anisotropy

FAS – Controlled Oral Word Association F-A-S test

FCT – Fundação para a Ciência e a Tecnologia

FEDER – European Regional Development Fund

FGF – fibroblast growth factor

fMRI – functional Magnetic Resonance Imaging

FoV – field of view

FOXP2 – forkhead box P2

FSV – fast-scan cyclic voltammetry

FWE – family-wise error

FWHM – full-width at half-maximum

G

GDS – Geriatric Depression Scale

GLM – General Linear Model

GM – gray matter

GRIN2B - glutamate ionotropic receptor NNMDA type 2B subunit

H

HAROLD – Hemispheric Asymmetry Reduction in Older Adults

HERA – hemispheric encoding/retrieval asymmetry

HG – Heschl's gyrus

HPLC – high-performance liquid chromatography

I

ICV – intra-cranial volume

ICVS – Life and Health Sciences Research Institiute

IL – interleukin

K

K – number of tapers

L

L – left

LD – laterality degree

LFP – local field potential

LI – laterality index

LRRTM - Leucine-rich repeat transmembrane neuronal

M

M – moment

ML – medial-lateral

MMSE – Mini-Mental State Examination

MNI – Montreal Neurological Institute

m.o. – month-old

MPRAGE – magnetization-prepared rapid gradient echo

MRI – Magnetic Resonance Imaging

N

NAcc – nucleus accumbens

NMDA – N-methyl-d-aspartate

O

OCD – obsessive-compulsive disorder

OFC – orbital frontal cortex

OPFC – orbital prefrontal cortex

P

PANINI – Physical Activity and Nutrition INfluences In ageing

PCA – Principal Component Analysis

PCSK – proprotein convertase subtilisin/kexin

PET – positron emission tomography

PFC – prefrontal cortex

PhDOC – Inter-University Doctoral Programme in Aging and Chronic Disease

PR – premature response

PrI – prelimbic córtex

PSD – post-synaptic density

PT – planum temporale

R

R – right

Resp - response

ROI – region of interest

rTMS – repetitive transcranial magnetic stimulation

S

SD – Sprague-Dalley

Shh – Sonic hedgehog

SPECT – single photon emission computed tomography

SNP – single-nucleotide polymorphism

SPM – Statistical Parametric Mapping

SRT – Selective Reminding Test

Stroop-c – colors component of the Stroop test

Stroop-w – words component of the Stroop test

Stroop-wc – words and colors component of Stroop test

T

tDCS – transcranial direct current stimulation

TE – echo time

Tp - timepoint

TR – repetition time

TW – time-bandwidth product

V

VBM – voxel-based morphometry

VDS – Variable Delay-to-Signal

W

WH – Wistar Han

WM – white matter

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FOREWORD

Hemispheric asymmetries have been known to be relevant for function since the XIXth century, when Marc Dax (1770-1837) (Dax, 1865) and Paul Broca (1824-1880) (Broca, 1861) described speech impairments due to lesions in the left frontal region, now known as Broca's area (see also (Manning and Thomas-Anterion, 2011)). Since then, brain laterality has been observed at multiple levels, from gross structural asymmetries such as the counterclockwise torque (Lyttelton et al., 2009; Raz et al., 2004; Toga and Thompson, 2003), to microanatomic differences in the columnar organization of the planum temporale (Buxhoeveden et al., 2001). Functionally, lateralized recruitment of areas involved in language has been observed in functional imaging studies, with a leftward predominance therefore confirming the initial observations by Dax and Broca (Vigneau et al., 2011; Westerhausen et al., 2014).

Brain asymmetry is overall thought to be a beneficial characteristic and several hypotheses in this direction have been put forward: (i) increase of brain's ability to perform multiple tasks at once (Vallortigara et al., 2011); (ii) avoidance of unnecessary duplication of functions and thus maximization of available space for neural tissue (Denenberg, 1981; Vallortigara et al., 2011); (iii) increased processing speed due to reduction in inter-hemispheric communication through the corpus callosum (Ringo et al., 1994); and (iv) prevention of initiation of incompatible responses in the two hemispheres (Bisazza et al., 1998; Cantalupo et al., 1995)).

In fact, expansion of the field within the past decades and particularly with the advent of new techniques such as Magnetic Resonance Imaging has made clear that brain asymmetries in both morphological and functional dimensions is commonplace. Associations with visuo-spatial attention (Badzakova-Trajkov et al., 2010; Hougaard et al., 2015), impulsivity (Gordon, 2015), risk-taking (Fecteau et al., 2007; Knoch et al., 2006) or face processing (Badzakova-Trajkov et al., 2010; Meng et al., 2012) among others have been found. Also, asymmetries are presented in atypical forms in various diseases such as dyslexia (Galaburda et al., 1985), attention deficit and hyperactivity disorder (Schrimsher et al., 2002) or schizophrenia (Kawasaki et al., 2008; Oertel et al., 2010; Okada et al., 2016), but also vary in physiological conditions such as sex-related differences (Good et al., 2001; Plessen et al., 2014) and even plastic changes with age (Plessen et al., 2014; Zhou et al., 2013). However, the association between brain laterality and functional outcomes is still not well understood.

The main objective of this thesis is therefore to clarify the associations between brain laterality and behavior and cognition. More specifically we will:

- Characterize the older brain in terms of structural asymmetries using Magnetic Resonance Imaging (MRI);
- Determine the functional relevance of these asymmetries by assessing their associations with cognition in transversal and longitudinal manners;
- Assess the association between on-task activations (functional MRI) and working memory in aged individuals;
- Determine the functional correlates of impulsivity in the Variable Delay-to-Signal (VDS) test by assessing local field potentials (LFPs) bilaterally in specific regions of interest.

In order to achieve these goals, we will start by revising the state of the art in brain asymmetries in **CHAPTER I**. A general view is provided in terms of historical and evolutionary context and asymmetry development, while more detailed information regarding structural and functional asymmetries and their associations with cognition in health and disease is shown.

Due to the above-mentioned sex- and age-specificity of brain laterality, its assessment requires extensively characterized populations. We will therefore evaluate a well described older population (Costa et al., 2013; Marques et al., 2016; Santos et al., 2013; Santos et al., 2014) that presents high variability in terms of cognitive performance. **CHAPTER II,** contains the paper published in the journal NeuroImage entitled "Structural laterality is associated with cognitive and mood outcomes: An assessment of 105 healthy aged volunteers". It provides characterization of the older brain's structural asymmetries and associates them with cognition and mood. **CHAPTER III**, which consists on the submitted manuscript "Asymmetrical subcortical plasticity and cognitive progression in older individuals", will then assess the same population in a longitudinal approach, evaluating the co-evolution of structural laterality and cognition. Additionally, lateralized activations during the performance of a working memory task (N-Back) will be assessed in **CHAPTER IV**, in the manuscript accepted for publication in the journal Frontiers in Aging Neuroscience "Functional hemispheric (a)symmetries in the aged brain – relevance for working memory" (in preparation).

Considering the limitations of human studies, specifically the difficulty in determining causal associations and the limited time definition of functional MRI, there is a need for the utilization of animal models. We have therefore analyzed brain activity in left and right regions of interest in

order to predict impulsive behavior in the VDS task in **CHAPTER V's** ongoing work "Functional encoding of inhibitory control in rats in the Variable Delay-to-Signal task".

Finally, **CHAPTER VI** discusses and integrates the general conclusions of each chapter and summarizes future perspectives of the field.

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CHAPTER I

Unmasking the functional relevance of hemispheric asymmetries: Break on through (to the other side)

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Title: Unmasking the functional relevance of hemispheric asymmetries – break on through (to the other side)

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Abstract:

The pioneer works of Marc Dax and Paul Broca on the association between left hemisphere injuries and speech impairments, revealed one of the most intriguing properties of the brain – asymmetry. Since then, lateralized features have been observed in virtually all phylogenetic branches, suggesting evolutionary conservation, although its adaptive role is still not clear. In humans, the field remains greatly shaped by early observations on language, but the advent of brain imaging revealed that functional and structural laterality is not only widespread, extending to memory, decision-making and emotion, but also that is plastic. In this review, we systematize information regarding structural and functional hemispheric asymmetries of the healthy brain and their associations with cognition and behavior. We briefly explore evolutionary theories and the pathways for asymmetry development, but mostly focus on central nervous system asymmetries of the developed human, bridging towards the laboratory rodent for mechanistic explanations.

Keywords: laterality, hemisphere, cognition, behavior

Contents:

1. Introduction

Brain asymmetry can be defined as a characteristic and/or property that differs between hemispheres either at structural or functional levels. Evolutionary, genetic, environmental and even pathological factors are known to contribute to laterality (Bisazza et al., 1998; Gunturkun and Ocklenburg, 2017; Rogers, 2014; Toga and Thompson, 2003; Vallortigara et al., 2011), although its adaptive value remains controversial (Badzakova-Trajkov et al., 2015; Rogers, 2000; Vallortigara, 2006).

Functional hemispheric specialization was first described in the XIXth century and it revolutionized the epoch's concept of symmetrical frontal location of speech. Marc Dax observed that soldiers who presented difficulties in speech displayed left hemisphere lesion as well as right hemiparesis, concluding that language was left lateralized. However, this notion found extreme resistance upon submission of his posthumous paper in 1863. In fact, Louis Françisque Lélut, head of the evaluation commission, withheld the manuscript for 21 months and finally concluded from his review that, not only was the suggestion of functional laterality ridiculous, but that it was a throwback into phrenology (Manning and Thomas-Anterion, 2011).

Paul Broca himself was resistant to this idea and first considered the left lesions of his eight speech-impaired patients as a coincidence. Eventually, he concluded in the same direction as Marc Dax's and the two papers were published at such similar timing, that the discussion on who should take credit for discovery of this phenomena prevails to this day (Manning and Thomas-Anterion, 2011). Regardless of such discussion, the lesioned region, which was located in the inferior frontal gyrus was designated as Broca's area and defined as the main region for language production. Not long after, Carl Wernicke determined that left posterior temporal (Wernicke's area) lesion induced difficulties in language comprehension (Sun and Walsh, 2006; Wernicke, 1874), further advancing the idea that language-related functions are predominant in the left hemisphere.

Ever since, much scientific work has been produced regarding body and brain asymmetries as well as its cognitive correlates. While most data initially arose from lesion studies and lateralized stimuli (assuming contralateral activation), a big advance in the field came from the study of patients submitted to surgical disconnection of the corpus callosum in the 60s and 70s (Sperry, 1982). Later, the introduction of techniques such as fMRI and EEG provided rapid expansion of the field.

In this review, we systematized information relative to brain asymmetries and its cognitive and behavioral implications. We went beyond the classical language and handedness correlates and explored other expressions of laterality. We provide brief reviews on evolutionary and developmental data but mostly focus on human and rodent asymmetries of the developed healthy brain and their cognitive correlates. Only reported data in which left/right differences were directly assessed (in opposition to effects found on one hemisphere but not the other) was included in this review. Additionally, data that includes associations with cognition or behavior was preferentially reported. It should be noted that (i) asymmetries are normally graded rather than binary (e.g. functional left lateralization for a given task does not mean that only the left hemisphere is engaged, but rather that it is more involved than the right hemisphere) (Badzakova-Trajkov et al., 2015; Bartolomeo and Thiebaut de Schotten, 2016); and (ii) lateralized function can be found at the individual or population levels, meaning that a population presents an equal amount of left and right biased subjects or that more than 50% of the individuals possess the same direction of asymmetry, respectively (Bisazza et al., 1998).

2. The origins of laterality – evolutionary hints from the animal kingdom

Asymmetries have for long been thought to be a human characteristic (Bisazza et al., 1998; Corballis, 2009). However, it is now clear that many behavioral side biases are present in animals including mammals, reptiles or fish (Bisazza et al., 1998; Corballis, 2009; Vallortigara et al., 2011). These are thought to reflect a morphofunctional asymmetric organization of the central nervous system and might provide important hints regarding evolution and, in many aspects, conservation of asymmetry.

2.1. Animal asymmetries

2.1.1. Paw preference

The most visible form of behavioral lateralization in humans is handedness, as approximately 90% of the population shows preference for the right hand (Gilbert and Wysocki, 1992) (see section 4 for further information). However, a variety of species show similar biases. Non-human primates, for example, have shown in a large meta-analysis by Hopkins and colleagues (2006) (total of 1524 great apes), that although there was a population left hand preference in the termite fishing task, when taking into account a wide range of tasks, a general right-handedness was found in chimpanzees and bonobos, but not in gorillas and orangutans (Hopkins, 2006). From an

evolutionary point of view, it is argued that the population bias in non-human primates is very small in comparison with the human. However true for simple tasks such as scratching, assessment of more complex behaviors such as throwing or retrieving food from a tube shows an increase in this bias (Vallortigara et al., 2011), which is in accordance with data coming from human prehistoric cultures. In traditional societies the majority of individuals show mixed handedness for the majority of the tasks, only exhibiting population right handedness when utilizing precision-grasping tools (Marchant et al., 1995). The question of whether human/ape handedness distribution differences are due to social evolution or genome changes thus remains to be answered (Vallortigara et al., 2011).

Other animals who use their paws for fine manipulation of food, such as parrots and cockatoos,, show in general a bias towards left pawedness (Rogers and Workman, 1993). Nonetheless, animals that do not show this form of fine utilization of the limbs, still present forms of paw preference. Cats (Tan, 1993; Tan and Kutlu, 1991; Wells and Millsopp, 2012) and dogs (Quaranta et al., 2004; Wells, 2003) show sex-mediated lateralization for food retrieval, with increased right and left preferences in females and males respectively. This lateralization seems to be associated with cognition as dogs have shown concordance between direction of visuospatial attention and paw preference (Fig. 1) (Siniscalchi et al., 2016). It may also provide advantages, as cats who present paw preference react faster to a moving light when compared with animals with decreased level of preference (Fig. 1) (Fabre-Thorpe et al., 1993). Similarly, paw preference in dogs has been associated with immune response. Left-pawed dogs present basal higher lymphocyte percentage (Quaranta et al., 2004), interleukin (IL)-2 and IL-6 levels (Quaranta et al., 2008) and decreased γ-globulin levels (Quaranta et al., 2004) in comparison with right-pawed and ambidextrous animals. After administration of the rabies vaccine, titers of anti-rabies antibodies and IFN-γ are lower in left-pawed animals in comparison with the remaining groups (Quaranta et al., 2006; Siniscalchi et al., 2014). On the other hand, ambidextrous dogs show a higher increase in epinephrine levels (Siniscalchi et al., 2010) (although this result failed to be replicated (Siniscalchi et al., 2014)) and a smaller decrease in IL-2 and IL-6 levels (Quaranta et al., 2008) in comparison with left- and right-pawed animals.

Importantly, paw preferences is also visible in laboratory rodents. Data on rats has reported populational biases of right preference that range from 50 to 80% (Cunha et al., 2017; Elalmis et al., 2003; Guven et al., 2003; Pence, 2002; Wu et al., 2010), while mice mostly show a 50/50

left/right distribution (Betancur et al., 1991; Cabib et al., 1995; Signore et al., 1991; Takeda and Endo, 1993) despite some strain-dependent differences (Betancur et al., 1991; Biddle et al., 1993; Takeda and Endo, 1993; Waters and Denenberg, 1994). This paw preference has been associated with dopamine asymmetries in the brain (Barneoud et al., 1990; Budilin et al., 2008; Cabib et al., 1995), but so far no clear associations with other behaviors have been found (Cunha et al., 2017). On the other hand, a sex-pawedness interaction has been found in anxiety-like behavior, with left-pawed female mice showing decreased exploratory behavior in comparison with left-pawed males, while right-pawed animals showed no sex-differences (Mrabet et al., 2000). Additionally, as in dogs, mice paw preference has shown implications at the immunological level, with left pawed animals presenting higher mitogen- (Neveu et al., 1988) and lower LPS-induced (Delrue et al., 1994) T lymphocyte proliferation, increased IL-1beta and decreased IL-6 response to LPS (Gao et al., 2000; Shen et al., 2005) and increased IL-6 asymmetry in the brain (Shen et al., 2005), although sex and strain seem to play a role in these associations (Fride et al., 1990; Neveu et al., 1991). Additionally, right-pawed and ambidextrous, but not left-pawed animals, presented LPS-induced increase in hypothalamic serotonin turnover and plasma adrenocorticotropin levels (Delrue et al., 1994), while no differences in the HPA axis have been found (Betancur et al., 1992).

Fig. 1. Laterality tests used in cats, dogs and rats. (left) Visuospatial attention in dogs (Siniscalchi et al., 2016), (middle) response to a moving light in cats (modified from (Fabre-Thorpe et al., 1993), with permission from Elsevier), and (right) pawedness and dexterity assessment in rats (Cunha et al., 2017).

2.1.2. **Eye specialization**

Chicks present specialization of the right and left eyes for discrimination of grain from pebbles and for predator monitoring, respectively (Mench and Andrew, 1986; Rogers et al., 2004). Also, toads are more likely to attack conspecifics on their left side (Robins et al., 1998; Vallortigara, 2006; Vallortigara et al., 1998) but preferentially use the right visual hemifield to direct attack on prey

(Robins and Rogers, 2004; Vallortigara et al., 1998). Similarly, lizards (Hews and Worthington, 2001) and baboons (Casperd and Dunbar, 1996) follow a similar trend for usage of the left visual field during aggressive behavior and pigeons present better food discrimination ability when using the right than the left eye (Gunturkun et al., 2000). Direction of lateralization for response to threat and feeding thus seems to be highly conserved across species.

2.1.3. **Language**

Despite current knowledge of integrative systems for language production and recognition, peri-Sylvian regions remain as core in these functions (Chang et al., 2015; Hagoort, 2014; Mirman et al., 2015) and present marked asymmetries in chimpanzees (Yeni-Komshian and Benson, 1976), which seem to be conserved in humans (LeMay, 1976; Lyttelton et al., 2009; Rubens et al., 1976; Takao et al., 2011a). Also, functional left lateralization of language, which can be found in humans (Broca, 1861; Vigneau et al., 2011; Westerhausen et al., 2014), is also present in vocalizations emitted by frogs (Bauer, 1993) and canaries (Halle et al., 2003), while dogs present a leftward bias for processing of meaningful words (Andics et al., 2016), suggesting evolutionary conservation.

2.1.4. **Visual/olfactory processing**

Epithalamic asymmetries, namely in the habenula, are present in many vertebrates as reviewed by Concha and Wilson (2001) and its structural laterality was even recently demonstrated to be conserved in humans (Ahumada-Galleguillos et al., 2016). The importance of such asymmetry was demonstrated in zebrafish (see section 3.2 for information on this model's asymmetry development), in which the majority of the habenular neurons that respond to visual and olfactory stimuli are found in the left and right hemispheres, respectively (Dreosti et al., 2014). In fact, left dorsal habenula activity mediates light-preference behavior (Zhang et al., 2017) while right dorsal habenula activation is associated with presentation of olfactory social cues (Krishnan et al., 2014). Additionally, inversion of habenular laterality during development produced inversion of this asymmetry, while manipulation that produced left-left or right-right fish induced loss of response to odor or light, respectively (Dreosti et al., 2014).

2.2. Advantages and disadvantages of lateralization

The evolutionary explanation for asymmetries may be adaptive. This seems to be the case for chimpanzees, in which individuals with higher individual hand preference for termite fishing seem

to be more efficient in this task (McGrew and Marchant, 1999), for pigeons (Gunturkun et al., 2000) and chicks (Rogers, 2000; Rogers et al., 2004), whose increased visual asymmetry provides advantage in discriminating grains from pebbles, and for fish, whose lateralization is associated with improved spatial reorientation (Sovrano et al., 2005) and who show stronger lateralization in high *vs* low predation areas (Brown et al., 2004).

Theoretical advantages of lateralization have been put forward including: (i) increase of brain's ability to perform multiple tasks simultaneously (Vallortigara et al., 2011); (ii) avoidance of unnecessary duplication of functions and thus maximization of available space for neural tissue (Denenberg, 1981; Vallortigara et al., 2011); (iii) increased processing speed due to reduction in inter-hemispheric communication through the corpus callosum (Ringo et al., 1994); and (iv) prevention of initiation of incompatible responses in the two hemispheres (Bisazza et al., 1998; Cantalupo et al., 1995). On the other hand, symmetry can also confer benefits, facilitating left-right coordination in behaviors such as swimming (Concha et al., 2012) and asymmetries can bring disadvantages. Behavioral asymmetry in terms of escape direction may increase predictability and render the animals more susceptible to predator attack (Vallortigara et al., 1999), while extreme central asymmetries associated with decreased plasticity may make individuals more vulnerable to consequences of unilateral lesions (Saur et al., 2006; Thulborn et al., 1999).

While individual asymmetries are spread-out in the animal kingdom, population asymmetries are more common in social species. In schooling fish for example, the security in the prediction of escape direction may outweigh the downfalls of prediction by the predator (Vallortigara et al., 1999). On the other hand, a subpopulation may benefit from opposite asymmetry as long as the percentage of contralateral animals is maintained low. This can still be seen in the modern human society in which in some sports such as boxing, being a minority (left-hander) remains an advantage (Loffing and Hagemann, 2015). It can also happen that population asymmetries continuously evolve in some species. The scale eating fish has its mouth on the left or right side and uses it to laterally attack other fish. The populational bias reverts roughly every five years, possibly to overcome prediction by its preys (Hori, 1993).

2.3. The evolutionary perspective

The mechanism for how these asymmetries developed has also been widely discussed. Badzakova-Trajkov and colleagues (2015) have recently reviewed evidence on two proposed models for this

process. The causal theory poses that the lateralization of one function "pushes" another function to the contralateral hemisphere, while the statistical hypothesis suggests that each function would have equal probability of being placed in one or the other hemisphere (Badzakova-Trajkov et al., 2015). Other theories are mainly centered in language lateralization. For example, Toga and Thompson (2003) suggested that the volume expansion of the language cortices on the left hemisphere may have driven the evolution of other structures important for speech production, perception and motor dominance in a lateralized fashion (Toga and Thompson, 2003). Others such as Vallortigara and colleagues (1999) focus on lateralization driven by laterally placed eyes (Vallortigara et al., 1999). On the other hand, Ringo et al. (1994) state a more functional theory, in which development of hemispheric laterality is mostly due to a delay in interhemispheric conduction that ultimately leads to specialization (Ringo et al., 1994). Corballis (2017) explains brain evolution through expansion and fission of ancestral systems, creating more specialized circuits that would increase the pressure towards lateralization in an effort to increase efficiency (Corballis, 2017) as well as social pressure towards population laterality (i.e. human tools are produced for right hand use, thus encouraging right handedness) (Corballis, 2009). Similarly, Hugdahl (2011) defends that evolutionary pressure towards more efficient cognitive function "pushed" towards a division of labor between the two hemispheres (Hugdahl, 2011). While it is clear that we are a long way from explaining the evolutionary progression of brain asymmetries, these ideas are in general not mutually exclusive and it is possible that they have interacted in the creation of current lateralized function.

3. Asymmetry development

Current knowledge indicates that asymmetry development is based on multiple genetic and environmental factors. Because the ontogenesis of this process has been recently reviewed (Gunturkun and Ocklenburg, 2017), we will only briefly mention the most relevant processes and models.

3.1. Theories on handedness heritability

Several theories for hereditary transmission of handedness have been proposed. Marian Annet (Annett, 1972) has originally suggested the existence of a human-specific gene, which she named right-shift, and which would "shift" hand preference towards the right. Absence of this gene would lead to random placement of this function with approximation of probabilities for left-, right-, and

mixed-handedness (Annett, 1972). Similarly, Chris McManus (1985) proposed that two alleles: D (dextral) and C (chance) determined handedness. Homozygous DD individuals would have a 100% chance of being right handed, which would be reduced to 50% and 75% in CC and DC subjects respectively (McManus, 1985). However, a large genome-wide association study has estimated the involvement of approximately 40 loci in the development of handedness (McManus et al., 2013), thus disproving single allele models.

3.2. Main pathways – the zebrafish model

The zebrafish has been regarded as an essential model for the study of asymmetry development. In addition to a number of characteristics – e.g. simple genetic manipulation, transparent embryos which facilitate sorting of live animals, high throughput behavioral analysis, etc (Roussigne et al., 2012) –, it shows a clear lateralization of the epithalamus and asymmetrical behavioral displays (Barth et al., 2005; Concha et al., 2009; Gunturkun and Ocklenburg, 2017; Roussigne et al., 2012). The dorsal habenula is composed by a medial and a lateral part, which are enlarged on the right and left sides respectively. Also, the other area that constitutes the epithalamus, the parapineal nucleus, is only present on the left hemisphere, resulting in lateralized connections with the habenula (Gunturkun and Ocklenburg, 2017). Several pathways are involved in the development of these asymmetries, but fibroblast growth factor (FGF) mediates their initial development. FGF controls the expression of transcription factors that are associated with repression of the nodal antagonist lefty1 in the brain and reduction the first leads to bilateral expression of the latter (Neugebauer and Yost, 2014). Fgf8, in particular, has been shown to be crucial for parapineal lateralization and its absence precludes the development of epithalamic asymmetries (Regan et al., 2009). Local provision of this factor leads to migration of parapineal cells, but, in the absence of Nodal, this migration is directed towards the source of Fgf8 (Regan et al., 2009), highlighting the role of Nodal for direction of laterality. However, absence of Nodal does not lead to symmetry, but rather to a randomization of direction of asymmetry (Concha et al., 2000). These Nodal genes, starting with the flow sensor charon, are expressed on the left lateral plate mesoderm due to cilia-dependent movement of fluid inside Kupffer's vesicle (Blum et al., 2014; Lopes et al., 2010; Sampaio et al., 2014) and, in fact, humans with ciliary dyskinesia have a 50% chance of developing situs inversus, a condition in which normal left/right organ placement is altered (Honore and Burgel, 2016).

Ciliogenesis thus plays an important role in asymmetry development. The Wnt/β-catenin pathway has been shown to regulate this process in Kupffer's vesicle via foxj1a expression. Wnt signaling reduction is associated with reduced foxj1a expression, loss of cilia function and ultimately disruption of left/right pattern (Caron et al., 2012). On the other hand, overexpression of this pathway during late gastrulation has been associated with bilateral expression of Nodal genes, suggesting that Wnt signaling also plays a role in repression of Nodal repression (Carl et al., 2007). Additionally, Notch signaling seems to be associated with total number of motile cilia (Sampaio et al., 2014; Tavares et al., 2017) and its impairment also reduces cilia length (Lopes et al., 2010), making this pathway essential for lateralized Nodal expression (Lopes et al., 2010; Raya et al., 2003; Sampaio et al., 2014).

3.3. Gene expression – human

Genes associated with the above-mentioned pathways have been hypothesized to be involved in the clearest form of human laterality – handedness. Single-nucleotide polymorphisms (SNPs) in proprotein convertase subtilisin/kexin type 6 (PCSK6), which encodes a protein involved in Nodal cleavage into its active form, have been associated with hand skill in dyslexic patients, but not in healthy individuals (Brandler et al., 2013; Scerri et al., 2011; Shore et al., 2016). Nonetheless, polymorphisms in this gene in terms of variable-number tandem repeats have been associated with degree of handedness in healthy subjects (Arning et al., 2013). Similarly, genes involved in glutamatergic function have been reported. Leucine-rich repeat transmembrane neuronal 1 (LRRTM1) has also been associated with handedness in a dyslexic population when the haplotype was inherited from the father (Francks et al., 2007). In healthy individuals this association was not found, however, levels of methylation in the putative promotor region of this gene were higher in individuals with mixed-handedness, further confirming its importance (Leach et al., 2014).

Also, sex differences found in handedness proportions, with males showing higher tendency for being left-handed (Papadatou-Pastou et al., 2008) gave rise to theories such as the above-mentioned idea regarding in utero testosterone sensitivity (Geschwind and Galaburda, 1985). The androgen receptor gene (AR) is placed in the X chromosome and its function varies according to the number of CAG repeats, which have been suggested to be associated with these sex-related differences. In fact, it has been shown that females with higher number of repeats have a higher probability of being left-handed (Arning et al., 2015; Medland et al., 2005), while males have been reported to show this increase in probability if they have a decreased number of repeats (Medland

et al., 2005) and to have higher probability of mixed-handedness with higher number of repeats (Arning et al., 2015).

Research in terms of genetic correlates of language lateralization is sparser. However associations with variations in glutamate ionotropic receptor N-methyl-d-aspartate (NMDA) type 2B subunit (GRIN2B) (Ocklenburg et al., 2011), forkhead box P2 (FOXP2) (Ocklenburg et al., 2013a; Pinel et al., 2012) and cholecystokinin-A receptor (CCKAR) (Ocklenburg et al., 2013b) genes in healthy subjects have been found.

3.4. Environmental *vs* genetic factors

Geschwind and Galaburda (1985), proposed that development of left-handedness was associated to *in utero* increased levels of testosterone and/or higher sensitivity to its action, which would increase the probability of atypical lateralization in male fetuses (Geschwind and Galaburda, 1985). On the other hand, Laland and colleagues (1995) suggested that, without disregarding genetic influence in the development of handedness, environmental influence during childhood such as parental stimulation will play a determinant role (Laland et al., 1995). In fact, despite all the above-mentioned molecular evidence, a large twin study encompassing more than 25000 families determined that genetics could only explain 25% of the handedness variance (Medland et al., 2009) which is in accordance with data showing that 23% of homozygotic twins present opposite handedness (Sicotte et al., 1999). Similarly, structural brain asymmetries, namely at the level of region volume (Eyler et al., 2014) and fiber tract characteristics' (Jahanshad et al., 2010) in adult twins, show small genetic influence.

Also, individuals with the developmental anomaly situs inversus, show an inversion of the petalia (Kennedy et al., 1999) (characterized in the majority of the population as right frontal and left occipital protrusions) and torque (Vingerhoets et al., 2018) (anticlockwise twisting), but normal left language (Ihara et al., 2010; Kennedy et al., 1999; Tanaka et al., 1999; Vingerhoets et al., 2018) lateralization and normal percentage of right handedness (Kennedy et al., 1999; McManus et al., 2004; Tanaka et al., 1999). However, when assessing only subjects without primary ciliary dyskinesia, the percentages become close to random, with 55% right-handers (Vingerhoets et al., 2018). There is therefore consistent evidence for both genetic and non-genetic factors involved in human laterality development, although a clear link is yet to be established.

It thus seems that environmental factors play a major role in asymmetry development. In fact, in the chick, light exposure of the egg is crucial for laterality development (Concha et al., 2012). The chick embryo is in general placed in the egg in such a way that the left eye is covered by its body while the right eye is exposed to light. Incidence of light induces lateralized connections in the visual pathways (Koshiba et al., 2003; Rogers and Deng, 1999). After hatching, chicks show the above-mentioned right/left preference for grain/predator discrimination respectively (Mench and Andrew, 1986; Rogers, 2000), which can be reversed by exposing the left (and not the right) eye to light (Rogers et al., 2007) or prevented by incubating the embryos in the dark (Dharmaretnam and Rogers, 2005) or exposing them to corticosterone (Rogers and Deng, 2005) or other steroid hormones (Rogers and Rajendra, 1993; Schwarz and Rogers, 1992). Curiously, this mechanism for light induction of laterality seems to be conserved in pigeons and zebrafish (Andrew et al., 2009).

Similarly, the rodent literature has shown that neonatal exposure to novel environments alters laterality development in terms of behavioral asymmetries (Tang and Reeb, 2004; Tang et al., 2003; Tang and Verstynen, 2002), hippocampal volume (Verstynen et al., 2001) and plasticity (Tang et al., 2008). These differences have been suggested to be partially due to epigenetic changes (Tang et al., 2008) and alterations in stress response (Guadalupe et al., 2015; Tang et al., 2003).

4. Handedness

The most commonly known form of asymmetry is handedness. In humans it shows an obvious populational bias, with around 90% of the individuals showing right-hand preference (Gilbert and Wysocki, 1992), a percentage that has been maintained stable over centuries (Coren and Porac, 1977). This preference can be found as early as at 15 weeks of gestation, in which a bias for sucking the right thumb was found (Hepper et al., 1991) and is a predictor of handedness at 10-12 years of age (Hepper et al., 2005). These motor asymmetries are not limited to preferred hand for writing or tool usage, but can also be found in other lateralized behaviors such as preferred foot for kicking (Carey et al., 2001) of preferred direction of head turning when kissing (Güntürkün, 2003).

Far from being a human characteristic, motor asymmetries are widespread throughout many species as discussed above (see section 2.1.1), including laboratory rodents. Large samples of

inbred mice present populational pawedness (although direction is dependent on the assessed measure) (Waters and Denenberg, 1994), while rat studies show discordant data that range from an inexistence of populational but clear individual pawedness (50% right, 50% left) (Cunha et al., 2017) to rightward preferences of 80% (Elalmis et al., 2003).

Without aiming to be exhaustive, we will, in this section, assess the correlates of human handedness in brain laterality and in behavior and cognition.

4.1. Associations with brain laterality

4.1.1. Gray matter

Associations of handedness and structural asymmetries have been thoroughly assessed and, in fact, some authors report effects, including decreased asymmetry of the planum temporale (PT) (Steinmetz, 1996) or the cerebellum (Snyder et al., 1995) in non-dextral subjects. On the other hand, several studies using large cohorts do not support this notion, failing to find handedness effects on gray matter structural asymmetry (Good et al., 2001; Guadalupe et al., 2016; Guadalupe et al., 2014; Kavaklioglu et al., 2016; Watkins et al., 2001).

4.1.2. White matter tracts

To date, direction of handedness has not been associated with white matter tract asymmetries, namely in the posterior limb of the internal capsule (Westerhausen et al., 2007), the arcuate fasciculus (Allendorfer et al., 2016; Vernooij et al., 2007) or the corpus callosum (except for the small W22-39 region) (Gurd et al., 2013). On the other hand, Propper and colleagues (2010) showed that consistently-handed individuals showed increased arcuate fasciculus asymmetry, independently of the direction of hand preference (Propper et al., 2010), highlighting the importance of assessing handedness as a continuous, rather than a binary variable.

4.1.3. Resting-state

Stronger functional connectivity in right- than left-handers has been shown between the left primary cortex and the right dorsolateral premotor cortex. In fact, this connectivity cluster was able to contribute to individual handedness classification with 86.2% accuracy (Pool et al., 2015). Left-handers also seem to have higher probability of showing atypical intra-hemispheric connectivity: while the group of typical connectivity constituted by Joliot and colleagues (2016) comprised a 1:1

proportion of right and left-handers, the atypical group had 10 times more left- than right-handers (Joliot et al., 2016). Additionally, right lateralization of attention networks has been described as higher in right- than left-handers (Liu et al., 2009).

4.1.4. **Task-related function**

Handedness has classically been associated with language dominance (Sun and Walsh, 2006; Toga and Thompson, 2003). Mazoyer and colleagues (2014) have assessed these differences and determined that left dominance for language occurred in 88% of right handers, but also in 78% of left handers. Additional patterns included absence of lateralization for language (12% of right handers and 15% of left handers) and right lateralization, which was only found in left-handers (7%) (Mazoyer et al., 2015). Similar results have been described in other cohorts (Allendorfer et al., 2016; Nenert et al., 2017; Powell et al., 2012), including a population of adult monozygotic twins discordant for handedness, in which left-handed individuals were found to be less left-lateralized for verbal processes than their right-handed siblings (Gurd et al., 2013). Additionally, within right-handers, degree of handedness has been shown to modulate laterality during sentence comprehension (Newman et al., 2014). Semantic task performance has also highlighted handedness differences in connectivity: left-handed individuals showed relatively higher interhemispheric connections, increased effective connectivity between right middle occipital gyrus and bilateral insula and decreased effective connectivity between left insula and left precentral gyrus (Gao et al., 2015).

Also face discrimination seems to be mediated by handedness. While most regions are right lateralized independently of handedness, the fusiform face area, which is normally described as showing a rightward bias in the general population, exhibits a small leftward asymmetry in left-handers (Bukowski et al., 2013).

4.2. Associations with behavior and cognition

The handedness-cognition association has been hypothesized for long. However, studies in this matter are disperse in terms of assessed cognitive ability, balance of left/right/mix-handers and handedness evaluation method. Results should therefore be considered with caution. Here, we will not discuss cognitive skills that, to our knowledge, have only been reported once.

4.2.1. **Verbal ability**

A large study in eleven-year-old children has determined that ambidextrous children have relatively weaker skills in verbal, non-verbal inference, reading comprehension (Crow et al., 1998) and slightly reduced performance on school tests (Björk et al., 2012). To a lower extent, strong right-handers also showed decreased deficits in verbal inference and left-handers also showed a deficit in verbal inference (Crow et al., 1998). On the other hand, inconsistent handedness has been positively correlated with vocabulary learning of a foreign language in adults (Kempe et al., 2009).

Nonetheless, a cohort of adult monozygotic twins discordant for handedness revealed no differences in verbal fluency (Gurd et al., 2013) and a large meta-analysis determined the inexistence of direction of handedness effects in verbal ability (although a small right-hand benefit was seen in children) (Somers et al., 2015). It is thus possible that handedness-related verbal ability differences are associated with strength of preference, rather than direction.

4.2.2. **Spatial ability**

A large meta-analysis assessing the effects of handedness on spatial ability, mostly regarding the ability to mental rotate objects, reported a small right-hand benefit (Somers et al., 2015).

4.2.3. **Memory**

While left-handers have been reported to show lower working memory scores than right-handers (Powell et al., 2012), the majority of the literature shows effects of inconsistent vs consistent handedness. In fact, mixed-handers have shown an advantage in episodic memory (Christman and Butler, 2011; Lyle et al., 2012; Sahu et al., 2016), that does not seem to extend to working memory performance (Sahu et al., 2016).

4.2.4. **IQ**

A cohort of adult monozygotic twins discordant for handedness revealed no differences in IQ (Gurd et al., 2013). Nonetheless, in a large meta-analysis comprehending individuals with intellectual disability, normal development and high IQ, individuals with intellectual disability had a significant tendency towards atypical (non-right) handedness (Papadatou-Pastou and Tomprou, 2015).

4.2.5. **Personality**

Several personality traits have been associated with handedness consistency. Mixed-handers have been reported to present higher sensation seeking levels (which is associated with risk-taking) (Christman, 2014; Kuderer and Kirchengast, 2016), less authoritarian (Christman, 2014; Lyle and Grillo, 2014), less sensitive to disgust (Christman, 2014) and more creative (Badzakova-Trajkov et al., 2011).

5. Structural asymmetries

Structural asymmetries can be found at different levels in the brain. These include asymmetries at the macrostructural (volume or thickness) and microstructural (cell number, cell packing, dendritic arborization or spine density) levels. Data shown in this section is almost exclusively human and exceptions in which rodent data is reported are clearly identified. Additionally, we will only discuss reports in which some type of left/right statistical comparison was performed (in opposition to effects found only in one hemisphere). Nonetheless, measures of laterality in the literature are highly variable, ranging from direct left/right comparison, to calculation of laterality indices, or introduction of an "hemisphere" variable in the analyses.

5.1. Macrostructural asymmetries

Macrostructural gray matter asymmetries can be assessed using either volumetric or cortical thickness methods. Additionally, white matter tract asymmetries will also be discussed in this section.

5.1.1. **Gray matter volume**

Within volumetric assessments, segmentation methods and atlas references associated with region of interest (ROI)-based approaches vary according to study, which increases difficulty in systematization. Because ROI- and voxel-based morphometry (VBM)-based approaches have been reported to retrieve concordant data (Esteves et al., 2017), they will be discussed together in this section. Regional laterality will be discussed whenever multiple reports have shown its structural laterality or when laterality-cognition associations have been found.

5.1.1.1. **Whole hemisphere**

The overall right hemisphere volume has been reported to be larger than the left (Esteves et al., 2017; Raz et al., 2004).

In terms of sex- and age-related differences, neither has been found by either Raz (2004) or Esteves (2017) and colleagues.

Whole hemisphere total gray matter side-independent asymmetry showed an association with working memory in the digits-span test. This association was mediated by education, being positive and negative in individuals with higher and lower levels of education, respectively (Esteves et al., 2017).

5.1.1.2. **Torque and petalias**

One of the most well described asymmetries is the Yakovlevian torque. This is defined as frontal and occipital lateralized enlargements that induce an anticlockwise twisting effect with the right frontal and left occipital lobes surpassing the midline (Raz et al., 2004; Toga and Thompson, 2003). These lobes also protrude to the front and back respectively and create impressions in the interior of the skull named petalia (Good et al., 2001; LeMay, 1976; Narr et al., 2007; Raz et al., 2004; Takao et al., 2011a; Toga and Thompson, 2003; Watkins et al., 2001). Together these asymmetries lead to increased volumes (Toga and Thompson, 2003; Watkins et al., 2001; Weinberger et al., 1982) in the correspondent enlarged lobe.

No effects of sex (Good et al., 2001; Narr et al., 2007; Raz et al., 2004; Takao et al., 2011a; Watkins et al., 2001) or age (Raz et al., 2004) were found in the adult frontal-occipital asymmetry (Good et al., 2001; Narr et al., 2007; Raz et al., 2004; Takao et al., 2011a; Watkins et al., 2001)Also, when comparing fetuses and babies (20 weeks of gestation to 8 months of age) with children and adults (3 to 91 years old), no differences between groups were found (Weinberger et al., 1982).

5.1.1.3. **Planum Temporale**

The PT is located in the area of Wernicke, presents an impressive leftward asymmetry (Geschwind and Levitsky, 1968; Takami et al., 1993; Toga and Thompson, 2003; Watkins et al., 2001). In

fact, in some studies, the left PT has been reported to be up to ten times larger than the right (Geschwind and Levitsky, 1968; Toga and Thompson, 2003).

Regarding the effects of sex on PT asymmetry, data is not clear. Some authors report no effects (Chiarello et al., 2009; Sommer et al., 2008), while others have found reduced asymmetry in females (Guadalupe et al., 2015; Kulynych et al., 1994). Additionally, it has been shown that, in children (3-14 years old), females present increased asymmetry in comparison with males, despite the fact that no age effects on asymmetry have been observed (Preis et al., 1999)

This left-right difference does not show association with language dominance (Tzourio-Mazoyer et al., 2017). Similarly, total PT asymmetry is not associated with lexical performance, but posterior PT side-independent laterality was positively correlated with this task (i.e. symmetrical posterior PT associates with worst performance) (Tzourio-Mazoyer and Mazoyer, 2017). Additionally, its functional relevance is evidenced by musicians with perfect pitch, who present a higher bias (Steinmetz, 1996) mostly due to reduced right, rather than increased left volume (Keenan et al., 2001).

5.1.1.4. **Transverse temporal (Heschl's) gyrus**

The Heschl's gyrus (HG) houses the primary auditory cortex. The majority of the literature reports leftward asymmetries in terms of volume (Esteves et al., 2017; Goldberg et al., 2013; Good et al., 2001; Meyer et al., 2014; Takao et al., 2011a; Warrier et al., 2009), although others did not find left/right differences (Kulynych et al., 1994). Additionally, Marie and colleagues (2015) have reported that asymmetrical duplication, normally on the right hemisphere, is the most frequent pattern of this region (Marie et al., 2015).

Data on gender effects is not clear. While it has been reported that females present decreased asymmetry (Good et al., 2001), other studies did not find an effect of sex (Chiarello et al., 2009; Esteves et al., 2017; Goldberg et al., 2013; Kulynych et al., 1994; Takao et al., 2011a).

Regarding cognition, no associations between transverse temporal gyrus asymmetry and learning and memory, attention, working memory, cognitive flexibility/response inhibition, verbal fluency or depressive mood were found (Esteves et al., 2017). However, differential left/right function has been suggested. Warrier and colleagues (2009) showed that left but not right HG volume was positively correlated with extent of same-hemisphere activation during rapid-changing auditory

stimuli while right but not left correlation was observed with spectrally complex auditory stimuli (Warrier et al., 2009). Also in accordance with this data, Wong et al. (2008) demonstrated that individuals with increased difficulties in linguistic pitch learning, i.e. utilization of pitch patterns to distinguish word meaning, presented decreased left HG volume (note that pitch is determined by frequency of the tone and thus this data is in line with the above-mentioned study) (Wong et al., 2008).

5.1.1.5. **Superior frontal cortex**

The superior frontal cortex has commonly been reported as presenting L>R volume (Esteves et al., 2017; Goldberg et al., 2013; Good et al., 2001).

The assessment of sex and age influence in superior frontal asymmetry has not often been reported, but so far no effects of sex or age have been found (Esteves et al., 2017; Goldberg et al., 2013).

In terms of cognition, Esteves et al. (2017) found a sex-mediated association of superior frontal laterality and learning and memory in the Selective Reminding Test (Fig. 2). In females, L>R volume was associated with better performance in the task while this correlation was reversed (although non-significant) in males (Esteves et al., 2017).

5.1.1.6. **Parahippocampal gyrus**

The parahippocampal gyrus has repeatedly shown to present a leftward bias (Esteves et al., 2017; Good et al., 2001; Raz et al., 2004; Takao et al., 2011a).

To the best of our knowledge, only Esteves and colleagues (2017) reported the assessment of sex and age influence in the asymmetry of this specific region, although no effects were found (Esteves et al., 2017).

The same authors did not find any associations between parahippocampal gyrus asymmetry and learning and memory, attention, working memory, cognitive flexibility/response inhibition, verbal fluency or depressive mood were found (Esteves et al., 2017).

5.1.1.7. **Inferior parietal cortex**

R>L asymmetry has been described in the inferior parietal cortex (Esteves et al., 2017; Goldberg et al., 2013; Raz et al., 2004).

Similarly to above-described regions, no effects of sex (Esteves et al., 2017; Goldberg et al., 2013) or age (Esteves et al., 2017) were found.

Also, no associations with learning and memory, attention, working memory, cognitive flexibility/response inhibition, verbal fluency or depressive mood were found (Esteves et al., 2017).

5.1.1.8. **Lingual gyrus**

Also the lingual gyrus presents right lateralization (Esteves et al., 2017; Goldberg et al., 2013).

Few reports have explored effects of sex and age, and no influences were found (Esteves et al., 2017; Goldberg et al., 2013).

On the other hand, right ocular dominance has been associated with leftward asymmetry in a region of the inferior lateral occipital cortex (Jensen et al., 2015).

5.1.1.9. **Cingulate gyrus**

Given its high dimension and sub-regions, reports on cingulate gyrus' asymmetry vary between reports. It has been described as both right (Takao et al., 2011a) and left (Goldberg et al., 2013) lateralized, while other studies report lateralized sub-regions in both directions (Esteves et al., 2017; Good et al., 2001; Wang et al., 2013).

Regarding sex and age differences, limited data has not shown any effects (Esteves et al., 2017; Goldberg et al., 2013).

Regarding cognitive correlates, anterior cingulate cortex asymmetry was shown to be associated with aggressiveness in adolescent males, although direction of this correlation was dependent on the assessed sub-region. In the limbic and paralimbic portions, aggression was positively correlated with leftward and rightward asymmetry, respectively. This association was not found in females (Visser et al., 2014).

Esteves et al. (2017) showed an education-mediated association between posterior cingulate asymmetry and vocabulary score (Fig. 2). Individuals with higher and lower levels of education benefited from leftward and rightward biases in this region, respectively (Esteves et al., 2017).

5.1.1.10. **Orbitofrontal cortex**

The orbital frontal cortex (OFC) can be divided into its lateral and medial portions, and, although not commonly reported, both seem to be left lateralized (Esteves et al., 2017).

Similarly, neither sub-region has shown sex or age effects on laterality (Esteves et al., 2017).

As shown for the anterior cingulate cortex, greater rightward asymmetry of the OFC has been positively correlated with parent-reported aggression in teenagers (Visser et al., 2014).

5.1.1.11. **Insula**

Also the insula has shown a leftward volumetric bias (Biduła and Króliczak, 2015; Virupaksha et al., 2012).

Additionally, no effects of sex or age were found (Esteves et al., 2017).

Insula's leftward asymmetry has been demonstrated to be positively correlated with leftward biased activation of the supramarginal gyrus during gesture planning and Broca's area during silent word generation (Biduła and Króliczak, 2015). Additionally, insula asymmetry has shown a direction-independent association with increased mood in an older population (Fig. 2) (Esteves et al., 2017).

5.1.1.12. **Globus pallidus**

The globus pallidus has consistently shown a leftward asymmetry (Esteves et al., 2017; Kang et al., 2015; Wyciszkiewicz and Pawlak, 2014).

No effects of sex were found in the majority of reports (Esteves et al., 2017; Wyciszkiewicz and Pawlak, 2014). However, a large analysis of more than 15 000 individuals determined that males have increased leftward asymmetry in comparison with females (Guadalupe et al., 2016). Regarding age, no effects were found in several studies (Esteves et al., 2017; Wyciszkiewicz and Pawlak, 2014), including a large meta-analysis in which median age of the cohorts varied from 17 to 78 years old (Guadalupe et al., 2016).

No associations with learning and memory, attention, working memory, cognitive flexibility/response inhibition, verbal fluency or depressive mood were found (Esteves et al., 2017).

5.1.1.13. **Thalamus**

Also the thalamus has been reported as asymmetric, presenting L>R volume (Kang et al., 2015; Okada et al., 2016).

In terms of contributing factors, a large meta-analysis was unable to find effects of sex or age (Guadalupe et al., 2016).

Cognitive flexibility/response inhibition measured in the Stroop task showed an association with thalamus asymmetry in a sex-mediated fashion (Fig. 2). Males benefited from a leftward bias in this region, while a non-significant rightward trend was found in females (Esteves et al., 2017).

5.1.1.14. **Putamen**

Reports on putamen asymmetry have consistently shown a leftward bias in this region (Elkattan et al., 2017; Esteves et al., 2017; Kang et al., 2015; Okada et al., 2016; Wyciszkiewicz and Pawlak, 2014).

Wyciszkiewicz (2014) and Esteves (2017) and colleagues did not find an effect of sex on putamen asymmetry. However, a large meta-analysis comprising more than 15 000 subjects found increased rightward asymmetry in males (Guadalupe et al., 2016). On the other hand, effects of age are not clear. Studies have reported both an absence of effects in the 51-82 age range (Esteves et al., 2017), and an age-dependent rightward shift in the 7-21 age range (Wyciszkiewicz and Pawlak, 2014). However, a large meta-analysis in which median age of the assessed cohorts varied from 17 to 78 years old, found increased leftward asymmetry with age (Guadalupe et al., 2016).

Reports on the correlates of such asymmetry are exclusively behavioral, with human subjects in a virtual Morris Water Maze preferring to rotate in the direction of the largest putamen (Yuan et al., 2014).

5.1.1.15. **Lateral ventricle**

The lateral ventricle presents marked leftward asymmetry (Cherbuin et al., 2010; Esteves et al., 2017; LeMay, 1976; Okada et al., 2016; Toga and Thompson, 2003; Zipursky et al., 1990).

Regarding mediating factors, no effects of sex (Esteves et al., 2017) or age have been reported (Berardi et al., 1997; Esteves et al., 2017).

Esteves et al. (2017) have reported a sex- and education-mediated association with learning and memory in the Selective Reminding Test (Fig. 2), in which males and individuals with lower levels of education benefit from a rightward shift in asymmetry, while no associations are found in females and higher educated subjects. On the other hand, a benefit of side-independent asymmetry was found for working memory/executive function in the digits-span test in higher, but not lower, educated subjects (Fig. 2) (Esteves et al., 2017). Also, Berardi and colleagues (1997) determined that older individuals showed worse face than word memory in comparison with younger subjects. The association between these two memory types in the older population was correlated with lateral ventricle asymmetry, i.e. better face>word memory was associated with L>R volume and face<word memory was associated with higher symmetry (Berardi et al., 1997).

5.1.1.16. **Hippocampus**

The hippocampus has been shown to present rightward asymmetry in a variety of studies (Esteves et al., 2017; Okada et al., 2016; Rogers et al., 2012; Soininen et al., 1994; Woolard and Heckers, 2012), including a large meta-analysis (Pedraza et al., 2004), despite some contradicting reports (Good et al., 2001). Particularly for this region, it has been shown that, in rats, neonatal exposure to new environments is associated with increased asymmetry (Verstynen et al., 2001).

Esteves and colleagues (2017) found no effects of sex in an aged population (Esteves et al., 2017), but a large analysis of more than 15 000 individuals saw a trend for an effect of sex, with males having increased leftward asymmetry in comparison with females (Guadalupe et al., 2016). Regarding age, no effects of age were found (Esteves et al., 2017), including in a large meta-analysis (Guadalupe et al., 2016).

On the other hand, some behavioral associations seem to be age-dependent, with younger, but not older subjects in a virtual Morris Water Maze preferring to rotate in the direction of the largest hippocampus (Yuan et al., 2014). Additionally, several associations with memory have been found. Rightward hippocampal asymmetry has been shown to be positively correlated with overall memory performance assessed in the Screen for Cognitive Impairment in Psychiatry, mostly due to R>L volume of the anterior hippocampus (Woolard and Heckers, 2012) and, in fact, subjects with age-

associated memory impairment present decreased structural bias (Soininen et al., 1994). Asymmetry has been found to be correlated with visual memory in the Benton test (Soininen et al., 1994), immediate verbal learning and verbal fluency scores (Woolard and Heckers, 2012). Also, right hippocampal lesions determine worse performance in maze learning, object-location memory and positional memory than left lesions (no effects were found for working memory) (Kessels et al., 2001).

In rodents, this asymmetry also seems to be important, as inversus viscerum mice, who present a bilateral distribution of NR2B similar to the one in the right hemisphere of normal animals, present slower learning of a reference memory task and lower accuracy in a working memory test (Goto et al., 2010).

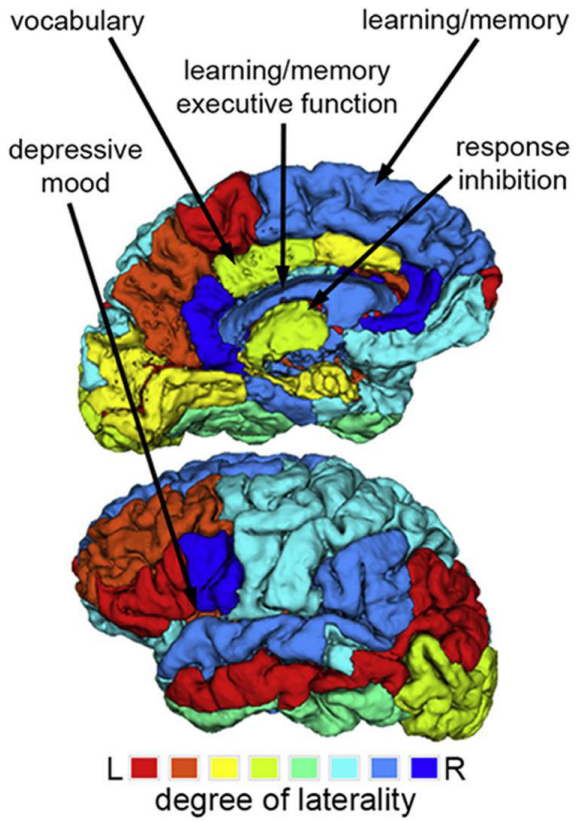

Fig. 2. Representation of hemispheric asymmetries of the older population and region-specific associations with cognition and mood. Hotter and colder colors represent increased leftward and rightward structural laterality, respectively. Insula, posterior cingulate, lateral ventricle, superior frontal and thalamus asymmetries were respectively associated with depressive mood, vocabulary, learning/memory/executive function, learning/memory and response inhibition scores respectively (Esteves et al., 2017).

5.1.2. **Cortical thickness**

Several studies have assessed cortical thickness asymmetries of the adult brain. When juxtaposing maps of these asymmetries, it seems clear that the mesial frontal cortex is the most prevalent leftward asymmetry (Chiarello et al., 2016; Kim et al., 2012; Luders et al., 2006; Plessen et al., 2014; Zhou et al., 2013). R>L thickness has been reported in the mesial occipital area (Chiarello et al., 2016; Kim et al., 2012; Luders et al., 2006; Plessen et al., 2014; Zhou et al., 2013), often extending to mesial temporal (Chiarello et al., 2016; Kim et al., 2012; Plessen et al., 2014; Zhou et al., 2013) and parietal (Kim et al., 2012; Luders et al., 2006; Plessen et al., 2014; Zhou et al., 2013) regions. Lateral frontal regions are also generally described as lateralized, although direction of lateralization is not clear, with both leftward (Kim et al., 2012; Plessen et al., 2014) and rightward (Chiarello et al., 2016; Zhou et al., 2013) asymmetries being reported.

Males and females show a similar pattern of asymmetries, although these tend to be more pronounced in males than in females. Nonetheless, there is some region-specificity, with females showing increased leftward asymmetry of the inferior temporal cortex and males presenting increased rightward bias of the inferior frontal gyrus and inferior occipital cortex (Chiarello et al., 2016; Plessen et al., 2014). Also increased age has been associated with asymmetry changes. Plessen and colleagues (2014) showed, in subjects 7-59 years old, a decrease in right thickness in the lateral surface, resulting in increased leftward asymmetry. Similarly, a decrease in leftward thickness of the mesial surface was found, leading to an increase in rightward bias (Plessen et al., 2014). On the other hand, 5-59-year-old subjects assessed by Zhou et al. (2013) showed an age-associated rightward shift in lateral inferior frontal, medial parietal and paracentral regions, as well as an increased leftward bias in lateral parietal, medial inferior and middle frontal areas (Zhou et al., 2013).

Regarding associations with cognition, R>L asymmetry of the lateral temporal lobe, posterior mesial wall and inferior surface of the brain was associated with better vocabulary scores, especially in males. These results were mostly due to an inverse correlation between vocabulary and left hemisphere cortical thickness. Similarly, working memory scores showed a positive correlation with R>L asymmetry of the posterior mesial surface, which was stronger in men (Plessen et al., 2014).

5.1.3. **White matter asymmetries**

White matter tracts as measured using tractography and diffusion-tensor imaging (DTI) have also shown lateralized features with functional correlates. We will here focus on general measures of tract diameter, volume and myelinization – fractional anisotropy (FA) -,as well as a measure of connectivity strength - number of streamlines – in major white matter tracts that have shown consistent asymmetries and/or associations of asymmetries with behavior and cognition.

5.1.3.1. **Arcuate fasciculus**

Leftward asymmetry of the arcuate fasciculus in terms of FA has been widely described (Buchel et al., 2004; Catani et al., 2007; Häberling et al., 2013; Lebel and Beaulieu, 2009; Ocklenburg et al., 2013c; Takao et al., 2011a), despite occasional reports of the absence (Allendorfer et al., 2016; Thiebaut de Schotten et al., 2011) or even inversion (Ocklenburg et al., 2014) of laterality. Leftward lateralization has also been reported for the number of streamlines (Allendorfer et al., 2016; Lebel and Beaulieu, 2009), although some authors do not find this left/right difference (Thiebaut de Schotten et al., 2011).

No effects of sex have been reported in terms of FA (Takao et al., 2011a; Thiebaut de Schotten et al., 2011) nor number of streamlines (Lebel and Beaulieu, 2009; Thiebaut de Schotten et al., 2011). Also, in this second measure, no age associations were found (Lebel and Beaulieu, 2009).

Increased leftward FA asymmetry was associated with increased functional leftward bias in the temporal lobe during silent verb generation (Ocklenburg et al., 2013c) and reading comprehension (Powell et al., 2006). A similar association was found with frontal lobe asymmetrical activation during verb generation (Powell et al., 2006). In terms of performance, however, lower FA asymmetry has been correlated with improved performance in word memory and semantic associations (Catani et al., 2007), while no associations with verbal fluency or complex ideation were found (Allendorfer et al., 2016). Similarly, asymmetries in the number of streamlines did not correlate with verbal fluency or complex ideation (Allendorfer et al., 2016). Nonetheless, children with higher leftward lateralization of the number of streamlines showed better performance in vocabulary and phonological processing tasks (Lebel and Beaulieu, 2009).

5.1.3.2. **Uncinate fasciculus**

Direction of asymmetry of the uncinate fasciculus is not clear. Studies assessing FA have found leftward (Hasan et al., 2009; Mohammad and Nashaat, 2017; Ocklenburg et al., 2013c), rightward (Madsen et al., 2012; Takao et al., 2011a; Takao et al., 2011b; Yasmin et al., 2009) and absence of laterality (Ocklenburg et al., 2014; Thiebaut de Schotten et al., 2011). To our knowledge, only one study evaluated asymmetry of the number of streamlines, without finding directional biases (Thiebaut de Schotten et al., 2011).

No effects have been found both in terms of FA in terms of sex (Mohammad and Nashaat, 2017; Takao et al., 2011a; Takao et al., 2011b; Thiebaut de Schotten et al., 2011) and age (Madsen et al., 2012). Similarly, the number of streamlines does not seem to be affected by the first (Thiebaut de Schotten et al., 2011) and second (Takao et al., 2011b) measures.

Increased FA leftward asymmetry was associated with increased functional frontal leftward bias during silent verb generation. Positive and negative associations with leftward activation in temporal regions in a passive listening task were also found, depending on the cluster of structural laterality (Ocklenburg et al., 2013c). Additionally, left and right FA were respectively positively and negatively correlated with cortisol awakening response, when modeled together but not individually (i.e. left or right volumes do not correlate with cortisol awakening response when the opposite side is not taken into account). This stress hormone response has been associated with neuroticism, but no correlation between FA and this trait was found (Madsen et al., 2012).

5.1.3.3. **Cingulum**

FA of the cingulum has been widely described as left lateralized (Gong et al., 2005; Madsen et al., 2012; Ocklenburg et al., 2013c; Takao et al., 2011a; Takao et al., 2011b; Yin et al., 2013), although Thiebaut de Schotten and colleagues (2011) have found no asymmetries both in terms of FA and number of streamlines (Thiebaut de Schotten et al., 2011).

No sex or age effects have been detected in terms of FA ((Takao et al., 2011a; Takao et al., 2011b; Thiebaut de Schotten et al., 2011) – sex; (Madsen et al., 2012) – age) or number of streamlines ((Thiebaut de Schotten et al., 2011) – sex; (Takao et al., 2011b) – age).

As shown for the uncinate fasciculus, left and right FA were respectively negatively and positively correlated with cortisol awakening response, when modeled together but not individually. Left and right FA were negatively and positively associated with neuroticism, respectively, when the two hemispheres were modeled together, but not individually (Madsen et al., 2012).

5.1.3.4. **Corpus callosum**

FA asymmetries of the corpus callosum seem to be region-dependent, with frontal (Ardekani et al., 2007), lateral (Takao et al., 2011b) and posterior (Ardekani et al., 2007; Takao et al., 2011a; Takao et al., 2011b) regions showing R>L FA, (Ardekani et al., 2007) while the medial level presents L>R asymmetry (Takao et al., 2011b). Direction of asymmetry of the body area is not clear and has been described both as right (Ardekani et al., 2007) and leftward (Ocklenburg et al., 2013c) biased.

No sex effects in the adult brain have been found (Takao et al., 2011a; Takao et al., 2011b). However, boys exposed in utero to higher levels of testosterone showed increased R>L asymmetry in the posterior corpus callosum (Chura et al., 2010), which may indicate a role of sexual hormones in the development of these asymmetries. Regarding age, the effects are not clear. Takao and colleagues (2011b) found no effects in a 25-85-year-old population (Takao et al., 2011b). On the other hand, Ardekani et al. (2007) found that middle-aged individuals (43-69 years old) presented reduced asymmetries in comparison with younger subjects (26-36 years old) (Ardekani et al., 2007).

In terms of associations with behavior and cognition, to the best of our knowledge, no associations have been found.

5.1.3.5. **Corona radiata**

The posterior corona radiata has been shown to present rightward asymmetry of FA (Takao et al., 2011a; Yin et al., 2013), while the superior region seem to present the opposite trend (Takao et al., 2011a).

Regarding mediating factors, no effects of sex on FA have been detected (Takao et al., 2011a) and no reports of age effects were found.

In terms of cognition, FA leftward asymmetry of inferior anterior corona radiata has shown a positive correlation with executive control of attention assessed in the attention network test (Yin et al., 2013).

5.2. Microstructural asymmetries

Post-mortem histological data on cytoarchitectonic asymmetries includes minicolumn organization, cell density or neuronal branching. However, the number of studies is reduced and the relevance for cognition and behavior is usually not explored. We will here provide a brief overview, in which we show the available data for each region.

5.2.1. **Planum Temporale**

Human data has evidenced that a higher number of minicolumns in the left PT (Chance et al., 2006), with increased width and neuropil space (Buxhoeveden et al., 2001), as well as measures that comprise both (Chance et al., 2006), are present on the left that on the right hemisphere. Similarly, cells on the left PT seem to be larger (Hutsler, 2003) and more linearly packed (Buxhoeveden et al., 2001) than on the right.

5.2.2. **Broca's area**

Broca's area presents neuronal branching asymmetry: while neurons on the left hemisphere present mostly higher order branches, right neurons are mostly constituted by lower order segments (Scheibel et al., 1985). This region is constituted by Brodmann's areas 44 and 45. In area 44, cell density (Amunts et al., 1999), number of neurons and volume (Uylings et al., 2006) are higher on the left hemisphere (although the latter was only shown in males). On the other hand, area 45 did not show asymmetries in any of these measures (Amunts et al., 1999; Uylings et al., 2006).

5.2.3. **Heschl's gyrus**

The amount of large layer III pyramidal cells was found to be larger on the left hemisphere (Hutsler, 2003), but no asymmetries in columnar spacing has been found (Chance et al., 2006).

5.2.4. **Fusiform gyrus**

The left fusiform gyrus has been shown to present wider minicolumns and larger pyramidal cells than the right. No asymmetries were found regarding size of non-pyramidal cells or cell density (Chance et al., 2013).

5.2.5. **Hippocampus**

In humans, CA2, but not CA1, CA3, CA4 and subiculum pyramidal neurons were found to be larger in the left than in the right side. On the other hand variability of neuronal orientation was higher on the left CA3 and right subiculum, when comparing with the opposing hemisphere.(Zaidel et al., 1997) No asymmetries of neuronal shape were observed (Zaidel et al., 1997). In rats, no equivalent parameters were measured, however higher cell number in left CA1 and CA2/3 regions than on the right has been described, while no effects on dentate gyrus or subiculum (Lister et al., 2006). Such data is in accordance with the higher pyramidal cell density found in the rat's left CA3, in comparison with the right (Ragbetli et al., 2002).

6. Functional asymmetries

Activation of brain regions often occurs in an asymmetrical fashion. Such can be assessed at resting-state or during task performance and both these measures have been associated with cognitive and behavioral performance.

6.1. Resting-state

Resting-state data, attained using either fMRI or EEG, can provide useful information regarding basal activity of the brain. Interestingly, in many cases, it is associated with cognition and behavioral dimensions. This section will be restricted to human reports.

6.1.1. **fMRI**

Functional connectivity has been described as over 95% symmetric (Raemaekers et al., 2018). Nonetheless, a number of asymmetries have been described, and these seem to be network dependent. The language network is mostly left-lateralized (Liu et al., 2009; Nielsen et al., 2013), although it has also been reported that, while Broca's area is left lateralized, Wernicke's region is rightward biased (Zhu et al., 2014). Similarly, the default mode network includes both left and right

biased regions (Joliot et al., 2016; Saenger et al., 2012), but it can be mostly seen as left-lateralized (Agcaoglu et al., 2015; Liu et al., 2009; Nielsen et al., 2013). Visual and attentional regions, on the other hand, are mostly right-lateralized (Agcaoglu et al., 2015; Liu et al., 2009; Nielsen et al., 2013; Yan et al., 2009), and sensorimotor networks include both right and left-biased areas (Agcaoglu et al., 2015; Dinomais et al., 2016; Joliot et al., 2016).

Left-right differences in terms of intra- and inter-hemispheric functional connectivity have also been reported. fMRI functional connectivity analysis showed that the left hemisphere presents stronger associations within itself rather than with the opposite hemisphere. These included regions related with language, social processing and communication and portions of the somatosensory and motor cortices. On the other hand, the right hemisphere mainly shows inter-hemispheric associations including regions related to visuospatial and attentional processing (Gotts et al., 2013).

Sex has shown a small but significant effect in functional connectivity asymmetries, with males showing, in general, higher lateralization than females (Agcaoglu et al., 2015; Liu et al., 2009; Tomasi and Volkow, 2012). Additionally, age has been shown to decrease functional connectivity asymmetry of sensorimotor, visual and frontal networks (Agcaoglu et al., 2015).

Different measures of resting-state asymmetry have been associated with different cognitive domains. Regions that are asymmetrically activated during semantic processing have similar (left) blood-oxygen-level dependent (BOLD) signal laterality in resting-state (McAvoy et al., 2016). Additionally, (2013)left segregation (i.e. higher intra-hemispheric associations) of language-related regions was associated with increased vocabulary score (Gotts et al., 2013). Such is in accordance with recent data showing that higher association between left language areas (as opposed to intra-hemispheric correlations on the right) was correlated with left lateralization during a language task (Raemaekers et al., 2018). This latter work also reported that higher correlation between left language regions and right default mode network further contributed for the association with language lateralization (Raemaekers et al., 2018).

In terms of visuospatial abilities, higher inter-hemispheric connectivity of visuospatial areas has been shown to correlate with block design score (Gotts et al., 2013). On the other hand, when comparing average-IQ and above average-IQ subjects, the first showed higher homotopic connectivity of visual and somatosensory cortices, supplementary motor area, rolandic operculum, and middle temporal gyrus (Santarnecchi et al., 2015).

6.1.2. **EEG**

6.1.2.1. Theta (4-7 Hz)

Economic risk-taking was positively correlated with resting-state theta rightward asymmetry, although this association was stronger in subjects with higher BIS (Behavioral Inhibition System) scores (i.e. higher sensitivity to punishment). Together, theta asymmetry in the prefrontal cortex (PFC) and BIS explained 25% of the risk-taking variance (Studer et al., 2013).

6.1.2.2. Alpha (8-12 Hz)

Alpha frontal rightward asymmetry has been associated with increased aggression levels both in the general population (Hofman and Schutter, 2012) and in imprisoned violent offenders (Keune et al., 2012), mainly associated with hostility (Hofman and Schutter, 2012), verbal aggression (Keune et al., 2012) and callousness (Keune et al., 2018) (although this last study did not replicate the effects on aggression). It has also been associated with higher susceptibility to social influence (Schnuerch and Pfattheicher, 2017) and increased anxiety, while no associations with BAS (Behavioral Approach Scale) or the fight-flight-freeze system were found (Neal and Gable, 2017). On the other hand, impulsivity has shown a positive correlation with leftward frontal asymmetry (Neal and Gable, 2017), which seems to be due to an association between laterality and sub-scales of this cognitive domain, i.e. leftward bias associates with decreased sensation seeking but increased lack of premeditation, lack of perserverance, and positive and negative urgency (Neal and Gable, 2016).

Parieto-occipital (van Bochove et al., 2016) and frontal (De Winter et al., 2015) leftward bias in alpha has also been associated with higher hedonic valuation of food, while whole hemispheric asymmetry in healthy subjects has been associated with trait approach motivation (Brookshire and Casasanto, 2012), elevated obsessive-compulsive symptoms and reduced trait anxiety but low worry (Smith et al., 2016). Curiously, while right-handed individuals showed a higher approach motivation with increased leftward asymmetry, left-handed subjects showed the opposite correlation (Brookshire and Casasanto, 2012).

6.1.2.3. Beta (13-30 Hz)

Rightward frontal beta asymmetry has been associated with higher aggression (especially in the hostility subscale) and higher percentage of errors in an impulsivity (Go/noGo) task (Hofman and

Schutter, 2012). Leftward parieto-occipital lateralization is positively correlated with hedonic valuation of food (van Bochove et al., 2016).

Additional, left lateralization of the beta/alpha ratio in the prefrontal cortex has been associated with improved inhibition of irrelevant information in a Stroop task (Ambrosini and Vallesi, 2017). On the other hand, left and right asymmetry in the middle frontal gyrus was positively correlated with ability exert phasic and sustained cognitive control, respectively (Ambrosini and Vallesi, 2016).

6.2. Task-related

Assessment of brain activity during task performance provides the most direct measure of region involvement in behavior. In this section, we will review data that as been mostly obtained using fMRI, PET (Positron Emission Tomography) or EEG, although complementary information regarding lesion studies or behavioral asymmetries that can add to the discussion has also been included. The prime model in this section is the human, although relevant data in rodent models and others is included.

6.2.1. **Memory**

It has been hypothesized that functional memory asymmetry is material-specific (i.e. verbal/non-verbal). Frontal activation has been shown to be left-lateralized during encoding and retrieval of verbal stimuli (Kelley et al., 1998; Wagner et al., 1998), right-lateralized for non-verbal stimuli (Kelley et al., 1998; Wagner et al., 1998) and bilateral during presentation of namable objects (Kelley et al., 1998). Similar verbal/non-verbal recognition in association with respectively left/right activations has been found in the medial temporal cortex (Dalton et al., 2016).

On the other hand, the hemispheric encoding/retrieval asymmetry (HERA) model states that the involvement of the PFC in episodic memory is asymmetric while complementary. The left PFC is involved in episodic memory encoding while the right is involved in retrieval (Babiloni et al., 2006; Tulving et al., 1994). Regarding incompatibilities with the above-mentioned material-specific lateralization, Habib and colleagues (2003) suggest that these are mainly due to differences in calculation and that, when directly comparing encoding and retrieval processes, the HERA model is sustained over both verbal and non-verbal stimuli (Habib et al., 2003).

Animal data also seems to support the HERA model for encoding and retrieval of spatial memory, applied to the hippocampus. Mice subjected to callosotomy and forced to use only the left eye have better accuracy during retrieval than animals forced to use the right eye (Shinohara et al., 2012). Additionally, spatial memory is impaired by reversible inactivation of the mouse right (but not left) or bilateral hippocampus before retrieval, but not acquisition (Klur et al., 2009).

On the other hand, left (but not right) and bilateral inactivation before each acquisition session also impairs spatial memory (Klur et al., 2009), although data on callosotomized animals does not support this idea, as no differences were found in acquisition (Shinohara et al., 2012). Additionally, optogenetic silencing of left (but not right) CA3 during acquisition of long-term memory, has been shown to impair performance (Shipton et al., 2014). This has been attributed to a higher ability for long-term potentiation induction in CA3-CA1 synapses when input originates from the left CA3 (Kohl et al., 2011; Shipton et al., 2014).

In terms of sex, no effects have been described. However, several theories regarding the lateralization pattern throughout aging have arisen. Cabeza's Hemispheric Asymmetry Reduction in Older Adults (HAROLD) model suggests that brain activity tends to be less lateralized in older individuals. This has mainly been shown for performance in memory-related tasks, but the model also has potential applications in other cognitive domains at simple (such as face matching) and complex (e.g. inhibitory control) levels. Additionally, while HAROLD construction was mostly based in PFC activation, the authors did not exclude a potential application in other regions (Cabeza, 2002). This HAROLD effect seems to be present in high-, but not low-performing older individuals during a memory task (Cabeza et al., 2002), as well as correlate with better repetition priming and semantic processing (Bergerbest et al., 2009), which suggests that this lateralization reduction is a compensatory mechanism for age-related decrease in efficiency, allowing maintenance of cognitive performance.

On the other hand, a recent study has shown that, in older subjects, asymmetric (leftward) activation of the superior parietal lobule during performance of a working memory task (N-Back) correlated with higher accuracy (Esteves et al., 2018), suggesting that the HAROLD model may not apply to all regions. Additionally, in older adults with comparable memory performance to younger adults, bilateral hippocampal activation has been shown to be reduced and negatively correlated with activation of the middle frontal cortex. This suggests that the middle frontal cortex is being involved as compensation for the reduced activation of the hippocampus due to aging (Gutchess

et al., 2005). Thus, the Compensation Related Utilization of Neural Circuits Hypothesis (CRUNCH) states that additional resources are recruited in older adults in order to attain similar performance (Reuter-Lorenz and Cappell, 2008), irrespectively of hemisphere. Additionally, this phenomenon can also be seen in younger individuals, with increased activations associated with higher memory loads (Cappell et al., 2010), supporting the idea of compensatory activations.

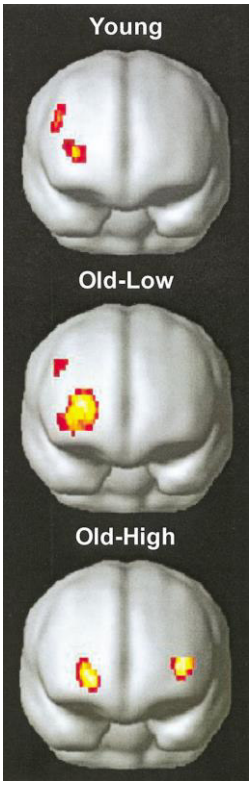

Fig. 3. Depiction of the HAROLD model. Older individuals with high memory performance (bottom) show bilateral PFC activation as opposed to younger (top) and older individuals with decreased memory performance (middle) (Cabeza et al., 2002).

6.2.2. **Language**

Multiple works have attributed language production to the Broca's area, in the anterior left region, while language comprehension occurs in the area of Wernicke in the left posterior-parietal region (Toga and Thompson, 2003). Westerhausen and colleagues (2014) for example, assessed fMRI in a common paradigm for assessment of language lateralization, the dichotic listening task. In this task, consonant/vowel sounds are simultaneously delivered to the left and right ears. The authors saw symmetrical activation in temporal, parietal, inferior frontal, and medial superior frontal regions, L>R activations in posterior superior temporal gyrus and exclusive left activation in the

peri-Sylvian region, post-central and medial superior frontal gyri. Despite this essentially left-biased pattern, R>L activations were also found in middle temporal and middle frontal gyri (Westerhausen et al., 2014). Similarly, Vigneau et al. (2011) saw that most activation peaks associated with language processing occurred on the left hemisphere, while the homotopic right region did not show activation. On the other hand, the majority of activation peaks that were found in the right hemisphere were also present in the left hemisphere, suggesting that the right hemisphere role in language processing is close to the dominant left hemisphere (Vigneau et al., 2011). Interestingly, this lateralization seems to be evolutionarily conserved, as it has been found in frogs (Bauer, 1993), canaries (Halle et al., 2003) and dogs (Andics et al., 2016) (see section 2.1.3)

When assessing verbal comprehension in individuals right- and left-dominant for language, no differences were found (Powell et al., 2012). However, it has been suggested that ear advantage in dichotic listening performance is U-shaped, meaning that higher lateralization is associated with higher accuracy independently of direction (Hirnstein et al., 2013a). Accordingly, Mellet et al. (2014) saw that subjects without clear language lateralization as assessed with fMRI showed disadvantage in verbal domains when comparing with both left- and right- lateralized subjects (Mellet et al., 2014). Additionally, language dominance may affect other domains, as Powell and colleagues (2012) showed that right verbal lateralization was associated with reduced working memory (Powell et al., 2012).

Nonetheless, left dominance for language does not exclude some right language-related capacities. Although the right hemisphere of split-brain patients was not able to name objects pictured in cards, when asked to match the ones that rhymed or the ones that were homonyms, it maintained that capacity. However, these individuals were unable to do this type of matching between written words or between a written word and a picture. This suggests that the right hemisphere is able to directly recognize words but not to produce grapheme-to-phoneme processing (Zaidel and Peters, 1981). On the other hand, it is also possible to have independent written and spoken language. This was shown by a left-handed split-brain patient who was left-dominant for language. She was able to produce spoken language from her left but not right hemisphere, but was only able to produce written language (with her left hand) from her right but not left hemisphere (Baynes et al., 1998).

Further evidence for the complexity of left/right balance in language processing comes from one study that assessed event-related potentials (ERPs) using low-resolution electromagnetic tomography during a lexical decision task. These authors determined that, while there is an initial

peak of activity on the left hemisphere (which is higher than on the right), subsequent peaks over a 300 ms time-course subsequently alternate between R>L and L>R activation, suggesting complementary processing of the two hemispheres (Sinai and Pratt, 2003). Additionally, it has been shown that a specific Turkish language, which is based in whistling sounds is asymmetrically processed (Güntürkün et al., 2015), which may be related with the left year (right hemisphere) advantage for musical processing (Hugdahl et al., 1999).

Regarding sex, the majority of data does not show effects on dichotic listening (Sommer et al., 2008) or BOLD lateralization (Hirnstein et al., 2013b; Nenert et al., 2017; Powell et al., 2012; Sommer et al., 2008). However, in a large dataset, Hirnstein and colleagues (2013b) showed that sex differences in the degree of right ear advantage were age-dependent. While there were no differences in children and adults, adolescent females showed increased lateralization than their male counterparts (Hirnstein et al., 2013b). These sex effects may be associated with hormonal levels. In fact, females have been shown to exhibit decreased side-independent ear advantage when estradiol levels are high (Hodgetts et al., 2015). Also effects of age on language-related laterality are not clear. BOLD data has shown both general maintenance (Powell et al., 2012) and decrease in asymmetry (Berlingeri et al., 2013) with aging, as well as a specific decrease in right-handed men (Nenert et al., 2017). Additionally, right ear advantage has been reported to increase with age, although this was mainly due to an increase that occurred from childhood/adolescence to adult ages, while no differences were found within adults (Hirnstein et al., 2013b).

6.2.3. **Pseudoneglect**

The majority of the population shows pseudoneglect in visuo-spatial tasks, e.g. when trying to determine the midpoint of a straight horizontal line, subjects tend to identify a point that is slightly to the left (Jewell and McCourt, 2000; Zago et al., 2017; Zago et al., 2016). These tasks have been shown to be associated with rightward asymmetries in brain activations, more specifically in the frontal, parietal and occipital lobes (Badzakova-Trajkov et al., 2010; Zago et al., 2017; Zago et al., 2016), which has elicited hypotheses regarding complementarity between language (left) and visuo-spatial (right) lateralization (Badzakova-Trajkov et al., 2010; Bryden and Bulman-Fleming, 1994). Such idea is supported by subjects who show atypical (right) dominance for language with complementary atypical (left) dominance for visuo-spatial stimuli (Cai et al., 2013; Powell et al., 2012), but, on the other hand, evidence of a significant correlation between these two asymmetries

is weak. Most authors do not find such quantitative association (Powell et al., 2012; Zago et al., 2017), while others can only find it in a small subset of the population (Zago et al., 2016).

Level of asymmetry does not seem to be associated with response time (Cai et al., 2013), but a weak correlation with error has been shown (i.e. increased relative rightward activation is associated with increased behavioral leftward bias) (Zago et al., 2017). This is consistent with data showing that right parietal lesion is associated to left neglect syndrome, i.e. the inability to acknowledge or manipulate an object that is within the individual's visual field (Brain, 1941; Halligan et al., 2003). Importantly, higher dissociation between language and visuo-spatial activation asymmetry has been associated with improved verbal comprehension and perceptual organization (Zago et al., 2016).

Sex does not seem to have an impact in pseudoneglect (Zago et al., 2017; Zago et al., 2016) (although a small increase in males has also been reported (Jewell and McCourt, 2000)), nor in the corresponding asymmetry in brain activation (Powell et al., 2012). Regarding age, older individuals have been reported to show an increased rightward bias in bisection tasks (Jewell and McCourt, 2000), although no changes in brain lateralization have been reported (Powell et al., 2012).

6.2.4. **Emotion and approach/withdrawal**

It is a prevailing idea in the literature that pleasant emotions are mainly processed on the left hemisphere while unpleasant ones are processed on the right hemisphere. This is consistent with fMRI data showing left and right dominant activations during viewing of emotionally positive and negative valenced images, respectively (Beraha et al., 2012; Canli et al., 1998). However, when emotional stimuli were auditive rather than visual, right lateralization was seen in the inferior parietal and dorsolateral PFC, regardless of valence (Wildgruber et al., 2002). Similarly, data on manipulation using non-invasive brain stimulation is not clear. Mondino and colleagues (2015) reviewed literature that showed lack of evidence of left PFC stimulation effect on mood (Mondino et al., 2015). On the other side, meta-analyses of randomized control trials utilizing repetitive transcranial magnetic stimulation (rTMS) (Schutter, 2009) and transcranial direct current stimulation (tDCS) (Brunoni et al., 2016) for enhancement of left PFC activity showed improvements in depressive symptoms (effects of drug resistance and "doses" depended on the study). Additionally, neurofeedback training was able to increase left PFC activity (and thus leftward

asymmetry), as well as improve depressive symptoms and executive function in depressive patients (Choi et al., 2011) and increase positive response (rating and biometrical) to emotionally positive and neutral films in healthy subjects (Allen et al., 2001).

Similarly, it is also frequently reported that left and right PFC differentially code approach and avoidance motivation respectively (Kelley et al., 2017). At first glance, these observations suggest an association with the above-mentioned lateralization of emotional valence, as approach would be directed at "happy" stimuli and avoidance would be directed at "sad" stimuli. While these two ideas are indeed impossible to separate in the majority of the literature, Berkmann and Lieberman (2010) were able to dissociate these two concepts. Participants were asked to memorize details of an article about a tribe believing that they would be performing a memory task. This tribe was said to enjoy eating insects and be disgusted by meat while their remaining tastes were similar to western's (liking desserts and being disgusted by fungus-contaminated foods). During fMRI acquisition pictures were shown and subjects were asked to determine if a tribe member would eat that food. Results showed greater left biased activation of dorsolateral PFC (but not orbitofrontal cortex) during approach than during avoidance regardless of stimulus valence (Sutton and Davidson, 1997).

Similarly, a leftward bias was found for anger-inducing pictures, which cannot be considered a "positive" emotion (Gable and Poole, 2014) and induction of R>L dorsolateral PFC asymmetry through non-invasive brain stimulation has been shown to reduce craving for food (Fregni et al., 2008; Goldman et al., 2011). A large meta-analysis also partially supports this idea, showing L>R asymmetries for approach, happiness and anger (no effects were found for withdrawal emotions) (Murphy et al., 2003). On the other hand, approach motivation induced by erotic pictures induces the opposite (rightward) frontal asymmetry (Schone et al., 2016), while other authors did not find asymmetries in approach-avoidance activations (Uusberg et al., 2014). Such contradicting results may be partially explained by non-controlled mediating effects. In fact, approach motivation (BAS) has been associated with increased approach-related left-lateralization (Berkman and Lieberman, 2010; Gable and Poole, 2014; Sutton and Davidson, 1997). Similarly, subjects with higher approach learning have shown L>R reward response (relative to punishment) in the NAcc (Aberg et al., 2015).

Lateralized lesions of the ventromedial PFC were shown to impair emotional processing in a sex-dependent manner, i.e. clear changes were seen in men and women after right and left lesion,

respectively (Tranel et al., 2005). Regarding specifically the response to fearful faces, laterality of the emotional response seems to be age-specific. In males, dorsolateral PFC activation was bilateral in children and adults, but right-lateralized in adolescents, while females decreased laterality with age (Killgore and Yurgelun-Todd, 2004).

6.2.5. **Impulsivity and risk-taking**

Impulsivity has been defined as a tendency to act prematurely and without foresight (Dalley et al., 2011). It comprises several dimensions, although the tendency towards choosing immediate/smaller over delayed/larger gains (Dalley et al., 2011) associates with drug-taking and risky decision-making. A meta-analysis of fMRI studies during impulsivity and drug craving tasks showed that impulsivity shows overall rightward biases for both Go/noGo and stop signal tasks, especially in anterior regions (Gordon, 2015). Drug craving, on the other hand, is associated with leftward asymmetries independently of drug type (Gordon, 2015). In light of the discussed approach and avoidance theory, these directional differences may be explained by the inhibition of impulsive responses (avoidance – right hemisphere) and approach motivation in drug craving (approach – right hemisphere).

Similarly, left alpha frontal asymmetry has been associated with increased risk-taking behavior (Telpaz and Yechiam, 2014), and right stimulation was shown to decrease risk-taking. Healthy individuals were shown to select the safe options more frequently than when the opposite stimulation was performed (Fecteau et al., 2007; Ye et al., 2015; Ye et al., 2016), while the opposite effect is seen when disrupting right activity (Knoch et al., 2006).

Data regarding unilateral lesions of the ventromedial PFC has highlighted a sex effect in laterality of risk taking. Men and women present reduced aversion to risk after right- and left-hemisphere damage, respectively (Sutterer et al., 2015; Tranel et al., 2005). To our knowledge, no effects of age have been reported.

6.2.6. **Face and word recognition**

The fusiform face and the visual word form areas, which are in close proximity within the fusiform gyrus, have been suggested to be complementarily lateralized to the right and left hemispheres, respectively (Dien, 2009). In fact, it has been shown that words are better recognized when presented to the right visual field (Leehey and Cahn, 1979; Vakil and Liberman, 2016), while faces'

recognition is improved in the opposite hemispace (Leehey and Cahn, 1979; Prete et al., 2015; Vakil and Liberman, 2016). Similarly, electrophysiological and fMRI data have associated word recognition with leftward activation (Izura et al., 2014) and face processing with rightward involvement (Badzakova-Trajkov et al., 2010; Yovel et al., 2008; Zhen et al., 2015).

Particularly regarding face recognition, the left fusiform seems to present "lower level" function (i.e. its activation gradually increases as images increase resemblance to faces) while right fusiform activation is associated with the decision (i.e. its activation in only increased with images that are ultimately decided to be faces) (Meng et al., 2012).

Callosal transfer in face recognition has been shown to be faster in left visual field presentations (i.e. right to left hemisphere) in males, than in the opposite direction. On the other hand, females did not present asymmetries in direction of conduction (Proverbio et al., 2012). Similarly, women in the lutheal fase (i.e. higher hormonal levels) did not show a left visual field advantage for face discrimination, which was present in females during the menses (i.e. lower hormonal levels), men and post-menopausal women (Hausmann and Gunturkun, 2000). In terms of age, comparison of EEG data from young and older adults showed that face processing's rightward asymmetry is lost with age (Daniel and Bentin, 2012).

7. Molecular asymmetries

Molecular asymmetries will be here described for regions in which more than one report has shown left/right differences at this level. These mostly include post-mortem and neurotransmitter manipulation data in rodents, as well as post-mortem and PET human data, showing asymmetries in serotonin (5-HT) and dopamine (DA) concentrations, metabolites and receptors. However, relevant data regarding additional asymmetries of the hippocampus is also included, and human data is reported when available.

7.1. Medial frontal cortex

7.1.1. 5-HT

In a PET study in human subjects has shown L>R 5-HT transporter binding in the superior medial frontal gyrus (Kranz et al., 2014). On the other hand, post-mortem levels of 5-HT and its major metabolite, 5-Hydroxyindoleacetic acid (5-HIAA), were found to be higher on the right medial frontal cortex (Arato et al., 1991).

7.1.2. DA

In humans, higher PET-assessed D2/D3 receptor binding on the left medial frontal has been correlated with higher sensitivity to positive vs negative feedback (i.e. better learning from reward than punishment feedback) (Tomer et al., 2014).

In rodent models, asymmetry of the ventromedial dopaminergic system has been associated with stress response. Unilateral DA depletion in the infralimbic cortex of non-handled animals induced an increase in adrenocorticotropic hormone (ACTH) and cortisol (CORT) levels (relative to sham) in response to acute exposure to this stressor independently of lesion side. On the other hand, a similar response in perinatally handled animals was achieved after right, but not left lesion. Similarly, post-mortem high-performance liquid chromatography (HPLC)-measured asymmetry of infralimbic 3,4-Dihydroxyphenylacetic acid (DOPAC) levels after chronic stress is dependent on handling. While handled animals show R>L levels, non-handled animals present inversion of this asymmetry (Sullivan and Dufresne, 2006).

Other factors, such as sex, contribute to ventromedial asymmetries' role in response to stress or anxiety. Using fast-scan cyclic voltammetry (FSCV), Sullivan and colleagues (2009) revealed that acute stress (predator odor or tail pinch) induces higher dopamine release in right and left infralimbic cortex in males and females, respectively (Sullivan et al., 2009). Also, DA depletion in the right, but not left, ventromedial prefrontal cortex increased/decreased risk assessment behavior in males and females, respectively, while anxiety-like behavior in the elevated plus maze (EPM) task was decreased by left lesion in females and increased by right lesion in males. Burying behavior in response to predator odor on the other hand, did not show sex differences, being increased in both males and females after left lesion (Sullivan et al., 2014).

7.2. Orbitofrontal cortex

7.2.1. 5-HT

Human 5-HT receptor asymmetries assessed in vivo, using PET, in the orbitofrontal cortex seem to be region-dependent. L>R levels have been found in the middle and inferior orbitofrontal gyrus, while the superior orbitofrontal cortex presents R>L bias (Kranz et al., 2014).

7.2.2. DA

Higher D2 receptor binding assessed with PET on the human left orbitofrontal cortex was correlated with higher sensitivity to positive vs negative feedback (i.e. better learning from reward than punishment feedback) (Tomer et al., 2014).

7.3. Middle frontal gyrus

7.3.1. 5-HT

In the middle frontal gyrus, 5-HT transporter presents L>R asymmetry in living humans (PET) (Kranz et al., 2014).

7.3.2. DA

PET analysis revealed an association between L>R D2 receptor binding in humans and stronger rightward orienting bias (i.e. bias towards choosing as overall darker a grayscale in which the darker side is on the right) (Tomer et al., 2013) and self-reported motivation in the BIS/BAS scale (Tomer et al., 2014).

7.4. Caudate

7.4.1. 5-HT

In humans, in vivo PET showed higher rightward asymmetry of 5-HT receptor binding in males than in females (Kranz et al., 2014).

7.4.2. DA

In humans, using PET, no asymmetries in dopaminergic activity were found, although increased rightward bias was associated with higher ability to perform bimanual movements (de la Fuente-Fernandez et al., 2000).

On the other hand, there seems to be a L>R asymmetry in human DA transporter (single photon emission computed tomography – SPECT) (van Dyck et al., 2002) and PET-measured D2/3 receptor availability has been described as right lateralized, although the latter decreases with age (Vernaleken et al., 2007). An increase in this receptor asymmetry has also been associated with stronger leftward orienting bias (Tomer et al., 2013).

7.5. Putamen

7.5.1. 5-HT

In humans, the 5-HT transporter levels have been described as lateralized to the right, when assessed in vivo using PET (Kranz et al., 2014).

7.5.2. DA

No PET-measured human DA uptake (de la Fuente-Fernandez et al., 2000) or D2/D3 receptor availability (Vernaleken et al., 2007) asymmetries have been found. On the other hand, SPECT analysis revealed leftward lateralization of DA transporter levels (van Dyck et al., 2002).

This neurotransmitter's relative uptake seems to be increased on the preferred hand's contralateral side (de la Fuente-Fernandez et al., 2000). Regarding D2/D3 PET-assessed receptor binding, a leftward bias has been associated with lower body mass index (BMI) (Cho et al., 2015), stronger rightward orienting bias (Tomer et al., 2013), higher sensitivity to positive vs negative feedback (Tomer et al., 2014) and higher achievement scores (associated with higher motivational disposition that comprises social dominance, enthusiasm, energy, assertiveness, ambitiousness, and achievement striving) (Tomer et al., 2008). All data refers to human measures.

7.6. Nucleus accumbens

7.6.1. 5-HT

Post-mortem HPLC measurements in rats have suggested that the right hemisphere presents higher 5-HT levels, while the left hemisphere shows higher 5-HT turnover (Rosen et al., 1984).

7.6.2. DA

Rodent post-mortem DA levels (Rosen et al., 1984) and D2 receptor binding (Adrover et al., 2007) have been shown to be higher on the right hemisphere, while D2, but not D1, receptor density is leftward biased (Giardino, 1996). Additionally, associations with pawedness have been found, with DA (Budilin et al., 2008; Cabib et al., 1995) and its metabolites DOPAC and 3-Methoxytyramine (3-MT)'s (Cabib et al., 1995) levels being higher on the side of the preferred paw.

7.7. Amygdala

7.7.1. 5-HT

In rats, higher post-mortem HPLC-assessed rightward asymmetry of 5-HT levels has been positively correlated with anxiety in the EPM task. (Andersen and Teicher, 1999).

7.7.2. DA

Stress response has been associated with DA asymmetry, although this relation seems to be mediated by sex. Acute stress has been shown to induce higher DA FSCV-assessed release in right and left hemisphere of males and females, respectively (Sullivan et al., 2009).

7.8. Hippocampus

Hippocampal cellular and molecular asymmetries have been recently reviewed by El-Gaby and colleagues (2015) and Shinohara and Hirase (2009). Nonetheless, an overview of this data is here provided.

7.8.1. 5-HT

In humans, a PET-measured leftward asymmetry of the 5-HT transporter has been described (Kranz et al., 2014). Additionally, L>R 5-HT1A receptor binding has been associated with better spatial memory (Glikmann-Johnston et al., 2015).

7.8.2. DA

In rats, post-mortem HPLC DA levels have been shown to be higher on the side ipsilateral to the animal's preference of turning in the T-maze (Diaz Palarea et al., 1987).

7.8.3. Glutamate

Histological data in mice has shown that CA1 spines that receive input from right CA3 have higher post-synaptic density (PSD) area, higher head volume and are more likely to be mushroom-shaped than spines with left inputs. PSD of contacts from the right also have higher synaptic density of GluR1, which increases with the size of the synapse. On the other hand, NR2B's concentration is higher when input is from the left and reduces with synapse size as its amount is maintained relatively constant (Kawakami et al., 2003; Shinohara et al., 2008). Additionally, CA3 input

produces L>R long-term potentiation at CA1 as a consequence of differential expression of NMDA receptors containing GluN2B subunits (Kohl et al., 2011).

7.8.4. Gene expression and proteomic analysis

Proteomic analysis of the rat hippocampus revealed asymmetry of several protein levels. R>L concentrations were mainly found for proteins associated with metabolism, while L>R levels occurred mostly in proteins that are mainly present in astrocytes (Samara et al., 2011). Regarding gene expression, genes involved mainly in signaling, transport and metabolism were shown to be modulated by a spatial reference memory task, especially in the right hemisphere (Klur et al., 2009).

8. Concluding remarks

The field of brain laterality has been until the present day greatly influenced by language asymmetries. In fact, these brain biases have repeatedly shown cognitive and behavioral correlates, solidifying their place as hallmarks of laterality. Nonetheless, multiple reports have shown that the brain is remarkably and ubiquitously asymmetric and, in fact, asymmetry rather than symmetry seems to be the norm in brain function. Such, at least theoretically, may have advantages including maximization of available space, higher processing speed and decreased inter-hemispheric competition. In fact, evolutionary advantage may explain the current associations with cognitive, emotional and behavioral outcomes, as well as the alterations found in neurodegenerative processes such as mild cognitive impairment and Alzheimer's disease (Cherbuin et al., 2010; Frings et al., 2015; Shi et al., 2009), and in neurodevelopmental pathologies including schizophrenia (Oertel-Knochel and Linden, 2011; Ribolsi et al., 2014), obsessive-compulsive disorder (Ischebeck et al., 2014; Rao et al., 2015), autism spectrum disorder (Dougherty et al., 2016; Gabard-Durnam et al., 2015) or attention deficit and hyperactivity disorder (Hale et al., 2014a; Hale et al., 2014b; Keune et al., 2015). Thus, if not necessary for, brain laterality seems to at least reflect proper brain function, and it is this exact question that remains unanswered. In fact, the great majority of laterality-cognition associations have arisen from correlational analyses that are unable to infer causality and animal studies, in which manipulation is commonly performed, normally assume complete equality of the hemispheres. Therefore, the field currently requires studies that include longitudinal and/or acute manipulations of function, which could elucidate the role of asymmetry in normal brain function.

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CHAPTER II

Structural laterality is associated with cognitive and mood outcomes: An assessment of 105 healthy aged volunteers

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ABSTRACT

The human brain presents multiple asymmetries that dynamically change throughout life. These phenomena have been associated with cognitive impairments and psychiatric disorders although possible associations with specific patterns of cognitive aging are yet to be determined. We have therefore mapped and quantified morphological asymmetries in a heterogeneous and aged population (65.2 ± 8.0 years old, 52 male and 53 female) to explore potential associations between the asymmetries in specific brain regions and cognitive performance. The sample was characterized in a battery of neuropsychological tests and in terms of brain structural asymmetries using a ROI-based approach. A substantial number of brain areas presented some degree of asymmetry. Such biases survived a stringent statistical correction and were largely confirmed in a voxel-based analysis. In specific brain areas, like the thalamus and insula, asymmetry was correlated with cognition and mood descriptors as the Stroop words/colors test or depressive mood scale, respectively. Curiously in the latter, the association was independent of its left/right direction. Altogether, results reveal that asymmetry is widespread in the aged brain and that area-specific biases (degree and direction) associate with the functional profile of the individual.

Introduction

The human brain is remarkably asymmetrical and gross distortions of brain symmetry like the Yakovlevian (anticlockwise) torque and the petalia have been recognized for decades (Galaburda et al., 1978; Hugdahl, 2011; Rogers, 2014; Sun and Walsh, 2006; Toga and Thompson, 2003). At the volumetric and cytoarchitectural levels, prominent asymmetries have been reported. A classic example is the marked leftward increase of the planum temporale in most individuals (Lyttelton et al., 2009; Takao et al., 2011; Watkins et al., 2001), while more recent subcortical asymmetries such as the leftward asymmetry of the habenula have been described (Ahumada-Galleguillos et al., In press). Left/right side differences in columnar organization (packing) (Chance et al., 2013) as well as in neuronal morphology (Scheibel et al., 1985) – size and dendritic arborization – have also been demonstrated. Dopamine (Glick et al., 1982) and noradrenaline (Oke et al., 1978) abundance is left and rightward lateralized respectively and marked differences in the expression of opioid receptors and respective ligands between the left and right anterior cingulate cortex were recently shown (Watanabe et al., 2015).

Some structural asymmetries seem to have a functional role. For example, the structural leftward imbalance of the planum temporale has been shown to be increased in musicians with perfect pitch (Keenan et al., 2001) and functional leftward asymmetry for language has been extensively described (see Toga and Thompson (2003)) for review). Moreover, a relevant body of literature describes abnormal structural lateralization associated with neuropsychiatric diseases. For example, right/left caudate volume quotient has been correlated with the manifestation of attention deficit and hyperactivity disorder (ADHD)-like symptoms in healthy subjects (Dang et al., 2016) and Eden et al. (2015) have shown an association between left or right prefrontal white matter pathways and reappraisal or trait anxiety, respectively. Brain asymmetries (or its lack) have also been recognized in obsessive-compulsive disorder (OCD) (Peng et al., 2015), autism (Conti et al., 2016; Herbert et al., 2005) and schizophrenia (Miyata et al., 2012; Narr et al., 2001; Park et al., 2013; Sun et al., 2015) - see also for review (Lindell and Hudry, 2013; Oertel-Knochel and Linden, 2011; Ribolsi et al., 2014; Ribolsi et al., 2009) - suggesting that

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asymmetry is crucial for (or at least reflects) proper functioning (Concha et al., 2012). Indeed, morphological asymmetries manifest early in development (Concha et al., 2012; Kasprian et al., 2011; Song et al., 2015) and seem to increase throughout life (Plessen et al., 2014; Zhou et al., 2013). However, while age-induced functional asymmetry changes seem to be associated with preservation of cognitive function as shown by models such as HAROLD (Cabeza et al., 2002) or CRUNCH (Reuter-Lorenz and Cappell, 2008), the association between the dynamic nature of structural laterality and the course of emotional and cognitive faculties throughout healthy aging is not entirely understood. In fact, the healthy aged population has not even been characterized in terms of hemispheric structural asymmetries.

We thus hypothesized that the older brain presents a high number of asymmetrical areas, which should be increased in comparison with younger subjects. Moreover, we hypothesized that many of these asymmetries should be relevant for neuropsychological profiles. In order to achieve these goals, we studied structural laterality in a thoroughly characterized and heterogeneous population of aged individuals and correlated these data with cognitive performance and mood classification.

Methods

Ethics statement

This study was performed in accordance with the Declaration of Helsinki (59th amendment) and approved by national and local ethics review boards (Comissão Nacional de Protecção de Dados, Hospital de Braga, Centro Hospitalar do Alto Ave and Unidade Local de Saúde do Alto Minho). All volunteers signed informed consent and all medical and research professionals who had access to participants' identity signed a Statement of Responsibility and Confidentiality.

Subjects

The population from the Switchbox project, a project that aims to study healthy aging in the population of northern Portugal, was used in the present study. Subjects' recruitment was performed in two phases. Initially, a large sample, representative of the older Portuguese population in terms of sex and education, was cognitively characterized [n=1051 after inclusion/exclusion criteria; subjects were randomly selected from the Guimarães and Vizela local area health authority registries (Costa et al., 2013; Santos et al., 2013, 2014)]. Then, in a second-phase, and based on the neurocognitive assessment, 120 subjects were selected from the previous sample in order to provide cognitive profiles of overall good cognitive performance (n = 60) and overall poor performance (n = 60) for further characterization, including magnetic resonance imaging (MRI) screening. Further details regarding formation of the groups are presented as Supplementary data.

Inability to understand the informed consent, participant's choice to withdraw from the study, incapacity and/or inability to attend the MRI session, dementia and/or diagnosed neuropsychiatric and/or neurodegenerative disorder (medical records) were the primary exclusion criteria. Regarding cognitive impairment, adjusted thresholds for the Mini-Mental State Examination (MMSE) test were calculated and applied, accounting for age and/or education (Busch and Chapin, 2008; Grigoletto et al., 1999). Following the MMSE validation for the Portuguese population (Guerreiro et al., 1994) the following thresholds were used: MMSE score < 17 if individual with ≤ 4 years of formal school education and/or ≥ 72 years of age, and MMSE score < 23 otherwise.

From the 120 subjects recruited for the Switchbox project, nine refused to perform the MRI screening in the assessment day, four had brain lesions/pathology detected through the MRI and two presented excessive motion/artifacts. This resulted in a final sample of 105 participants (description in Table 1 and Supplementay Table 1). All volunteers were right-handed.

Table 1
Population characterization. Distribution of the population included in the global analysis and respective cognitive and mood scores.

	Female	Male
Number of subjects	53	52
Age	66.5 ± 7.7 (51 to 82)	63.8 ± 8.1 (51 to 79)
Years of formal education	4.1 ± 2.8	6.7 ± 4.2
SRT-CLTR	14.0 ± 12.3	18.1 ± 12.7
SRT-DR	4.9 ± 3.2	5.8 ± 2.9
SRT-int	2.8 ± 4.4	2.1 ± 2.5
Dforward	7.2 ± 2.0	8.2 ± 2.4
Dbackward	3.8 ± 2.3	5.0 ± 2.7
Stroop-w	56.8 ± 19.8	71.6 ± 20.0
Stroop-c	46.6 ± 13.8	50.5 ± 15.2
Stroop-wc	26.1 ± 9.9	32.1 ± 14.2
FAS	14.7 ± 9.4	21.1 ± 12.8
GDS	13.2 ± 6.3	8.8 ± 6.3

Cognitive and mood assessment

Cognitive and mood evaluation was performed by a team of trained psychologists and all tests have been previously described (Santos et al., 2014). The Selective Reminding Test (SRT) (Buschke et al., 1995) was used as a verbal learning and memory test and evaluated the following components: consistent long term retrieval (CLTR), long term storage, delayed recall (DR) and intrusions. The Digits Span Test (Wechsler, 1997) was used in the forward (dforward) and reverse (dbackward) order as a measure of attention in the first case and working memory/executive function in the second. The Stroop test (Strauss et al., 2006), which is divided into three modules - words (w), colors (c) and words/colors (wc) - assessed selective attention, cognitive flexibility and response inhibition. Verbal fluency was evaluated through the Controlled Oral Word Association F-A-S (FAS) (Lezak et al., 2004) test, while depressive mood was assessed using the Geriatric Depression Scale (GDS) (Yesavage et al., 1982).

Image acquisition

Acquisitions were performed on a clinically approved Siemens MagnetomAvanto 1.5 T (Siemens Medical Solutions, Elangen, Germany) scanner, in Hospital de Braga, using a 12-channel receive-only Siemens head coil. A T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence with repetition time (TR)=2730 ms, echo time (TE)=3.5 ms, flip angle=7°, field of view (FoV)=256·256 mm, 176 sagittal slices, with isotropic resolution of 1 mm and no slice-gap was used.

ROI-based volumetric analysis

For the region-of-interest (ROI) based volumetric analysis, the structural data was processed with the semi-automated workflow implemented in FreeSurfer v5.10 (http://surfer.nmr.mgh.harvard.edu/). This pipeline implements a total of 31 processing steps which include the spatial normalization to Talairach standard space, skull stripping, intensity normalization, tessellation of gray matter (GM)-white matter (WM) boundary and segmentation of cortical, subcortical and WM regions. Results obtained with this pipeline were shown to be reliable across sessions, scanner platforms, updates, and field strengths (Jovicich et al., 2009) and were validated against manual segmentations (Fischl et al., 2002). Details regarding the procedures and improvements implemented throughout the years have been described in several publications (Desikan et al., 2006; Destrieux et al., 2010; Fischl et al., 2002). For the present work only volumes

resulting from the subcortical and cortical segmentation according to the Desikan atlas were considered (Desikan et al., 2006). ROI-wise measures of cortical thickness and surface area were not considered in order to facilitate the comparison between ROI-wise and voxel-wise laterality assessed with different techniques.

Laterality Index (LI) for structural segmentation-derived data was calculated using the following formula in an intra-individual analysis:

$$LI = (L - R)/(L + R)$$

where L=volume of the specified left region and R=volume of the specified right region. The absolute value of this result was used to determine the effects of side-independent asymmetries, i.e. the asymmetry index (AI).

VBM analysis

In order to control for possible atlas-derived biases, a complementary voxel-wise analysis was also performed using a modified version of the typical voxel based morphometry (VBM) analysis. The key aspect of the analysis is inter-hemispheric correspondence. In order to achieve this, we applied the typical VBM data processing pipeline (Mechelli et al., 2005) using two versions of the structural scans for each participant: a regular version of the structural scan and a flipped version in which the original scan was flipped along the midsagittal plane. All the VBM procedures were performed using Statistical Parametric Mapping 8 (SPM8; http://www.fil.ion.ucl.ac.uk/spm/). Initially all images (flipped and unflipped versions) were segmented into six different tissue classes (GM, WM, cerebrospinal fluid, skull, soft tissue and others) using the New Segmentation procedure. Then, the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) procedure was used in order to create a study specific template, which, by using both flipped and unflipped versions of the structural scans, originated a symmetric template. The GM tissue maps were then affine registered to the Montreal Neurological Institute (MNI) standard space for localization purposes. The GM maps were then scaled with the Jacobian determinants, also known as modulation, so tissue volumes could be compared and smoothed with a 10 mm full-width at half-maximum (FWHM) Gaussian filter.

LI images were created using imcalc from SPM8 according to the following formula:

$$LI = (Im_{unflipped} - Im_{flipped})/(Im_{unflipped} + Im_{flipped})$$

where $Im_{unflipped}$=unflipped image and $Im_{flipped}$=image flipped in the midsagittal plane. According to this formula, positive voxels in the left hemisphere of the brain reflect greater volumes on the left hemisphere and vice-versa for positive voxels in the right hemisphere.

Statistical analysis

All statistical analysis was performed on Matlab R2009b software. $P < 0.05$ was considered the threshold for statistical significance (Bonferroni-Holm correction was applied whenever multiple comparisons were performed (Holm, 1979)). Graphs were attained using Prism 6 software and, except for ROI-based laterality, only significant results are shown. Laterality pictogram was attained using 3D slicer (http://www.slicer.org) (Fedorov et al., 2012).

For ROI-based laterality evaluation values are presented as mean ± standard error of the mean and as normal distribution of the LI values could not be confirmed, non-parametric tests were used. Variables used for multiple regressions control are shown in the individual analyses.

Statistical analyses of VBM LI images were performed using SPM8. One sample *T*-Tests were performed and results were considered significant at $p < 0.05$ family-wise error (FWE) corrected at the voxel level and cluster size greater than five voxels. In the analysis, an explicit mask was applied by thresholding the gray matter tissue probability map suing a threshold of 0.2 in order to exclude voxels with low probability of corresponding to gray matter.

For determination of the factors that influence structural laterality, multiple linear regressions were conducted, in which the dependent variable was the ROI's LI and independent variables of interest were sex, age, group (good or poor performer) and education.

Multiple regression analyses were also used to determine the association between laterality-related data (LI or AI). The dependent variable was cognitive or mood score and variables of interest were either LI or AI and their interactions with sex or education. These analyses were controlled for sex, age, group and education.

In order to determine if the associations between the LI and the cognitive data were in fact due to the left/right balance or to the single left or right area volumes, a new analysis was run for each significant LI-cognition correlation. All model variables were similar, but LI was replaced with left or right area volume (percentage of intracranial volume, ICV) as independent variables of interest. As only areas of interest were analyzed, presented p-values were not corrected for multiple comparisons.

Results

Structural asymmetries - ROI-based analysis

Cortical GM segmentation revealed ubiquitous asymmetries. This lateralization was seen in both directions (left and right) with no obvious left or rightward trends in neighboring areas (Table 2 and Fig. 1). On the temporal lobe, leftward asymmetries were found in the entorhinal and transverse temporal cortices, parahippocampal and superior temporal gyruses and in the temporal pole; the middle temporal gyrus was the sole temporal area presenting a rightward volumetric asymmetry. Frontally, leftward asymmetries were seen in the superior frontal and caudal middle frontal gyri, pars opercularis and lateral and medial orbitofrontal cortices, contrasting with the rightward lateralization found in the rostral middle frontal gyrus, pars triangularis, pars orbitalis, frontal pole and paracentral lobule. Laterality in the parietal lobe was well distributed with two areas presenting L > R bias (postcentral and supramarginal gyruses) and two others presenting R > L asymmetry (inferior parietal and precuneus cortices). Rostral anterior and isthmus cingulate cortices were found to be left lateralized and the insula presented a rightward asymmetry. The only two occipital asymmetrical areas were the pericalcarine cortex and the lingual gyrus, both showing right lateralization.

Similarly, subcortical areas showed significant lateralization (Table 2 and Fig. 1). Lateral ventricle, accumbens, pallidum and putamen showed a leftward bias while the hippocampus, caudate and amygdala presented rightward lateralization. Interestingly, average LIs over the entire cortical gray, white matter and sub-cortical areas (Table 2 and Fig. 1) presented rightward laterality, even though only average gray matter LI reached statistical significance.

Structural asymmetries - VBM-based analysis

Aiming to provide validation to our ROI-based approach, VBM analysis of left and right gray matter differences was performed (Fig. 2). Leftward asymmetries were found in rolandic operculum, supramarginal gyrus, Heschl gyrus, inferior occipital, middle occipital, paracentral lobule, parahippocampus and fusiform gyrus. Rightward structural lateralization was found in calcarine, parahippocampus, middle frontal gyrus, middle temporal gyrus, inferior parietal gyrus, orbital part of the inferior frontal gyrus, supramarginal gyrus and fusiform gyrus. In order to facilitate comparisons, an Automatic Anatomical Labeling (AAL) – FreeSurfer (Desikan Atlas) correspondence was established. Left lateralized areas corresponded to supramarginal gyrus, superior temporal gyrus, lateral occipital cortex, precentral gyrus, parahippocampal gyrus and fusiform gyrus. Rightward shifts were found in cuneus cortex, entorhinal cortex, rostral

Table 2
Laterality statistics. Statistics for cortical gray matter, subcortical and general areas' LI. ROI=Region of Interest, LI=laterality index, corrected p-value=Bonferroni corrected p-value for 46 comparisons.

ROI	Mean LI	Standard Deviation	Corrected p-value	z-value	r (effect size)
Cortical Gray Matter					
Rostral Anterior Cingulate	0.1262	0.0951	< 0.0001	8.2789	0.8118
Transverse Temporal Cortex	0.1253	0.0952	< 0.0001	8.2526	0.8054
Pars Opercularis	0.0909	0.0815	< 0.0001	7.7730	0.7586
Isthmus Cingulate	0.0514	0.0704	< 0.0001	6.4013	0.6277
Caudal Middle Frontal Gyrus	0.0414	0.0709	< 0.0001	5.1009	0.5002
Entorhinal Cortex	0.0333	0.0844	0.0043	3.6741	0.3603
Parahippocampal Gyrus	0.0332	0.0658	0.0001	4.5794	0.4469
Supramarginal Gyrus	0.0304	0.0498	< 0.0001	5.4155	0.5310
Temporal Pole	0.0245	0.0678	0.0107	3.4030	0.3321
Banks of the Superior Temporal Sulcus	0.0239	0.0831	0.1187	2.6072	0.2557
Superior Temporal Gyrus	0.0235	0.0413	< 0.0001	4.9809	0.4908
Medial Orbitofrontal Cortex	0.0201	0.0531	0.0072	3.5245	0.3440
Postcentral Gyrus	0.0199	0.0489	0.0038	3.7163	0.3644
Superior Frontal Gyrus	0.0190	0.0315	< 0.0001	5.3659	0.5287
Lateral Orbitofrontal Cortex	0.0147	0.0338	0.0020	3.9081	0.3814
Fusiform Gyrus	0.0108	0.0562	0.5935	1.7854	0.1742
Precentral Gyrus	0.0077	0.0309	0.2142	2.3687	0.2334
Inferior Temporal Gyrus	0.0076	0.0568	1.1036	1.2228	0.1193
Superior Parietal Cortex	0.0021	0.0419	1.0224	0.6569	0.0641
Posterior Cingulate	−0.0031	0.0693	1.0224	0.5383	0.0528
Lateral Occipital Cortex	−0.0072	0.0472	0.8857	1.1966	0.1173
Cuneus Cortex	−0.0100	0.0566	1.0286	1.4504	0.1436
Precuneus Cortex	−0.0137	0.0287	0.0005	4.2675	0.4185
Lingual Gyrus	−0.0172	0.0490	0.0230	3.1682	0.3107
Insula	−0.0177	0.0385	0.0007	4.1638	0.4063
Rostral Middle Frontal Gyrus	−0.0244	0.0427	< 0.0001	5.1485	0.5024
Caudal Anterior Cingulate	−0.0334	0.1459	0.2447	2.2585	0.2204
Middle Temporal Gyrus	−0.0491	0.0473	< 0.0001	7.4780	0.7368
Pericalcarine Cortex	−0.0545	0.0461	< 0.0001	7.7396	0.7663
Paracentral Lobule	−0.0581	0.0659	< 0.0001	6.8747	0.6709
Pars Triangularis	−0.0779	0.0788	< 0.0001	7.4310	0.7252
Inferior Parietal Cortex	−0.0902	0.0512	< 0.0001	8.7394	0.8570
Pars Orbitalis	−0.1034	0.0740	< 0.0001	8.3859	0.8223
Frontal Pole	−0.1472	0.0921	< 0.0001	8.6170	0.8409
Subcortical					
Lateral Ventricle	0.0559	0.0885	< 0.0001	5.6101	0.5501
Accumbens	0.0467	0.0959	0.0002	4.5219	0.4413
Inferior Lateral Ventricle	0.0331	0.1984	0.2447	2.2862	0.2242
Pallidum	0.0320	0.0548	< 0.0001	5.2533	0.5151
Putamen	0.0143	0.0253	< 0.0001	5.0854	0.5111
Thalamus Proper	−0.0037	0.0264	1.1762	1.2929	0.1274
Hippocampus	−0.0088	0.0281	0.0230	3.1649	0.3118
Caudate	−0.0250	0.0273	< 0.0001	7.1050	0.6967
Amygdala	−0.0282	0.0685	0.0020	3.8979	0.3822
General Areas					
White Matter	−0.0014	0.0070	0.4688	1.9424	0.1905
Sub-Cortical	−0.0019	0.0151	1.1762	1.2246	0.1219
Gray Matter	−0.0023	0.0050	0.0006	4.2156	0.4134

middle frontal gyrus, banks of the superior temporal sulcus, middle temporal gyrus, superior temporal gyrus, supramarginal gyrus, pars orbitalis, inferior parietal cortex and fusiform gyrus.

Determining factors for structural laterality

As can be observed in the error bars shown in Fig. 1A, regional asymmetries were relatively homogeneous within the population. In order to confirm this, models with regional LIs as the dependent variable and sex, age, cognitive performance group (group) and years of formal education as independent variables were established. The only factor that had an influence in the LI was sex and only for the fusiform gyrus (p=0.0011, β=−0.0465, R^2=0.1379). In fact, post-hoc analysis showed that females presented increased (leftward) LI when compared to males (p=0.0001, t=4.0401, d=0.8001).

Correlates of laterality, cognition and mood

Memory

Memory, as evaluated by the SRT test in its various components, was associated with area-specific laterality while being strongly influenced by cognitive performance group. SRT-CLTR association with superior frontal gyrus LI was mediated by sex (Supplementary Table 2 - p=0.0062, β=−139.5240, R^2=0.7897). Post-hoc analysis showed that left lateralization in this area was correlated with better performance in female (p < 0.0001, R^2=0.4268), but not in male (p=0.4281, R^2=0.0150) subjects (Fig. 3A). SRT-DR's connection with lateral ventricle's LI was mediated by both sex and education (p=0.0203, β=−21.9812 and p=0.0137, β=3.4567, respectively R^2=0.4557 – Supplementary Table 2). In fact, a strong association between rightward lateralization of this area and increased SRT-DR score can be found for male, but not female subjects (Fig. 3B – p=0.0050, R^2=0.1653 and p=0.9690, R^2=0.0000 respectively) and for individuals with lower, but not higher education (Fig. 3C – p=0.0009, R^2=0.1453 and p=0.2300, R^2=0.0678 respectively).

Dbackward results were mostly correlated with group and age of the subjects and no associations with the LI were found. However, asymmetry of the lateral ventricle and cortical gray matter were linked to this outcome and mediated by education (Supplementary Table 3 – p=0.0375, β=3.3144, R^2=0.5821 and p=0.0477, β=59.8505, R^2=0.5491 respectively). Increased asymmetry of the lateral ventricle was connected with increased score in individuals with superior education (Fig. 3D - p < 0.0001, R^2=0.5883), although this was not true for lower educated subjects (p=0.4853, R^2=0.5883). Post-hoc analysis of the association between gray matter AI and dbackward showed that, in individuals with more years of formal education, increased asymmetry was associated with lower scores, while the opposite was true for lower levels of education (Fig. 3E – p=0.1735, R^2=0.0252 0-4 years of education and p=0.5369, R^2=0.0176 for > 4 years of education).

Attention, inhibition and cognitive flexibility

No associations of laterality/asymmetry with Stroop-w or Stroop-c were found. Stroop-wc, which presents increased cognitive demand and is mostly considered as a test that evaluates attention, inhibition and cognitive flexibility (Strauss et al., 2006) was mostly influenced by group. In the thalamus, a LI-sex association was found to be correlated with increased performance in the Stroop-wc (Supplementary Table 2 – p=0.0149, β=258.0564, R^2=0.5679). Post-hoc analysis (Fig. 4A) revealed that in male participants, a leftward volumetric lateralization of the thalamus was associated with increased number of named colors (p=0.0020, R^2=0.1977), while no correlation could be found for females (p=0.2445, R^2=0.0281).

Verbal fluency

Verbal fluency, assessed by the FAS, was associated with posterior

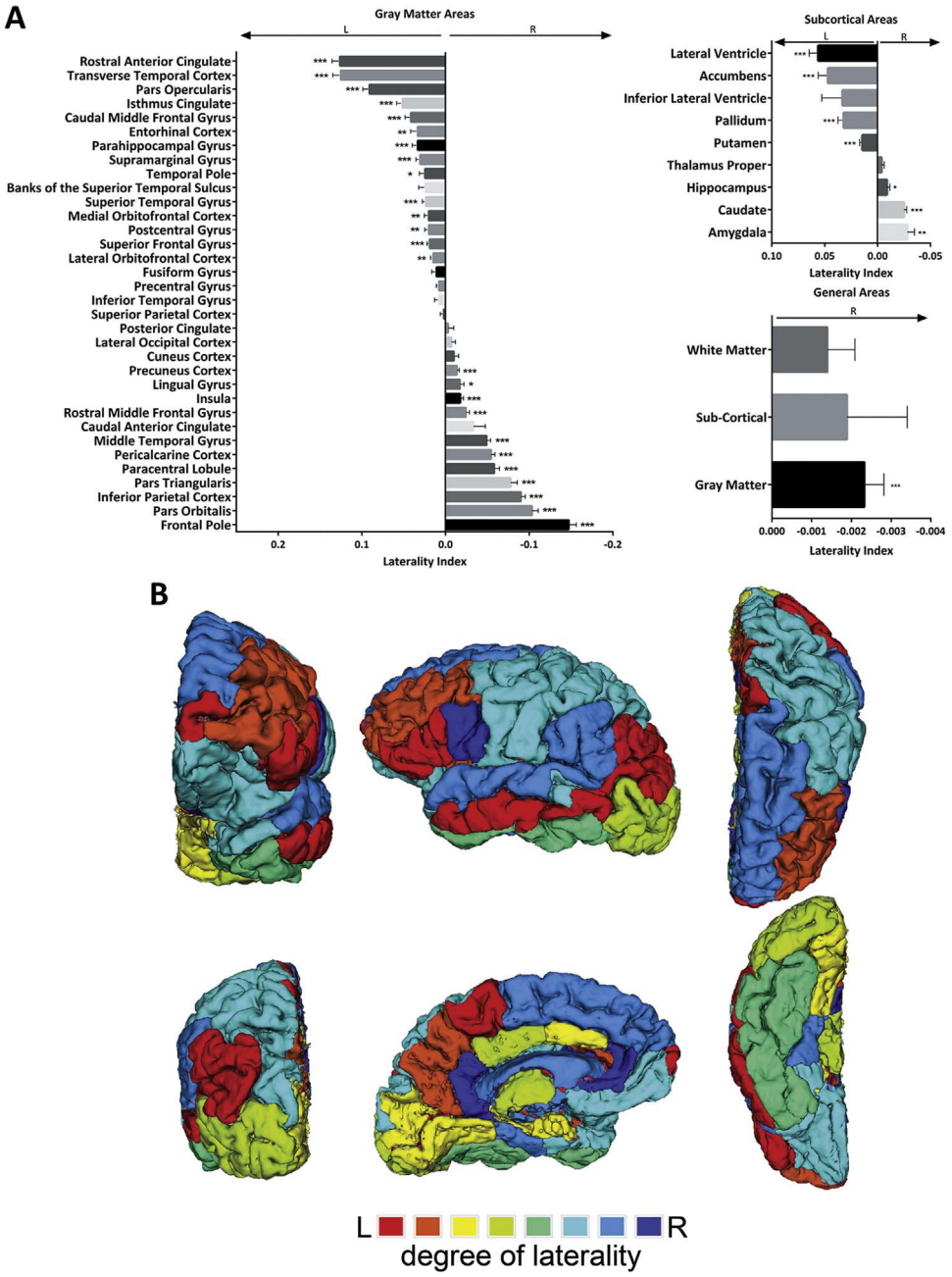

Fig. 1. Structural laterality indices evaluated using a ROI-based approach. (A) Structural LI evaluated through a ROI-based approach for cortical gray matter, subcortical and general areas. Positive and negative values represent leftward and rightward lateralization and are respectively represented on the left and right side of the graphs. **(B)** Color-coded pictographic depiction of significance of the degree of laterality in anterior, left, superior, posterior, right and inferior views (left to right, top to bottom). L=Left, R=Right, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in Wilcoxon signed rank analysis Bonferroni corrected for 46 comparisons.

cingulate laterality, but it was mostly influenced by cognitive performance group. Posterior cingulate laterality was correlated with this score in an education-mediated fashion (Supplementary Table 2 – p=0.0242, β=−11.2447, R^2=0.6064). Interestingly, the association between posterior cingulate LI and performance showed opposite trends in individuals with lower and higher education. In subjects with 0 to 4 (p=0.0533, R^2=0.0599) or more than 4 (p=0.0154, R^2=0.2846) years of formal education, a leftward or a rightward volumetric lateralization of the posterior cingulate are respectively associated with increased number of recalled words (Fig. 4B).

Mood

Depressive mood was evaluated through the GDS (higher scores are associated with increased depressive symptomatology) and although it was associated with group, the most striking correlation was found between the degree of asymmetry of the insula and the overall score of this scale (Supplementary Table 3 – p=0.0084, β=−91.0949, R^2=0.3501). Post-hoc analysis confirmed this result by revealing a very strong negative correlation between AI and GDS score (Fig. 4C – $p < 0.0001$, R^2=0.1477).

Volume-cognition correlates

The above-described LI-cognition associations could result from either an association between left/right balance and neuropsychological scores or from disproportionate influence of the left or right area. In order to clarify this question, multiple linear regression models were established.

cluster p(FWE-corr)	cluster size	peak p(FWE-corr)	peak T	Z	coordinates x	y	z	AAL anatomical region	Desikan anatomical correpondence	ROI direction
leftward asymmetry										
0	3975	0	13,56	Inf	-44	-30	16	Rolandic Operculum	Supramarginal Gyrus	L^{***}
		0	9,63	Inf	-64	-27	25	Supramarginal Gyrus	Supramarginal Gyrus	L^{***}
		0	8,05	7,08	-64	-12	7	Heschl Gyrus	Superior Temporal Gyrus	L^{**}
0	6560	0	11,07	Inf	-50	-73	-6	Inferior Occipital	Lateral Occipital Cortex	R^{NS}
		0	9,96	Inf	-50	-76	7	Middle Occipital	Lateral Occipital Cortex	R^{NS}
0	588	0	8,58	7,45	-9	-30	67	Paracentral Lobule	Precentral Gyrus	L^{NS}
0	350	0	7,05	6,36	-26	-33	-9	Parahippocampus	Parahippocampal Gyrus	L^{***}
		0	6,48	5,92	-27	-42	-12	Fusiform Gyrus	Fusiform Gyrus	L^{NS}
rightward asymmetry										
0	9225	0	13,82	Inf	15	-61	12	Calcarine	Cuneus Cortex	R^{NS}
		0	11,9	Inf	22	-9	-21	Parahippocampus	Entorhinal Cortex	L^{**}
0	2607	0	8,28	7,24	40	45	33	Middle Frontal Gyrus	Rostral Middle Frontal Gyrus	R^{***}
		0	7,6	6,76	27	56	28	Middle Frontal Gyrus	Rostral Middle Frontal Gyrus	R^{***}
0	626	0	8,78	7,57	49	-37	4	Middle Temporal Gyrus	Banks of the Superior Temporal Sulcus	L^{NS}
		0	8,21	7,19	51	-28	-3	Middle Temporal Gyrus	Middle Temporal Gyrus	R^{***}
		0	7,74	6,87	52	-19	-8	Middle Temporal Gyrus	Superior Temporal Gyrus	L^{**}
0	337	0	7,43	6,64	57	-31	52	Inferior Parietal Gyrus	Supramarginal Gyrus	L^{***}
		0,001	6,16	5,67	52	-42	55	Inferior Parietal Gyrus	Supramarginal Gyrus	L^{***}
0	178	0	6,76	6,14	51	20	-9	Orbital Part of the Inferior Frontal Gyrus	Pars Orbitalis	R^{***}
0,011	28	0,006	5,78	5,37	52	-43	40	Supramarginal Gyrus	Inferior Parietal Cortex	R^{***}
0,021	12	0,023	5,43	5,09	36	-9	-41	Fusiform Gyrus	Fusiform Gyrus	L^{NS}

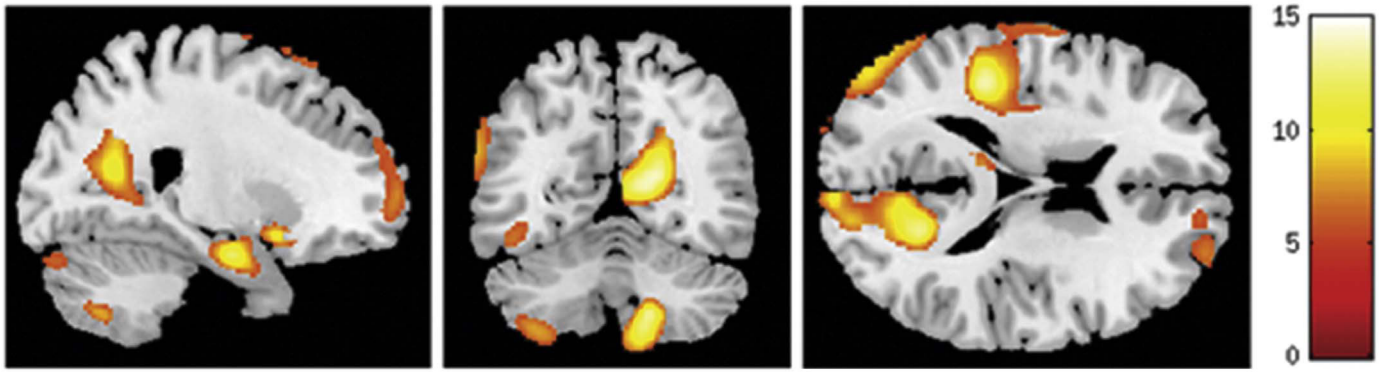

Fig. 2. Structural laterality indices evaluated using VBM analysis. Structural LI evaluated through a VBM analysis for gray matter areas. On the table, AAL-FreeSurfer correspondence and ROI concordance are also shown and asterisks represent level of significance of the ROI analysis. Clusters are also pictographically represented, where L > R and R > L asymmetries are represented on the left and right hemispheres respectively and darker to lighter colors represent increasing laterality. On the sagittal plane, only the left side is represented. L=Left, R=Right, **p < 0.01, ***p < 0.001, NS=non-significant in Wilcoxon signed rank analysis Bonferroni corrected for 46 comparisons.

SRT-CLTR was correlated with left, but not right, superior frontal gyrus volume in a sex-dependent way (Supplementary Table 4 – p=0.0017, β=−21.6295, R^2=0.7828 and p=0.6173, β=−3.9500, R^2=0.7125 respectively). This translated into a positive association between left volume and the score in female subjects (Fig. 5A – p=0.0219, R^2=0.1047), which could not be found for the right hemisphere volume (Fig. 5A – p=0.4702, R^2=0.0105). Relatable findings occurred in the FAS-posterior cingulate associations, which showed a statistically significant education-dependent connection with left, but not right volume (Supplementary Table 4 – p=0.0073, β=−21.8486, R^2=0.5768 and p=0.3069, β=10.2077, R^2=0.5517, respectively). The post-hoc test showed a similar trend, as left volume was negatively correlated with performance in individuals with more than 4 years of education (Fig. 5E – p=0.0650, R^2=0.1864) while no association was found for the right posterior cingulate volume (Fig. 5E – p=0.8507, R^2=0.0020).

The remaining analyses showed no associations between cognitive and volumetric data. In fact, SRT-DR score was not correlated with left or right lateral ventricle volume when considering mediation by either sex (Supplementary Table 4 – p=0.4830, β=0.9001, R^2=0.3823 and p=0.4322, β=1.1892, R^2=0.3869 respectively) or education (Supplementary Table 4 – p=0.3609, β=−0.1870, R^2=0.3823 and p=0.2427, β=−0.3869, R^2=0.3869 respectively). Also, thalamus left or right volumes were not associated with stroop-wc score (Supplementary Table 4 – p=0.8366, β=45.2554, R^2=0.4834 and p=0.6622, β=14.2356, R^2=0.4711 respectively for sex-mediated data).

Discussion

In the present study we investigated the association between structural asymmetries and cognitive/mood performance in an aged cohort. We demonstrated that most brain areas present some degree of structural asymmetry in either leftward or rightward directions. These were independent of age, cognitive status, years of formal education or sex (except for the fusiform area). Moreover, we were able to show that structural lateralization of specific areas was associated with memory, attention, verbal fluency and mood. These associations were mostly mediated by sex or education and most curiously, they were dependent on left-right relation but not on the absolute left or right volumes.

Structural asymmetries

A brain-wide ROI-based MRI approach with semi-automatic segmentation was employed to map brain structural asymmetries. This strategy avoided biases associated with area definition as well as potential issues associated with the left/right definition of voxel homotopy, while creating an easily readable output that facilitates structure-function inferences. Widely recognized asymmetries were found, such as a rightward frontal cortex lateralization (Goldberg et al., 2013; Good et al., 2001; Takao et al., 2011; Watkins et al., 2001) and a bigger left ventricle volume (Toga and Thompson, 2003). Concomitantly, we showed that brain-wide asymmetries were not only very consistent within the population, but also were not affected by age,

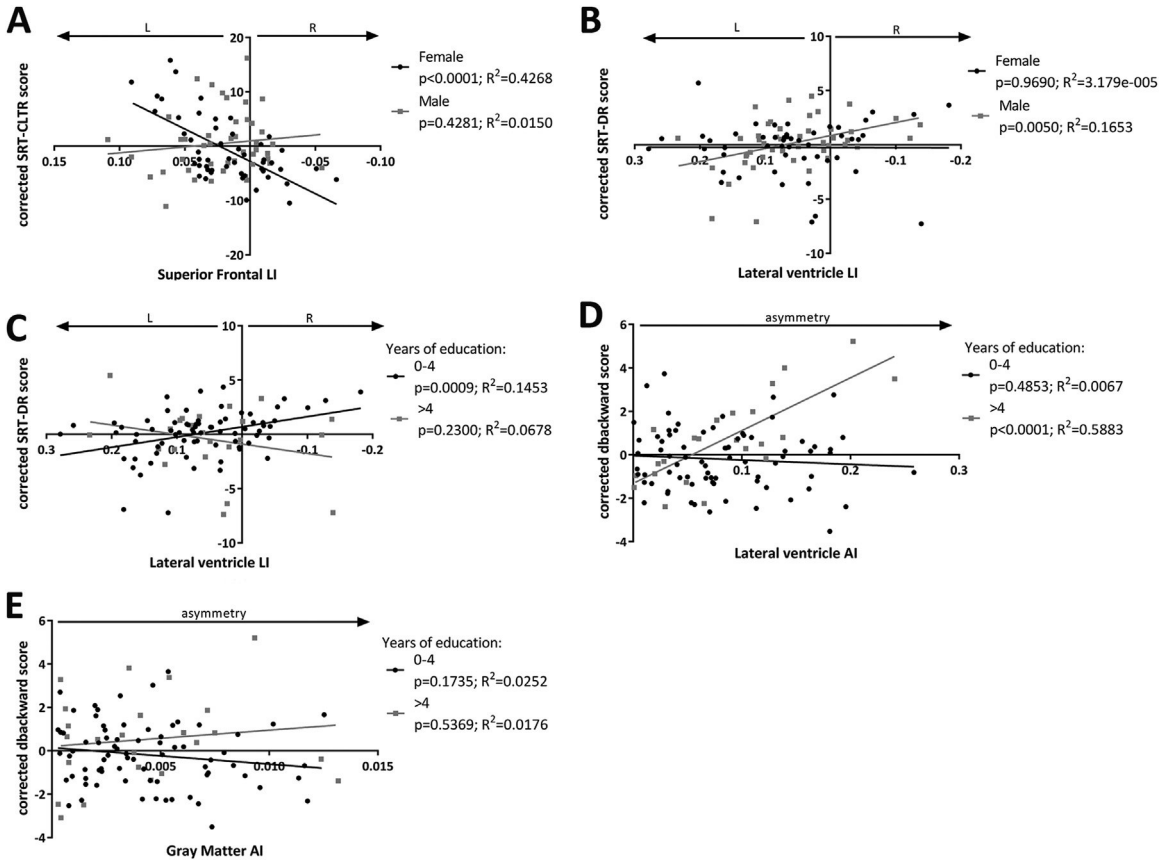

Fig. 3. Graphical representations of the models with significant correlations between structural laterality/asymmetry and learning and memory performance. Associations between learning and memory scores and LI **(A-C)** or AI **(D-E)**. **(A)** Sex-superior frontal gyrus LI interaction vs Consistent Long Term Retrieval (SRT-CLTR) score; **(B)** sex-lateral ventricle LI interaction vs Delayed Recall (SRT-DR) score; **(C)** education-lateral ventricle LI interaction vs SRT-DR; **(D)** education-lateral ventricle AI interaction vs Digit Span Test backward (dbackward) score; **(E)** education-gray matter AI interaction vs dbackward score. On A-C positive and negative values represent a leftward and a rightward lateralization and are respectively represented on the left and right side of the graphs while on D-E increased values on the xx axis represent increased asymmetry. L=Left, R=Right, LI=laterality index, AI=asymmetry index.

education, cognitive status or sex (except for the fusiform gyrus). These results were largely replicated in the VBM analysis, further validating our approach.

A similar distribution of morphological asymmetries was observed in a previous ROI study, despite differences in the population analyzed (39 adults; age range: 19–40 years old) (Goldberg et al., 2013). Concordance between the latter and our study seems to be higher in lateral rather than medial areas. Moreover, the proportion of asymmetries found in our study is higher, which may be related with differences in the segmentation process or with intrinsic characteristics of the evaluated population. Despite technical and sampling differences, other studies have reported comparable asymmetries such as leftward lateralization in the superior temporal gyrus (Good et al., 2001; Luders et al., 2006; Lyttelton et al., 2009), supramarginal gyrus (Lyttelton et al., 2009), putamen (Cherbuin et al., 2010; Okada et al., 2016; Wyciszkiewicz and Pawlak, 2014), pallidum (Cherbuin et al., 2010; Wyciszkiewicz and Pawlak, 2014) and lateral ventricle (Cherbuin et al., 2010; Okada et al., 2016); or a rightward lateralization of the anterior occipital cortex (Good et al., 2001; Luders et al., 2006; Lyttelton et al., 2009), frontal pole (Watkins et al., 2001), middle frontal gyrus (Watkins et al., 2001), inferior frontal gyrus (Luders et al., 2006; Lyttelton et al., 2009), caudate (Cherbuin et al., 2010; Wyciszkiewicz and Pawlak, 2014), hippocampus (Cherbuin et al., 2010; Okada et al., 2016) and amygdala (Cherbuin et al., 2010; Okada et al., 2016). However, some studies show opposite results (Raz et al., 1997; Takao et al., 2011).

Regarding laterality-influencing factors, no age-dependent differences were found. Despite previous reports describing age-related variations in laterality (Plessen et al., 2014; Zhou et al., 2013) the small age range of our population can largely explain its absence in our study. Additionally, our cohort showed no sexual dimorphism, except in the fusiform gyrus. This area presents functional hemispheric specialization for facial recognition (Ma and Han, 2012), which is known to be sex-dependent (Loven et al., 2014). Other brain-wide studies failed to find overall sex differences (Goldberg et al., 2013; Takao et al., 2011). Others, however, found slight differences on cortical thickness (Luders et al., 2006; Plessen et al., 2014) or VBM-measured asymmetries between male and female subjects (Good et al., 2001). Of note, to speculate that the post-menopausal state of the women included in our study may alter gender differences on brain structure and mitigate those differences (Eberling et al., 2004; Fukuta et al., 2013).

Cognitive and mood correlates

Laterality/asymmetry-cognition correlates were found in regards with learning, memory, response inhibition/cognitive flexibility, verbal fluency and mood. Importantly, these laterality associations could only be partially explained by the absolute left (or right) ROI volume and thus the left-right balance seems to be a main contributor in the established models. In summary, the superior frontal gyrus laterality showed a sex-mediated correlation with learning and memory (SRT-CLTR), posterior cingulate left-right balance was related with verbal fluency (FAS) in an education-mediated fashion and insula asymmetry was found to be associated with mood (GDS). Moreover, general cortical gray matter asymmetry had a correlation with working memory

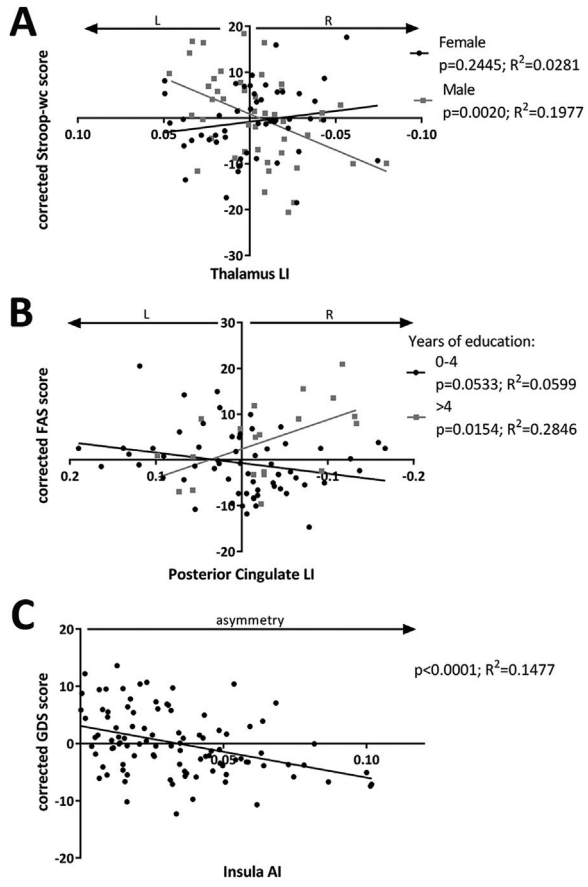

Fig. 4. Graphical representations of the models with significant correlations between structural laterality/asymmetry and cognitive and mood evaluations. Associations between cognitive scores and LI **(A-B)** and mood score and AI **(C). (A)** Sex-thalamus LI interaction vs Stroop-words/colors (Stroop-wc) score; **(B)** education-posterior cingulate LI interaction vs Controlled Oral Word Association F-A-S (FAS) score; **(C)** Insula AI vs Geriatric Depression Scale (GDS). On A-B positive and negative values represent leftward and rightward lateralization and are respectively represented on the left and right side of the graphs and on C increased values on the xx axis represent increased asymmetry. L=Left, R=Right, LI=laterality index, AI=asymmetry index.

(dbackward) that was mediated by education.

The PFC has a well-established role in working memory and sex-specific functioning has been shown (Goldstein et al., 2005). The superior frontal gyrus, in particular, seems to be involved in the performance of high (but not low) load working memory tasks (Rypma et al., 1999). Herein, we have shown a female-specific association between a leftward volumetric lateralization of this region and an improved performance in SRT-CLTR, which is partially (although not completely) explained by the left volume-score correlation. In fact, du Boisgueheneuc et al. (2006) have demonstrated that, in a slightly female-enriched population (5:3), left superior frontal gyrus lesion induces working memory deficits in the N-Back task.

The function of the posterior cingulate cortex has not yet been fully elucidated. However, its involvement in self-appraisal tasks has been described for both healthy and cognitively impaired subjects (Ries et al., 2006) and it seems to be involved in verbal fluency (Costafreda et al., 2011). A lateralized effect of posterior cingulate cortex volume in verbal fluency performance was, to the best of our knowledge, never reported and we may hypothesize that this is due to the mediation of education in this correlation, in which left volume seems to play a critical role.

We show a striking association between (side-independent) structural asymmetry of the insula and mood; i.e. irrespective of the direction, increased asymmetry was associated with lower GDS scores. Insular association with depressive symptoms is extensively described. In fact, left insular cortex volume is decreased in depressive patients, when comparing with healthy controls (Hatton et al., 2012; Soriano-Mas et al., 2011; Takahashi et al., 2010) and left and right volume reduction is found in depressive patients with suicide attempts (Hwang et al., 2010). The asymmetric involvement of this area in mood processing is still not fully understood. However, a meta-analysis of neural correlates of depression showed left deactivation of the insula in patients when comparing with healthy subjects, while treatment with antidepressants showed no effects on the left, but decreased activation of the right insula (Fitzgerald et al., 2008). Even though none of these authors explored the lateralized effects of their findings and here the assessment concerns depressive mood and not symptomatology, results seem to be in accordance with ours, showing an importance of insular asymmetry for depressive mood.

Interestingly, besides the above-mentioned cortical area-specific associations, general gray matter asymmetry also seems to play a role in cognition. In agreement with the present data, Savic (2014) have previously reported a R > L gray matter hemispheric lateralization. Gray matter volume has also been associated with cognitive profiles such as set-shifting (Tsutsumimoto et al., 2015), memory, mental flexibility and speed abilities (Steffener et al., 2013). However, to the best of our knowledge, this is the first description of an association between gray matter asymmetry and working memory score.

Subcortically, only thalamus laterality showed an association with cognition. This region has well-known functions in attention and executive function and decreased cingulo-thalamic connectivity has been associated with worse Stroop interference score (Wagner et al., 2013). Here, a correlation between this area's laterality and Stroop score was not unexpected. Moreover, right-left thalamic imbalances have been linked with language (Ojemann, 1977), memory (Hugdahl and Wester, 1997; Ojemann, 1977; Van der Werf et al., 2003) and complex speeded processing (Van der Werf et al., 2003). We further describe a sex-mediated association between thalamus laterality and the most demanding task of the Stroop test – words/colors –, in which L > R volume in men was associated with better performance. Although similar sex mediations were not yet described, sexual dimorphisms of the thalamus were previously reported (Menzler et al., 2011) and, importantly, left and right volumes could not explain this association.

Lateral ventricle enlargement and increase of its leftward asymmetry have been thoroughly correlated with aging (Berardi et al., 1997; Coffey et al., 1998; Fjell et al., 2009; Long et al., 2012) and neurodegenerative diseases such as Alzheimer's (Apostolova et al., 2012; Fjell et al., 2009). Here a leftward lateralization is also noted. Despite this, we also determined that rightward laterality of this area was associated with better delayed recall performance in male and lower educated subjects and importantly, this was exclusively due to left-right volume balance, as individual volumes did not impact the score. On the other hand, higher educated individuals benefited from a more asymmetrical lateral ventricle in the dbackward test. Memory-ventricle laterality correlates had been previously reported by Berardi et al. (1997) as elderly (but not in young) subjects, showed an association between laterality of the lateral ventricle and discrepancy between verbal and face memory. We hypothesize that ventricular enlargement is associated with atrophy of the surrounding areas and/or eventually with reduction of neurogenesis in the sub-ventricular zone and that the asymmetric evolution of this process leads to the described cognitive outcomes.

Conclusions

As brain asymmetries evolve with age (Plessen et al., 2014; Zhou et al., 2013), it is relevant to explore possible associations between laterality and cognitive performance. Our results show ubiquitous cerebral volumetric asymmetries in the older brain, some of which associated with cognitive performance and mood classification. Interestingly, in some cases a preferred left/right direction is noted,

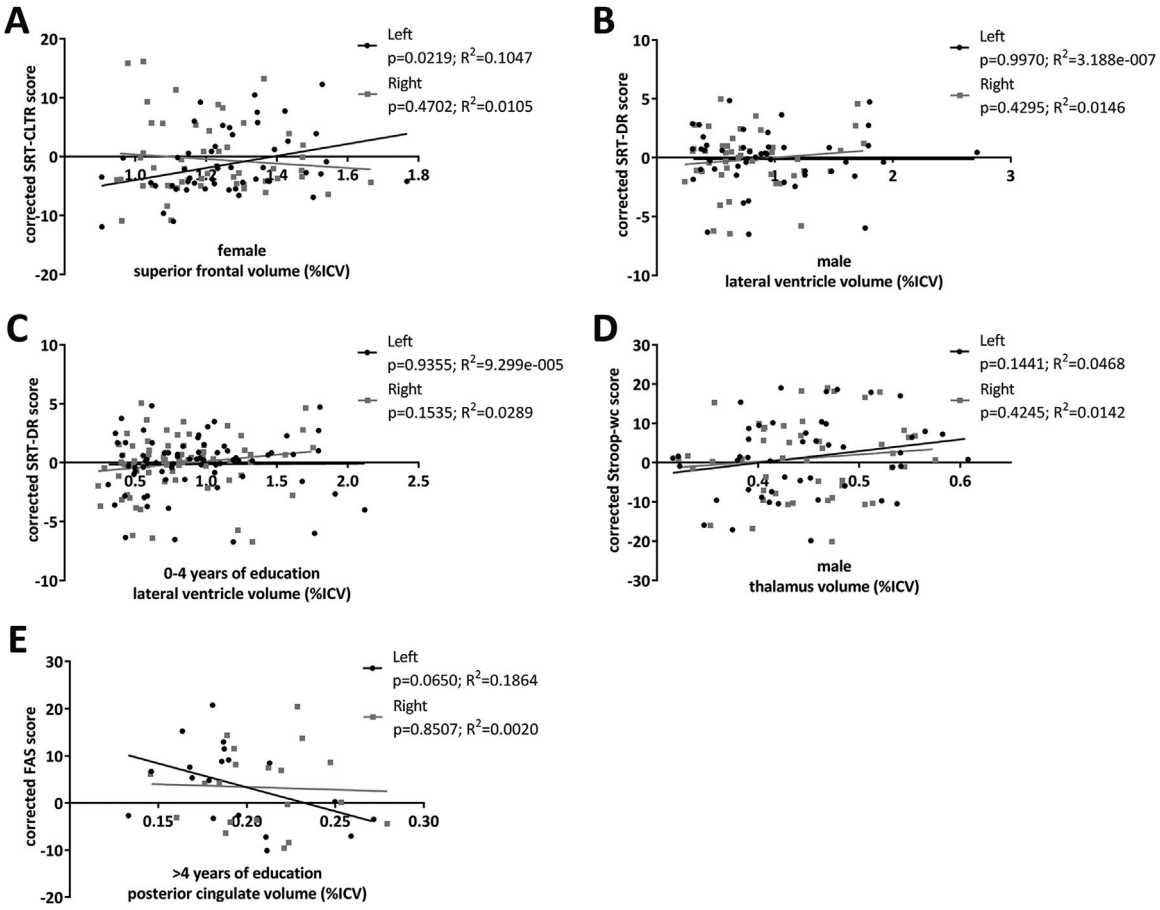

Fig. 5. Graphical representations of left and right volume correlations with cognitive scores. Volume-score correlations for subgroups in which laterality index (LI) vs score was found to be statistically significant; **(A)** female superior frontal volume vs Consistent Long Term Retrieval (SRT-CLTR) score; **(B)** male lateral ventricle volume vs Delayed Recall (SRT-DR) score; **(C)** 0-4 years of education lateral ventricle volume vs SRT-DR score; **(D)** male thalamus volume vs Stroop-words/colors (Stroop-wc) score; **(E)** > 4 years of education posterior cingulate volume vs Controlled Oral Word Association F-A-S (FAS) score. All volumes are depicted as percent of intracranial volume (ICV).

while in others the associations occurred without a preferred direction. In addition, in the dbackward task we observed an association with cortical gray matter asymmetry probably as a result of the involvement of a wide network in this type of processes. Despite well described age-dependent cortical atrophy (Hedden and Gabrieli, 2004), we were also able to demonstrate that directional (laterality) associations are mostly due to a left-right balance rather than to individual right or left volumes. Finally, redundancy (ROI vs VBM) and analysis stringency – all results have been corrected for multiple comparisons – enabled us to withdraw solid conclusions about the particularities of the aged brain. Our results point to the importance of both left/right and side-independent structural balance for neuropsychological performance. Further studies regarding functional asymmetries, as well as age differences are needed to better understand the lateralization of cognitive processes.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuroimage.2017.03.040.

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Sup Methods - group construction

Cognitive and mood evaluation was performed by a team of trained psychologists. In the initial phase of the project, the larger sample was characterized with an extensive battery of neuropsychological tests. The neuropsychological battery included the following tests: Digit-span forward (dforward) and backward (dbackward) test, Stroop words (w), colors (c) and words/colors (wc), Controlled Oral Word Association Test F-A-S (FAS; admissible words), Selective Reminding Test (SRT), Digit Symbol Substitution Test (DSST), Mini-Mental State examination (MMSE), Geriatric Depression Scale (GDS, long-version) (Santos et al., 2014).

Based the test scores, a Principal Component Analysis (PCA) was conducted, enabling the identification of two main dimensions of cognitive performance: MEM (memory) and GENEXEC (general executive functioning) (Santos et al., 2013). MEM was comprised by the long-term storage (LTS), consistent long-term retrieval (CLTR) and delayed-recall (DR) variables from the SRT. GENEXEC factor was constituted by the variables dforward, dbackward, stroop-w, stroop-c, stroop-wc, FAS. Based on these dimensions' scores, the MMSE and GDS scores, a cluster analysis was performed. The division into four clusters was identified as the best solution for the clustering algorithm and revealed four clusters ordered according to cognitive performance (C1>C2>C3>C4), with C1 and C4 corresponding to the best and worst cognitive profiles, respectively. For further details regarding the factor structure and cluster analysis please see (Santos et al., 2013).

For the Switchbox project, 120 subjects belonging to the best and poorest performance clusters (i.e. C1 and C4) were then selected, providing two groups of healthy older individuals with opposite cognitive profiles. The remaining participants were excluded and did not perform MRI (Marques et al., 2016).

Sup Tables

	Female		Male	
	Good performers	Poor performers	Good performers	Poor performers
Number of subjects	26	27	32	20
Age (y)	65.2±8.4 (51 to 82)	67.9±7.1 (54 to 82)	64.2±9.0 (51 to 79)	63.3±6.9 (52 to 77)
Education (y)	5.3±3.5	3.0±1.4	8.1±4.6	4.5±2.4
SRT-CLTR	24.4±7.9	4.0±5.9	26.1±8.9	5.2±5.4
SRT-DR	6.7±3.2	3.0±1.9	7.0±2.9	3.8±1.7
SRT-int	3.8±5.3	1.8±3.1	2.2±2.6	2.0±2.6
Dforward	8.2±2.1	6.3±1.3	9.1±2.4	6.7±1.7
Dbackward	5.4±2.1	2.3±1.2	6.4±2.6	2.8±1.0
Stroop-w	70.9±14.7	42.7±13.6	80.8±15.5	56.8±18.0
Stroop-c	56.5±11.7	36.7±7.4	59.3±10.6	36.5±10.5
Stroop-wc	32.3±9.2	19.9±6.1	38.8±10.7	21.3±12.7
FAS	20.9±8.7	8.5±5.6	28.5±10.4	8.6±4.4
GDS	10.7±5.5	15.7±6.4	6.6±4.9	12.3±7.0

Sup Table 1. Population characterization according to sex and group. Distribution of the population included in the global analysis and respective cognitive and mood scores. y - years.

		SRT-CLTR	SRT-DR	Stroop-wc	FAS
		Superior Frontal Gyrus	Lateral Ventricle	Thalamus	Posterior Cingulate
corrected p-value	const	0.0537	5.6686	3.9716	0.3307
	LI	0.6297	0.3200	2.3601	13.5379
	sex	15.6802	18.8267	1.6655	11.7058
	age	1.4902	3.6557	1.0562	6.6829
	group	<0.0001	<0.0001	<0.0001	<0.0001
	school	18.8794	10.9988	0.5194	0.2012
	sex.*LI	0.0062	0.0203	0.0149	7.6713
	school.*LI	12.0990	0.0137	3.0375	0.0242
beta	const	-1.8201	-0.1238	-1.4181	-2.0559
	LI	45.4250	-7.5718	61.9743	3.3817
	sex	1.0095	0.3784	1.0050	1.4336
	age	-0.1569	-0.0326	-0.2554	-0.1423
	group	18.3303	3.8247	13.8707	13.0318
	school	-0.1273	-0.0667	0.8482	0.6265
	sex.*LI	-139.5240	-21.9812	258.0564	22.9118
	school.*LI	-3.4596	3.4567	-23.2285	-11.2447
R^2		0.8054	0.4958	0.5998	0.6400
adj R^2		0.7897	0.4557	0.5679	0.6064

Sup Table 2. Multiple regression models showing an effect of the LI on cognitive outcomes. Corrected p-value=Bonferroni corrected p-value for 46 comparisons, adj R^2=adjusted R^2, const=model constant, LI=laterality index, sex.*LI=sex-LI interaction, school.*LI=school-LI interaction.

		dbackward	dbackward	GDS
		Lateral Ventricle	Gray Matter	Insula
corrected p-value	const	2.0957	3.4332	14.9878
	AI	4.3313	12.9042	0.0084
	sex	6.2109	2.2158	0.2868
	age	0.0469	0.0289	1.8382
	group	<0.0001	<0.0001	0.0322
	school	5.0233	5.0233	0.3009
	sex.*AI	10.0376	10.9346	3.7620
	school.*AI	0.0375	0.0477	9.5703
beta	const	-0.1554	-0.2053	0.4454
	AI	4.3645	-32.9890	-91.0949
	sex	0.2059	0.0872	-3.0215
	age	-0.0611	-0.0696	-0.1416
	group	2.7565	2.9613	-4.0662
	school	0.0922	0.0964	-0.3887
	sex.*AI	-2.7001	-80.3817	3.4106
	school.*AI	3.3144	59.8505	2.8473
R^2		0.6122	0.5813	0.3961
adj R^2		0.5821	0.5491	0.3501

Sup Table 3. Multiple regression models showing an effect of the AI on cognitive and mood outcomes. Corrected p-value=Bonferroni corrected p-value for 46 comparisons, adj R^2=adjusted R^2, const=model constant, AI=asymmetry index, sex.*AI=sex-AI interaction, school.*AI=school-AI interaction.

		SRT-CLTR		SRT-DR		Stroop-wc		FAS	
		Superior Frontal Gyrus		Lateral Ventricle		Thalamus		Posterior Cingulate	
		Left	Right	Left	Right	Left	Right	Left	Right
p-value	const	0.0081	0.0114	0.5110	0.5403	0.0618	0.1103	0.0258	0.0766
	volume	0.8526	0.1759	0.6242	0.7077	0.5812	0.5548	0.3008	0.1884
	sex	0.2233	0.3432	0.6359	0.4842	0.5310	0.4863	0.4398	0.1475
	age	0.0154	0.1283	0.1099	0.0426	0.0820	0.1252	0.1402	0.2879
	group	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	school	0.3585	0.7897	0.1208	0.1897	0.0381	0.0468	0.0032	0.0336
	sex.*vol	0.0017	0.6173	0.4830	0.4322	0.1477	0.6622	0.1747	0.1590
	school.*vol	0.4623	0.6582	0.3609	0.2427	0.8366	0.7356	0.0073	0.3069
beta	const	-1.5835	-1.7128	-0.1650	-0.1558	-1.7899	-1.5477	-1.8440	-1.5070
	volume	0.5938	-4.9126	-0.2998	0.2940	8.2199	9.4545	-25.5892	-32.9492
	sex	1.5026	1.3254	-0.2592	-0.3890	1.2191	1.3765	1.3069	2.5033
	age	-0.1829	-0.1281	-0.0589	-0.0743	-0.2095	-0.1871	-0.1584	-0.1124
	group	20.2515	19.4428	3.3040	3.2444	13.5235	13.6949	13.1928	13.3237
	school	-0.1977	-0.0649	0.1465	0-1299	0.7154	0.6744	0.8733	0.6703
	sex.*vol	-21.6295	-3.9500	0.9001	1.1892	45.2554	14.2356	73.3988	-76.2312
	school.*vol	0.7953	0.6011	-0.1870	-0.3412	-1.0122	1.8600	-21.8486	10.2077
R^2		0.7988	0.7334	0.4283	0.4335	0.5203	0.5089	0.6133	0.5900
adj R^2		0.7828	0.7125	0.3823	0.3869	0.4834	0.4711	0.5768	0.5517

Sup Table 4. Multiple regression models showing the effects of left or right volumes on cognitive outcomes. adj R^2=adjusted R^2, const=model constant, sex.*vol=sex-volume interaction, school.*vol=school-volume interaction.

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CHAPTER III

Asymmetrical subcortical plasticity and cognitive progression in older individuals

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Abstract:

Structural brain asymmetries have been associated with cognition. However, it is not known to what extent neuropsychological parameters and structural laterality co-vary with aging. Seventy-five older subjects were evaluated in terms of MRI and neuropsychological parameters at two moments (M1 and M2), 18 months apart. In this time frame, asymmetry as measured by structural laterality index (ΔLI) was stable regarding both direction and magnitude in all areas. However, a significantly higher dispersion for this variation was observed in subcortical over cortical areas. Increased rightward lateralization of the caudate was associated with higher Stroop interference scores, but also with a worsening of general cognition (MMSE). In contrast, increased leftward lateralization of the thalamus was associated with higher Stroop interference scores. In conclusion, while a decline in cognitive function was observed at the population level, regional brain asymmetries were relatively stable. At the individual level neuropsychological trajectories were associated with laterality changes particularly in subcortical regions.

Keywords: aging, cognition, MRI, structural laterality

1. Introduction

The human brain presents marked population-wide asymmetries. These include laterality of columnar organization (Buxhoeveden et al., 2001) and neurotransmitter distribution (e.g. dopamine (Glick et al., 1982) and noradrenaline (Oke et al., 1978)), as well as asymmetries that can be macroscopically perceived such as the Yakovlevian torque (Hugdahl, 2011; Toga and Thompson, 2003) or the left ventricle's increased relative size (Toga and Thompson, 2003). Area-specific structural laterality has also been vastly described (Esteves et al., 2017; Guadalupe et al., 2016; Wyciszkiewicz and Pawlak, 2014; Yamashita et al., 2011), as well as associations with factors such as sex (Good et al., 2001; Guadalupe et al., 2016) and musical training (Amunts et al., 1997; Li et al., 2010). The planum temporale, for example, shows clear leftward asymmetry (Toga and Thompson, 2003; Watkins et al., 2001), which seems to be reduced in females (Guadalupe et al., 2015; Kulynych et al., 1994) and increased in musicians with perfect pitch (Steinmetz, 1996).

In aging studies, most research has focused on changes that happen at a functional level where increased activation accompanied by decreased lateralization has systematically been reported. Such alterations have been observed in word encoding/retrieval (Cabeza et al., 1997; Madden et al., 1999), working memory (Reuter-Lorenz et al., 2000), face recognition (Grady et al., 2002), inhibitory control (Nielson et al., 2002), risk-taking (Lee et al., 2008) and error processing (Zhu et al., 2010). The bilateral activity pattern seems to result from a compensatory recruitment (Cabeza, 2002); in fact, as cognitively efficient older performers recruit additional contralateral networks (Cabeza et al., 2002; Erickson et al., 2007), it is postulated that symmetrical activation should be a correlate of good cognitive aging.

Age-dependent structural changes have also been described, including a non-linear alteration of basal ganglia asymmetries (Guadalupe et al., 2016; Wyciszkiewicz and Pawlak, 2014). For example, the putamen, which shows a leftward bias (Esteves et al., 2017; Wyciszkiewicz and Pawlak, 2014), presents decreased asymmetry in males and in younger subjects (Guadalupe et al., 2016), while the globus pallidus suffers a rightward shift with age (Wyciszkiewicz and Pawlak, 2014). The importance of these structural asymmetries arise from associations with neurodegenerative processes like mild cognitive impairment (Cherbuin et al., 2010; Long et al., 2013), Alzheimer's (Derflinger et al., 2011; Kim et al., 2012; Long et al., 2013) and Parkinson's (Lee et al., 2015) diseases, which typically develop at older ages. In fact, structural biases have

been correlated with cognitive outcomes such as memory (Berardi et al., 1997; Esteves et al., 2017; Plessen et al., 2014), vocabulary (Esteves et al., 2017; Plessen et al., 2014) and cognitive flexibility (Esteves et al., 2017).

Nonetheless, so far evidence of cognition-laterality association has been mostly driven from correlational analysis, and causality inferences have been difficult to obtain. One way to surpass this limitation is the utilization of longitudinal approaches, in which a more causal link may be established. Additionally, considering the effects of age on laterality and cognition, specific ranges of ages have to be considered. We have thus explored for the first time the longitudinal association between structural laterality and cognitive traits in an older population. Summarily, neuroimaging and cognitive data were acquired at two time points, 18 months apart. It was hypothesized that variations in cognition would be associated with area-specific alterations in structural laterality.

2. Methods

2.1. Ethics statement

Procedures were performed according to the Declaration of Helsinki and were approved by national and local ethics committees. All volunteers signed informed consent.

2.2. Subjects

Subjects were evaluated at two time points 18 months apart. The sample used in this study was withdrawn from the Switchbox project and selection for the first moment of evaluation (M1) has been previously described (Esteves et al., 2017; Marques et al., 2016). Briefly, a sample representative of the older Portuguese population was selected from the Guimarães and Vizela health authority registries (n=1051) (Costa et al., 2013; Santos et al., 2013; Santos et al., 2014). Primary exclusion criteria (at both time points) included incapacity to understand the informed consent, choice to withdraw from the study and/or diagnosed dementia, neuropsychiatric or neurodegenerative disorder. Cognitive data was used in order to perform Principal Component Analysis followed by cluster analysis, in which four clusters were identified. 120 subjects belonging to the two extreme groups (best and poorest cognitive performers) and balanced for sex and age were then selected for further characterization at M1, including Magnetic Resonance Imaging (MRI). All subjects were right handed. At the second time point (M2), two individuals could not be contacted, six were unable to attend the assessment and 26 met exclusion criteria (17 by decision

to withdraw from the study). In total, 86 subjects agreed to participate in the study. Nine refused to perform MRI (at either the first or second time points), one had brain lesions detected at MRI M2 and one was excluded due to movement artifacts at M2. The final population for longitudinal assessment thus included 75 individuals, from which 36 were females, 47 belonged to the good performers group, average education was 5.9±4.1 years (mean±standard deviation) and average age at M1 was 64.6±7.8 years old.

2.3. Cognitive assessment

A team of trained psychologists applied and scored all neuropsychological tests as previously described (Santos et al., 2014). The Stroop test (Strauss et al., 2006) aimed at assessing selective attention, cognitive flexibility and response inhibition. Two control trials consisted of reading from a list of color names written in black ink and naming the color of the ink in which a neutral set of letters (XXX) was written in. The incongruent part of the test consisted in a list of color names written in a different color ink (e.g. the word blue written in red ink) in which the ink color had to be named. Interference was assessed using the Golden (Lansbergen et al., 2007) and Chafetz (Chafetz and Matthews, 2004) indices, which evaluate the number of correct colors named in 45 seconds on the incongruent trial in comparison with the control trials (higher score means decreased Stroop interference). The Mini-Mental State Examination (MMSE) evaluated general cognition (Guerreiro et al., 1994) via a short assessment of orientation, memory, attention, language, verbal comprehension, writing and visual construction.

2.4. Image acquisition and analysis

A clinically approved Siemens MagnetomAvanto 1.5 T (Siemens Medical Solutions, Elangen, Germany) with a 12-channel receive-only Siemens head coil was used to perform all acquisitions at Hospital de Braga (Braga, Portugal). A scan using a T1 weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence with the following parameteres: repetition time (TR) = 2730 ms, echo time (TE) = 3.5 ms, flip angle = 7°, field of view (FoV) = 256·256mm, 176 sagittal slices, isotropic resolution of 1 mm and no slice-gap. Structural data was processed using the semi-automated workflow implemented in FreeSurfer v5.10 (http://surfer.nmr.mgh.harvard.edu/) which has been thoroughly described and continuously updated (Desikan et al., 2006; Destrieux et al., 2010; Fischl et al., 2002). The 31 processing steps were run, including spatial normalization to Talairach standard space, skull stripping, intensity normalization, tessellation of gray matter

(GM)-white matter (WM) boundary and segmentation of cortical, subcortical and WM regions. This pipeline has been shown to be reliable (Jovicich et al., 2009) and it was validated against manual segmentation (Fischl et al., 2002). Only subcortical and cortical gray matter (GM) volumes according to the Desikan atlas were considered (Desikan et al., 2006).

2.5. Data analysis

All statistical analyses were performed on Matlab R2009b software (The MathWorks, Inc., Natick, Massachusetts, United States). A threshold of $p<0.05$ for statistical significance was considered and Bonferroni-Holm (Holm, 1979) multiple comparison correction was applied when whole brain analyses were performed to control for the family wise error rate. Whenever normality assumptions were not met, non-parametric testing was performed. All graphs were attained using Prism 6 software (GraphPad Software, Inc., La Jolla, USA). For each cortical GM and subcortical area, a laterality index (LI) was calculated as $LI=(L-R)/(L+R)$, where L corresponds to left hemisphere area volume and R corresponds to right area volume. Positive values indicate L>R and negative values indicate L<R, while the denominator provides normalization for total area volume. Variation of LI (ΔLI) was defined as $\Delta LI=(LI\_M2-LI\_M1)/\left|LI\_M1\right|$, where LI_M2 and LI_M1 correspond to LI on the second and first moment of evaluation, respectively, and $\left|LI\_M1\right|$ is the absolute value of LI_M1. Positive values indicate variation to the left (i.e. at M2 the area was more asymmetric to the left, when comparing with M1) and negative values indicate variation to the right. The denominator provides normalization to basal laterality levels. Variation of left and right volumes (Δvol) was defined in a similar fashion: $\Delta vol=(vol\_M2-vol\_M1)/vol\_M1$, Variation of neuropsychological scores was defined as cog_M2-cog_M1, where cog_M2 and cog_M1 correspond respectively to score at M2 or M1. Positive and negative values indicate an increase and decrease of neuropsychological score respectively. Dispersion was assessed using the interquartile range. Groups for ordinal logistic regression were also based on percentiles and included the lower (right variation), middle (no variation) and higher (left variation) 25% of ΔLI (right, nil and left categories, respectively). Left variation was always the reference category. Logistic regressions where the independent variable of interest was neuropsychological variation were always corrected for variation of total gray matter as a proxy for aging.

3. Results

3.1. Neuropsychological alterations

Moment (M)1 and 2 cognitive data is shown in Table 1. From M1 to M2, a statistically significant decline in MMSE score was found (Z=4.096, Cohen's d=0.362, p<0.001), while no changes were found in either Stroop interference score (Golden - Z=1.707, Cohen's d=-0.131, p=0.088; Chafetz - Z=1.559; Cohen's d=-0.146; p=0.119).

3.2. Changes in laterality

Analysis of the laterality index (LI) at both M1 and M2 revealed ubiquitous asymmetries in both directions (Fig. 1A/B, Sup Table 1) with no differences in average laterality between the two moments (Sup Table 1). In fact, among 41 areas, only six were found to be lateralized at M1 but not at M2, namely the insula, parahippocampal, postcentral and lingual gyri, while temporal pole and hippocampus were found to be lateralized at M2 but not at M1 (Sup Table 1).

Average ΔLI was thus approximately 0 in all areas (Fig. 2A/B). Nonetheless, dispersion of values was area-dependent and interquartile range was higher in subcortical rather than cortical GM areas (Fig. 2B vs 2A - Z=3.586; Cohen's d=2.185; p<0.001). Further analysis was therefore focused in subcortical regions.

3.3. Left/Right volumes equally contribute to variation of subcortical laterality

The contribution of left and right volumes variation to ΔLI in individuals whose LI evolved to the left, to the right or maintained unaltered (left, right and nil categories respectively) was evaluated. In all areas, variation of left and right volumes significantly contributed to this categorization in the same order of magnitude but in inverse direction, i.e. an increase in right area volume increased the probability of being placed in the right category and vice-versa for increase in left area volume (Table 2). This is graphically represented in Fig.3, which shows the similar average left and right volume variations in the extreme (left and right) categories (ΔR=0.8546*ΔL-0.001; R^2=0.972; p<0.001).

3.4. Neuropsychological changes associate with subcortical variation of laterality

The association between M1 to M2 neuropsychological variation and ΔLI was assessed. As stated above, on average M1 to M2 LI was stable and therefore extreme variants on each direction (left

and right) and non-variants (nil) were analyzed in a logistic regression approach. Better M1 to M2 performance in the Stroop test (increased Chafetz interference score) was associated with leftward variation of thalamus' volume. Leftward variation of the caudate was associated with worst (lower) Stroop interference scores and better (higher) general cognition in the MMSE. Data can be seen in Table S2 and Fig. 4: (i) an increase of 1 point on Stroop's Golden index was associated with a 6% increase in the probability of caudate's LI varying to the right (negative ΔLI) (Fig. 4A - OR=0.935; CI=0.886 to 0.988; p=0.016); (ii) a similar association was found with the Stroop's Chafetz index (Fig. 2B - OR=0.940; CI=0.891 to 0.992; p=0.025) while the same index variation was associated with a 5% increase in the probability of thalamus' LI varying to the left (positive ΔLI) (Fig. 4B - OR=1.051; CI=1.002 to 1.102; p=0.040); and (iii) a point increase in the MMSE score was associated with a striking 49% probability of left (positive) variation in the caudate LI (Fig. 4C - OR=1.491; CI=1.105 to 2.014; p=0.009). Importantly, all these results were maintained when controlling the analyses for sex and age: Stroop Golden - OR=0.932, CI=0.881 to 0.987, p=0.016 for caudate; Stroop Chafetz - OR=0.933, CI=0.882 to 0.988, p=0.018 for caudate and OR=1.050, CI=1.001 to 1.102, p=0.045; MMSE - OR=1.522, CI=1.110 to 2.086, p=0.009.

3.5. Neuropsychological changes do not associate with subcortical left/right volume variations

Associations between neuropsychological changes and left/right volume variations were verified for regions in which correlations with laterality were found in the above section. This aimed to determine if these results could in fact be attributed to laterality rather than individual volumes. Because M1 to M2 volume variation did not differ from 0 (thalamus left p=0.237; thalamus right p=0.099; caudate left p=0.132; caudate right p=0.378), a similar percentile strategy was applied: reduction, maintenance or increase in volume were predicted in a logistic regression analysis (Fig. 5).

In all analyses, the trend followed the results found in the laterality results, i.e. whenever increased in neuropsychological score was associated with rightward laterality variation, a trend towards right increase and left decrease was found (and vice-versa for associations with leftward variation). However, none of the associations with individual left/right volumes achieved statistical significance: Stroop's Golden Index and caudate – OR=0.965, CI=0.909 to 1.024, p=0.243 for left volume and OR=1.031, CI=0.977 to 1.089, p=0.269 (Fig. 5A); Stroop's Chafetz Index and thalamus – OR=1.034, CI=0.979 to 1.092, p=0.229 for left volume and OR=0.994, CI=0.944 to

1.047, p=0.830 (Fig. 5B); Stroop's Chafetz Index and caudate – OR=0.965, CI=0.913 to 1.020, p=0.210 for left volume and OR=1.027, CI=0.976 to 1.080, p=0.307 (Fig. 5B); and MMSE and caudate – OR=1.009, CI=0.774 to 1.314, p=0.949 for left volume and OR=0.800, CI=0.627 to 1.022, p=0.074 (Fig. 5C).

4. Discussion

Aiming to study asymmetrical plasticity and respective neuropsychological correlates, herein, we evaluated 75 older individuals in two different moments. Data analysis indicates that, in older individuals, an 18 month time window was sufficient to observe a general cognitive decline, but no average changes in structural laterality. In subcortical areas, individuals were more heterogeneous regarding LI variation between the two moments. Interestingly, counterpart areas in the left and right hemisphere contributed nearly equally for this variation (varying in opposing directions) suggesting some degree of organization in the phenomena and excluding potential local neuropathological events. Importantly, in the caudate and thalamus laterality variations (M1 to M2) were associated with the course of mental flexibility and general cognition, which could not be attributed to individual left and right volume variation.

With aging, there is a general atrophy of gray matter (see (Hedden and Gabrieli, 2004) for review). The scale of these reductions is area-dependent; for instance, per decade, lateral pre-frontal cortex reduces its volume in 5% (Raz et al., 2004a), striatum atrophies 3% (Gunning-Dixon et al., 1998) and hippocampus may reach a 6% reduction at higher ages (Raz et al., 2004b) (see also (Du et al., 2003; Fjell et al., 2009; Raz et al., 2005; Scahill et al., 2003) for estimation of atrophy in shorter temporal scales). These reductions may translate into age-dependent changes in laterality but results in this domain have been inconsistent. Both asymmetry reductions (Long et al., 2013) and increases (caudate (Yamashita et al., 2011) and cortical thickness (Plessen et al., 2014)) have been reported, while other authors have found no effects of age on brain asymmetries (Abedelahi et al., 2013; Ota et al., 2007; Raz et al., 2004a; Raz et al., 2004b; Smeets et al., 2010; Takao et al., 2010). Two important factors contributing to these disparities may be the strategy used to assess laterality (Taki et al., 2011) and on the range of ages evaluated (i.e. it may not be a linear evolution (Guadalupe et al., 2016; Zhou et al., 2013). Considering, the small time-window between the 2 evaluations of our study, it is not surprising that we were unable to find differences in volumetric laterality. However, the striking difference between dispersion of cortical and subcortical laterality indices was not expected. This showed that, although the average was maintained, a

higher number of individuals experienced variations in subcortical asymmetries, suggesting a higher plasticity in these regions. In fact, some subcortical areas were previously shown to have high rates of atrophy during healthy aging (Fjell et al., 2009). Variations in side-specificity of this atrophy may be associated with increased dispersion laterality values' trajectory. Of importance, we were able to show that left or right variation of subcortical laterality was not due to local phenomena, but was rather associated with opposite patterns in the two hemispheres (i.e. left and right volumes evolution equally contributed for the laterality index variation).

It is widely accepted that aging induces a decline of cognitive functions such as the encoding of episodic memories and processing speed, while others like semantic memory and emotional processing remain mostly unaltered (see (Hedden and Gabrieli, 2004; Tromp et al., 2015) for review). Accordingly, in the time window of this study we observed a general decline in MMSE, which was negatively correlated with leftward evolution of caudate's LI (i.e. MMSE increase was associated with increased leftward lateralization). This area has been vastly shown to be reduced in diseases associated with cognitive decline such as Parkinson's disease (Apostolova et al., 2012), Alzheimer's disease (Barber et al., 2002; Ferrarini et al., 2008; Looi et al., 2008; Madsen et al., 2010), dementia with Lewy bodies (Barber et al., 2002), mild cognitive impairment (Madsen et al., 2010) and frontotemporal dementia (Looi et al., 2008). Additionally, both left and right caudate stroke was shown to induce cognitive decline (Bokura and Robinson, 1997) but side-specific associations have been found. In fact, and in accordance with the overall rightward asymmetry of the caudate in our healthy cohort, right volume (Apostolova et al., 2010) and rightward asymmetry of this area (Madsen et al., 2010) have been previously described as higher in non-demented rather than demented patients Also, other authors have described reduced left (but not right) caudate volume in demented patients (Barber et al., 2002) and a positive correlation between left (but not right) caudate volume and MMSE score, when assessing different types of cognitive decline (Looi et al., 2008). It is important to notice that we were, to the best of our knowledge, the first to assess a longitudinal index that measures left and right differences rather than absolute volumes. In fact, in our cohort, caudate's left/right balance, rather than the absolute volumes, better associated with cognitive decline and we may speculate that it should be relevant for disease onset.

No alterations in either measure of Stroop interference effect (Golden and Chafetz) were observed between M1 and M2. Regarding these tests, the literature presents conflicting evidence of an age effect. Indeed, while most studies show an interference increase with age (Comalli et al., 1962;

Davidson et al., 2003; Houx et al., 1993; Salthouse and Meinz, 1995; Spieler et al., 1996; Troyer et al., 2006), others (Langenecker et al., 2004; Zysset et al., 2007), including a meta-analysis (Verhaeghen and De Meersman, 1998), found no evidence of such effect. It is important to stress that these are cross-sectional studies, using wider age ranges than the 18-month interval used in our study. However, at the individual level, we showed that increased Stroop interference score (i.e, decreased interference) was associated with caudate and thalamus rightward and leftward trajectories, respectively. Performance in Stroop test has been classically associated with activation of frontal, cingulate and temporal areas (Langenecker et al., 2004; Leung et al., 2000; Peterson et al., 1999; Taylor et al., 1997), although relatively consistent findings in caudate and thalamic regions have also been described (Langenecker et al., 2004; Van Der Werf et al., 2001; Wagner et al., 2013) – see also (Peterson et al., 1999) for comparison of different studies. Additionally, left but not right caudate has been shown to be activated in incongruent vs congruent Stroop contrast (Langenecker et al., 2004), which may be related to its role in the switch between these two conditions, as the left (but not right) caudate head reduces its BOLD signal during this transition (Ali et al., 2010). On the other hand, Cai and colleages (Cai et al., 2016) have shown in individuals with internet gaming disorder that increased errors in incongruent Stroop are positively correlated with right caudate volume. Regarding the thalamus, our group has recently observed an association between Stroop words and colors and thalamus laterality (Esteves et al., 2017) in a transversal analysis of this same population. Our current results seem to reinforce this previous finding. Altogether, the sparse literature in the matter seems to agree with our data, showing a differential role of left and right caudate and thalamus in the Stroop interference effect.

5. Conclusions

In conclusion brain asymmetries (Plessen et al., 2014; Zhou et al., 2013) and cognitive performance (Hedden and Gabrieli, 2004) change with age, raising the hypothesis that these two phenomena could be associated. However, as these changes do not seem to follow a linear evolution (Guadalupe et al., 2016; Zhou et al., 2013), assessment of a stringent age category is necessary and the characteristic cognitive decline of aged individuals makes them prime candidates for such evaluation. Here, despite the absence of change in average structural laterality in the 18 months time-frame, it is shown that intra-individual variability in this measure was higher in subcortical rather than cortical areas. Additionally, caudate and thalamus laterality evolution were associated with changes in mental flexibility and general cognition.

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8. Figures

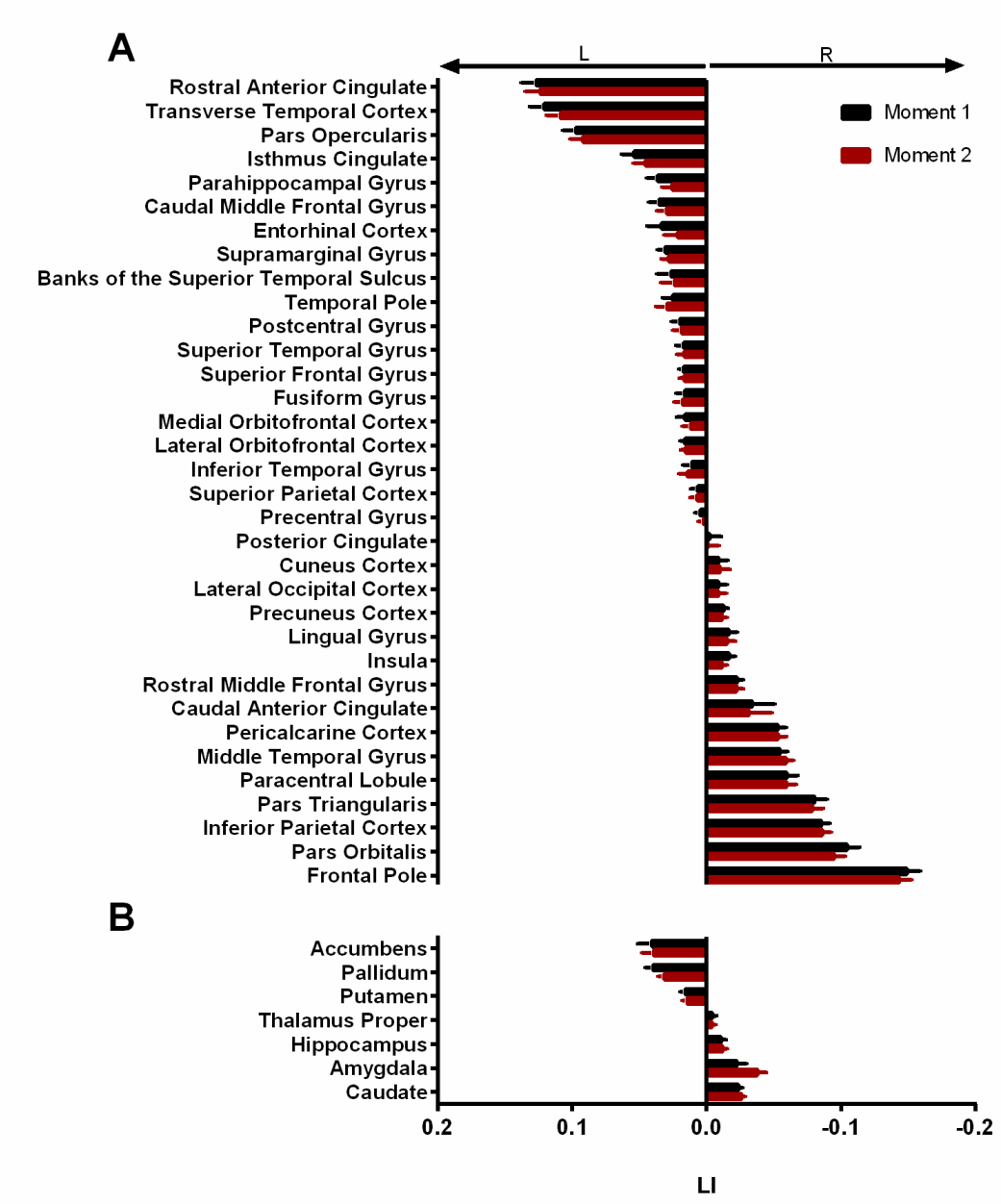

Fig. 1. Average structural laterality at M1 and M2. Structural laterality of cortical gray matter (A) and subcortical (B) areas at M1 and M2. Bar graphs show mean and standard error of the mean (SEM) and are organized from highest to lowest LI at M1. Positive and negative values represent left and rightward laterality respectively and are represented on the left and right side of the graphs. L=left, R=right, LI=laterality index, M1=moment 1, M2=moment 2.

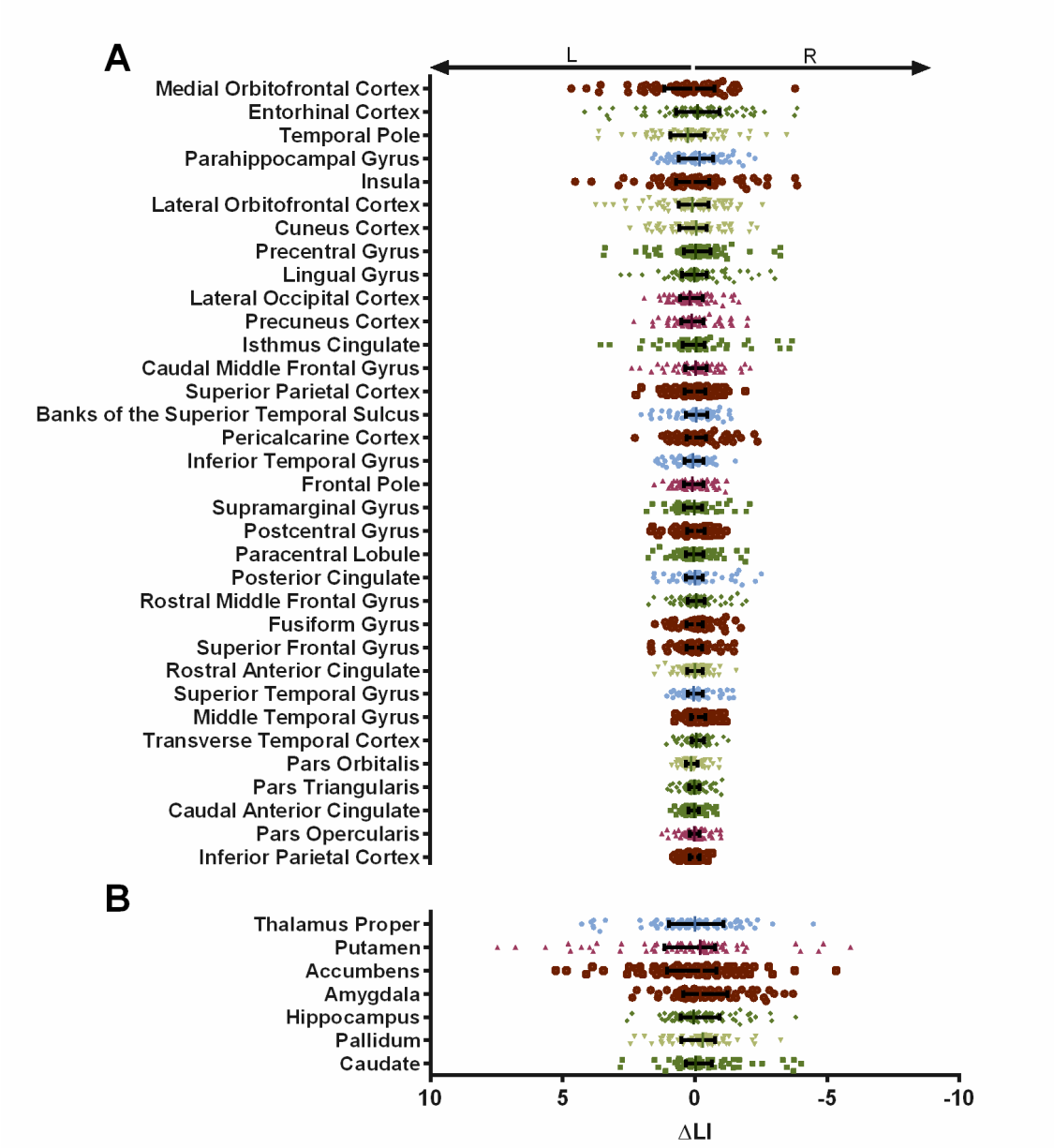

Fig. 2. Individual values of structural laterality variation. Individual values of ΔLI for cortical gray matter (A) and subcortical (B) areas. Dots represent individual values, and lines represent mean and interquartile range. Areas are organized from highest to lowest dispersion. Positive and negative values represent left and rightward evolution respectively and are represented on the left and right side of the graphs. L=left, R=right, ΔLI=variation of laterality index.

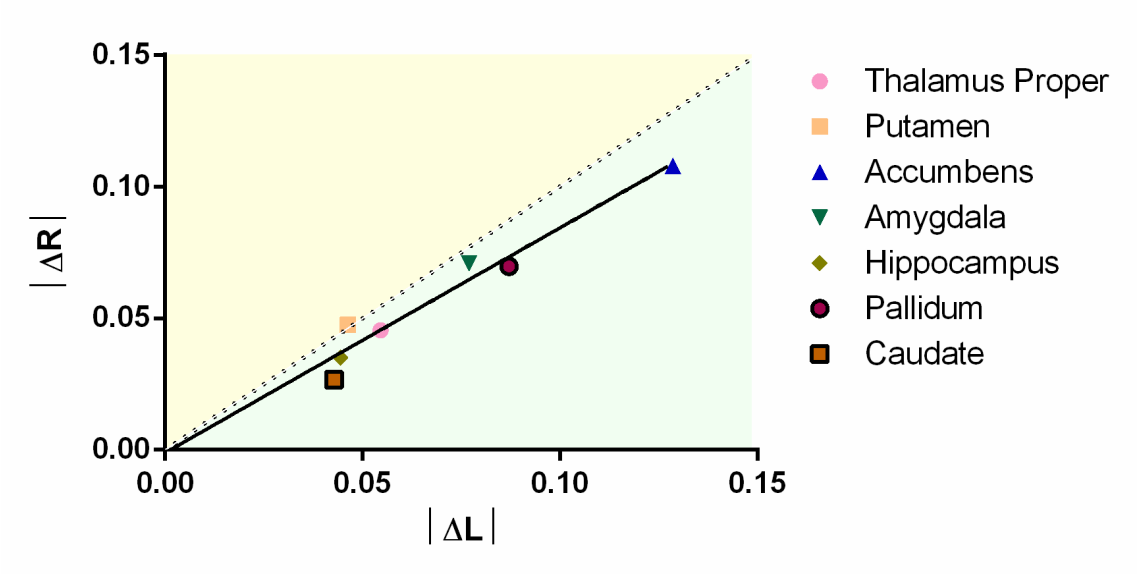

Fig. 3. Graphical representation of left and right variation influence for ΔLI. Representative graph of similar left and right subcortical volume variation in the right and left categories. Individual dots represent average absolute variation of left and right area volume in the extreme (right and left) categories. Full line represents the linear regression for these values and dotted line represents perfect $|\Delta L| - |\Delta R|$ correlation (slope=1). Blue and red areas represent respectively areas of higher $|\Delta L|$ or $|\Delta R|$. $|\Delta L|$ =absolute value of M1 to M2 left area volume variation, $|\Delta R|$ =absolute value of M1 to M2 right area volume variation.

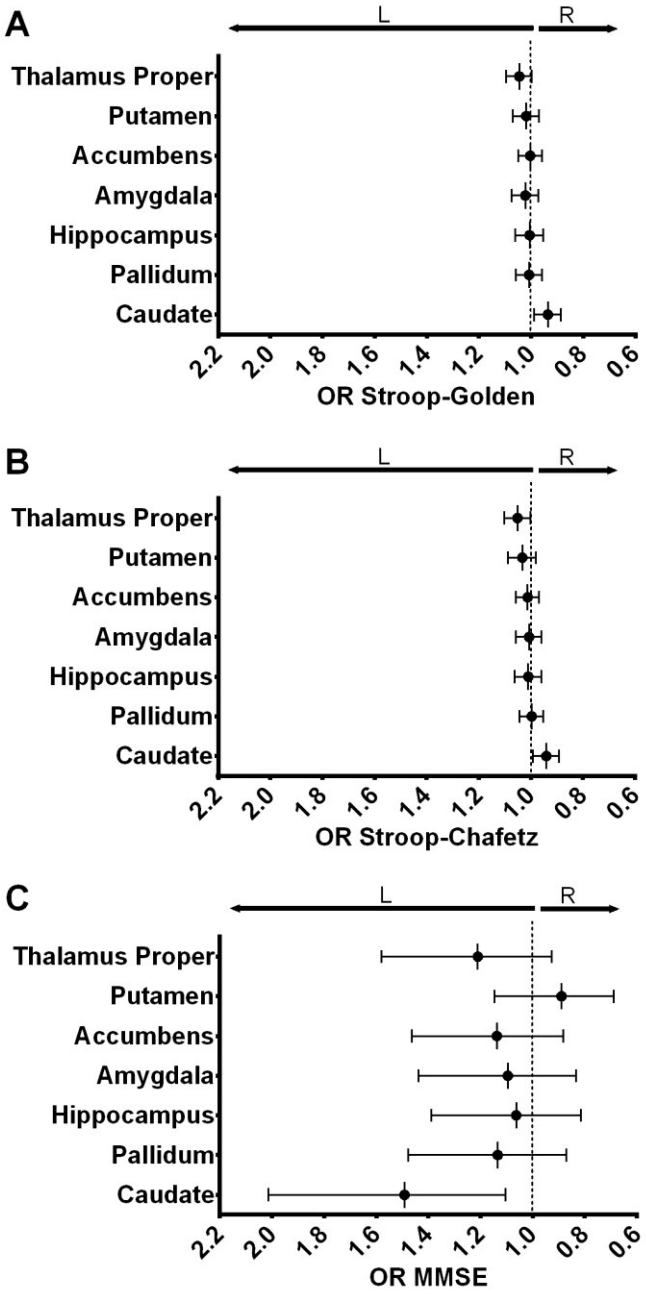

Fig. 4. Graphical representation of the neuropsychological M1 to M2 variation influence in subcortical ΔLI. The graphs depict OR and 95%CI of (A) Stroop's Golden Index, (B) Stroop's Chafetz Index, and (C) MMSE M1 to M2 variation's influence on ΔLI categorization for each subcortical area. OR higher and lower than 1 represent leftward and rightward evolution of ΔLI and are respectively represented on the left and right side of the graphs. Increased Stroop (Golden or Chafetz indices) and MMSE scores means lower Stroop interference effect and higher general cognition, respectively. L=left, R=right, OR=odd's ratio, MMSE=Mini-Mental State Examination, CI=confidence interval.

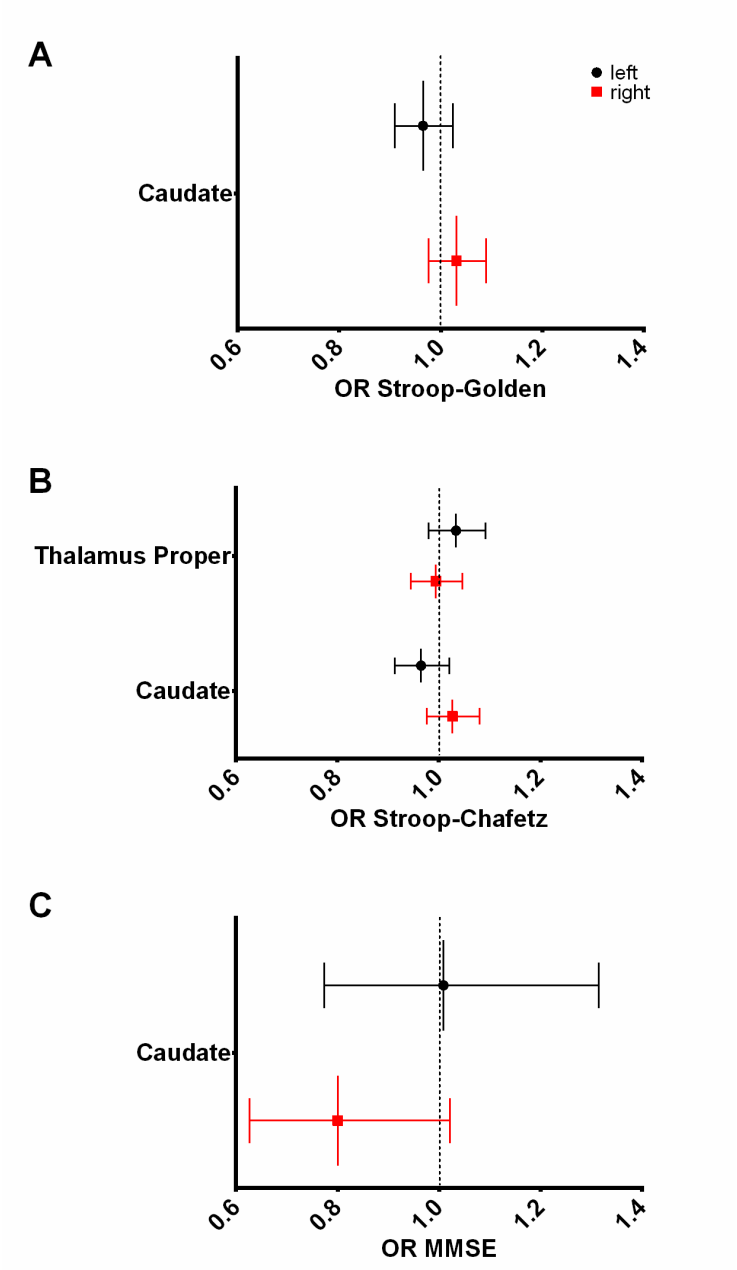

Fig. 5. Graphical representation of the neuropsychological M1 to M2 variation influence in subcortical left and right volume changes. The graphs depict OR and 95%CI of (A) Stroop's Golden Index, (B) Stroop's Chafetz Index, and (C) MMSE M1 to M2 variation's influence on volume categorization for each subcortical area, i.e. decrease, maintenance or increase in volume. Increased Stroop (Golden or Chafetz indices) and MMSE scores means lower Stroop interference effect and higher general cognition, respectively. Associations with left and right volume variations are depicted in black and red respectively. OR=odd's ratio, MMSE=Mini-Mental State Examination, CI=confidence interval.

9. Tables

	Stroop - Golden	Stroop - Chafetz	MMSE***
M1	2.050±7.553	-6.548±8.835	27.085±3.193
M2	3.082±8.174	-5.288±8.422	25.932±3.181

Table 1. Neuropsychological characterization of the population at both moments of evaluation (M1 and M2). Data is shown as mean±standard deviation. Asterisks represent statistically significant differences between M1 and M2. M1=moment 1, M2=moment 2, MMSE=Mini-Mental State Examination, ***p<0.001.

		OR	95% CI		p-value
			lower	upper	
Thalamus Proper	ΔR	1.581E-56	2.772E-83	9.022E-30	<0.001
	ΔL	2.362E+55	1.454E+29	3.836E+81	<0.001
Putamen	ΔR	3.228E-33	2.344E-48	4.445E-18	<0.001
	ΔL	2.734E+45	1.478E+24	5.059E+66	<0.001
Accumbens	ΔR	9.720E-21	4.795E-31	1.970E-10	<0.001
	ΔL	7.878E+25	2.155E+12	2.880E+39	<0.001
Amygdala	ΔR	1.352E-37	4.367E-60	4.183E-15	0.001
	ΔL	1.630E+40	9.522E+16	2.792E+63	0.001
Hippocampus	ΔR	2.858E-73	2.623E-111	3.113E-35	<0.001
	ΔL	2.648E+75	5.559E+34	1.261E+116	<0.001
Pallidum	ΔR	2.994E-18	9.164E-27	9.785E-10	<0.001
	ΔL	2.435E+20	4.299E+10	1.380E+30	<0.001
Caudate	ΔR	3.078E-97	1.400E-130	6.769E-64	<0.001
	ΔL	2.229E+98	1.618E+65	3.071E+131	<0.001

Table 2. Left and right subcortical volume variation influence in the establishment of left, right and nil categories. ΔR=variation of right volume (M1 to M2), ΔL=variation of left volume (M1 to M2), OR=odd's ratio, CI=confidence interval.

10. Supplementary data

area		LI vs 0						LI M1 vs M2		
		M1			M2					
		Z	effect size (r)	corrected p-value	Z	effect size (r)	corrected p-value	Z	effect size (cohen's d)	corrected p-value
Cortical GM	Rostral Anterior Cingulate	7.011	0.815	<0.001	7.018	0.810	<0.001	0.539	0.030	10.557
	Transverse Temporal Cortex	6.791	0.784	<0.001	6.849	0.791	<0.001	2.158	0.134	1.053
	Pars Opercularis	6.706	0.774	<0.001	6.886	0.806	<0.001	1.119	0.065	6.581
	Isthmus Cingulate	5.239	0.609	<0.001	4.008	0.463	0.002	1.463	0.107	4.163
	Parahippocampal Gyrus	3.765	0.435	0.004	2.783	0.321	0.086	2.028	0.167	1.403
	Caudal Middle Frontal Gyrus	3.702	0.427	0.004	3.638	0.420	0.006	1.751	0.088	2.399
	Entorhinal Cortex	2.820	0.328	0.067	1.848	0.213	0.517	0.729	0.134	10.256
	Supramarginal Gyrus	4.721	0.545	<0.001	4.156	0.480	0.001	0.702	0.062	10.256
	Banks of the Superior Temporal Sulcus	2.350	0.271	0.207	2.044	0.236	0.410	0.644	0.030	10.394
	Temporal Pole	2.804	0.324	0.067	3.464	0.400	0.010	1.109	0.062	6.581
	Postcentral Gyrus	2.962	0.342	0.046	2.387	0.276	0.204	0.388	0.020	10.322
	Superior Temporal Gyrus	3.824	0.448	0.003	3.257	0.381	0.020	0.312	0.013	9.016
	Superior Frontal Gyrus	4.684	0.545	<0.001	4.140	0.478	0.001	0.477	0.018	10.136
	Fusiform Gyrus	2.191	0.253	0.256	2.429	0.280	0.204	0.380	0.027	9.777
	Medial Orbitofrontal Cortex	2.302	0.266	0.213	2.443	0.284	0.204	0.544	0.079	10.557
	Lateral Orbitofrontal Cortex	3.770	0.435	0.004	3.844	0.444	0.003	0.256	0.015	6.301
	Inferior Temporal Gyrus	1.288	0.149	0.790	1.901	0.220	0.516	1.177	0.053	6.218
	Superior Parietal Cortex	1.547	0.179	0.731	1.537	0.177	0.519	0.073	0.011	2.760
	Precentral Gyrus	1.447	0.167	0.740	0.840	0.097	0.745	1.212	0.079	6.088
	Posterior Cingulate	0.444	0.052	0.847	0.892	0.104	0.914	0.305	0.020	8.309
	Cuneus Cortex	1.161	0.135	0.790	1.026	0.119	0.914	0.191	0.020	4.972
	Lateral Occipital Cortex	1.845	0.214	0.475	1.770	0.206	0.537	0.053	0.007	1.884
	Precuneus Cortex	3.553	0.413	0.007	3.059	0.358	0.038	1.102	0.039	6.419
	Lingual Gyrus	3.181	0.370	0.023	2.704	0.312	0.103	0.288	0.026	7.603
	Insula	3.406	0.393	0.012	2.329	0.269	0.219	1.299	0.144	5.430
	Rostral Middle Frontal Gyrus	4.156	0.480	0.001	4.145	0.479	0.001	0.317	0.006	9.149
	Caudal Anterior Cingulate	1.885	0.218	0.475	1.626	0.188	0.540	0.401	0.015	10.322
	Pericalcarine Cortex	6.521	0.758	<0.001	6.220	0.718	<0.001	0.100	0.008	3.546
	Middle Temporal Gyrus	6.419	0.746	<0.001	6.759	0.780	<0.001	1.965	0.099	1.580
	Paracentral Lobule	5.719	0.660	<0.001	5.840	0.674	<0.001	0.143	0.006	4.242
	Pars Triangularis	6.426	0.742	<0.001	6.585	0.760	<0.001	0.217	0.024	5.586
	Inferior Parietal Cortex	7.356	0.855	<0.001	7.297	0.848	<0.001	0.269	0.017	6.959
	Pars Orbitalis	7.102	0.820	<0.001	6.902	0.797	<0.001	1.888	0.130	1.829
	Frontal Pole	7.424	0.857	<0.001	7.414	0.856	<0.001	0.618	0.070	10.394

Subcortical	Accumbens	3.945	0.455	0.002	3.871	0.447	0.003	0.121	0.017	2.705
	Pallidum	5.320	0.618	<0.001	5.740	0.667	<0.001	1.139	0.176	1.782
	Putamen	4.284	0.516	<0.001	3.807	0.440	0.003	0.337	0.036	2.945
	Thalamus Proper	0.800	0.093	0.847	1.695	0.196	0.540	0.116	0.008	1.807
	Hippocampus	2.790	0.327	0.066	3.472	0.404	0.010	0.568	0.037	3.419
	Amygdala	3.216	0.371	0.022	4.510	0.521	<0.001	2.185	0.262	0.289
	Caudate	5.925	0.684	<0.001	6.532	0.759	<0.001	0.568	0.088	3.419

Sup Table 1. Laterality statistics. Statistics of cortical gray matter and subcortical areas' LIs at M1 and M2 and comparisons between the two moments. LI=Laterality Index, M1=Moment 1, M2=Moment 2, GM=gray matter, corrected p-value=Bonferroni-Holm corrected p-value for 41 comparisons.

		OR	95% CI		p-value
			upper	lower	
Stroop-Golden	Thalamus Proper	1.045	1.096	0.997	0.068
	Putamen	1.018	1.070	0.969	0.475
	Accumbens	1.003	1.050	0.959	0.889
	Amygdala	1.022	1.076	0.971	0.408
	Hippocampus	1.006	1.060	0.954	0.838
	Pallidum	1.008	1.059	0.959	0.765
	Caudate	0.935	0.988	0.886	0.016
Stroop-Chafetz	Thalamus Proper	1.051	1.102	1.002	0.040
	Putamen	1.033	1.089	0.980	0.232
	Accumbens	1.012	1.058	0.968	0.594
	Amygdala	1.007	1.057	0.959	0.781
	Hippocampus	1.010	1.063	0.959	0.712
	Pallidum	0.997	1.044	0.952	0.890
	Caudate	0.940	0.992	0.891	0.025
MMSE	Thalamus Proper	1.210	1.579	0.927	0.160
	Putamen	0.889	1.147	0.689	0.365
	Accumbens	1.137	1.464	0.883	0.318
	Amygdala	1.095	1.438	0.834	0.512
	Hippocampus	1.063	1.388	0.814	0.653
	Pallidum	1.134	1.476	0.870	0.353
	Caudate	1.491	2.014	1.105	0.009

Sup Table 2. Neuropsychological variation (Δcog) influence in laterality categorization for each subcortical area. MMSE=Mini-Mental State Examination, OR=odd's ratio, CI=confidence interval.

CHAPTER IV

Functional hemispheric (a)symmetries in the aged brain – relevance for working memory

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Title: Functional hemispheric (a)symmetries in the aged brain – relevance for working memory

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Abstract:

Functional hemispheric asymmetries have been described in different cognitive processes, such as decision-making and motivation. Variations in the pattern of left/right activity have been associated with normal brain functioning, and with neuropsychiatric diseases. Such asymmetries in brain activity evolve throughout life and are thought to decrease with aging, but clear associations with cognitive function have never been established. Herein, we assessed functional laterality during a working memory task (N-Back) in a healthy aging cohort (over 50 years old) and associated these asymmetries with performance in the test. Activity of lobule VI of the cerebellar hemisphere and angular gyrus was found to be lateralized to the right hemisphere, while the precentral gyrus presented left>right activation during this task. Interestingly, 1-Back accuracy was positively correlated with left>right superior parietal lobule activation, which was mostly due to the influence of the left hemisphere. In conclusion, although regions were mostly symmetrically activated during the N-Back task, performance in working memory in aged individuals seems to benefit from lateralized involvement of the superior parietal lobule.

Keywords: laterality, n-back, Aging, fMRI, superior parietal lobule

1. Introduction

The human brain presents several marked structural asymmetries such as the Yakovlevian torque (Hugdahl, 2011; Toga and Thompson, 2003) or the asymmetry of the planum temporale (Takao et al., 2011), as well as chemical left/right unbalances in dopaminergic (Glick et al., 1982), noradrenergic (Oke et al., 1978) and opioidergic (Watanabe et al., 2015) systems.

It is known since the early observations by Mark Dax and Paul Broca (Broca, 1861) – see also (Manning and Thomas-Anterion, 2011; Roe and Finger, 1996) that the brain also distributes its functional load in an asymmetrical fashion. Numerous imaging studies have confirmed these observations reporting an asymmetric recruitment of brain regions not only for language processing but also for emotion (Costanzo et al., 2015), motivation (Hughes et al., 2015; Poole and Gable, 2014), memory (Brambilla et al., 2015; Otsuka et al., 2008) and even general intelligence (Santarnecchi et al., 2015). A relevant body of literature also describes abnormal lateralization associated with neuropsychiatric diseases such as autism (Conti et al., 2016), schizophrenia (Royer et al., 2015) and dyslexia (Altarelli et al., 2014; Leonard and Eckert, 2008) suggesting that fine-tuned asymmetry is fundamental for, or at least reflects, proper functioning. In aged individuals, marked structural asymmetries have been identified (Esteves et al., 2017) andfunctional studies have systematically reported decreases in lateralization; such has been observed in word encoding/retrieval (Cabeza et al., 1997; Madden et al., 1999; Stebbins et al., 2002), working memory (Reuter-Lorenz et al., 2000), face recognition (Grady et al., 2002), inhibitory control (Nielson et al., 2002), risk-taking (Lee et al., 2008) and error processing (Zhu et al., 2010). Such bilateral activity pattern has been hypothesized to result from a compensatory recruitment (Cabeza, 2002) and, in fact, as cognitively efficient older performers recruit additional contra-lateral networks (Cabeza et al., 2002; Erickson et al., 2007) symmetrical activation could be a correlate of good cognitive aging.

Nonetheless, the differential left/right hemisphere involvement in working memory, both in the general population and in distinct age groups, is still not clear. We hypothesize that left and right areas are involved in working memory in different degrees in an older population. Herein, we evaluated functional laterality in an aged cohort using a cognitively stringent working memory task – N-Back (Vermeij et al., 2012) – in 2 different memory loads (1- and 2-back tasks), aiming to assess the validity of our hypothesis.

2. Methods

2.1. Ethics statement

This study was performed in accordance with the Declaration of Helsinki (59th amendment) and approved by national and local ethics review boards (Hospital de Braga, Centro Hospitalar do Alto Ave and Unidade Local de Saúde do Alto Minho) and by the national data protection entity (Comissão Nacional de Protecção de Dados). All volunteers signed informed consent and all medical and research professionals who had access to participants' identity signed a Statement of Responsibility and Confidentiality.

2.2. Subjects

The sample used in the present study was recruited from the Switchbox project. Briefly, a large sample, representative of the older Portuguese population in terms of sex and education, was cognitively characterized [n = 1051, after inclusion/exclusion criteria; subjects were randomly selected from the Guimarães and Vizela local area health authority registries (Costa et al., 2013; Santos et al., 2013; Santos et al., 2014)]. For this study, we started with a subcohort of 60 participants; 31 participants were selected because they showed above-minimum accuracy in the N-back task (see below); these subjects displayed high overall cognitive performance (for further detail regarding the participants' selection process, tests used for the cognitive assessment and the group formation, please consult (Marques et al., 2016)). All participants were right-handed.

2.3. Task design and minimum performance definition

During the fMRI session, participants performed a modified version of the verbal N-Back task. The task was composed by four different conditions: rest condition (Fig. 1, top), 0-Back (Fig. 1, second row from the top – control condition), 1-Back (Fig. 1, third row from the top – low working memory load) and 2-Back (Fig. 1, bottom – high working memory load). Four blocks of every condition (16 blocks in total) were pseudo-randomly distributed throughout the experiment. Each block was initiated by the presentation of an instruction card indicating the block condition (6000 ms) followed by 16 trial cards and ended with a pause card (10 000 ms). Each trial card contained a letter which was presented for 500 ms, followed by a fixation cross card for 2000 ms. Response was allowed up to the first 1900 ms of the fixation cross card, totaling 2400 ms of response time.

Participants were instructed to press a button if the trial card presented was a target card during the control and experimental conditions (0-, 1- and 2-Back). On 0-Back blocks, the target letter was presented in the instruction card and participants were instructed to press the button in trial cards showing the same letter. On 1-Back, the target was any letter equal to the one presented one trial before and on 2-Back, subjects should press the button if the letter corresponded to the one presented two trials before. Finally on the rest condition blocks participants were instructed to remain still, looking at the screen, without pressing any button while the letter "X" was presented in trial cards.

Inclusion of subjects in the fMRI analysis was dependent on N-Back performance. Accuracy (Acc) per block was calculated as Acc=1-(inc/4), where inc = number of incorrect answers in each block (omissions and non-target button presses) and 4 is the maximum number of possible correct responses per block. The worst trial of each condition was excluded and subjects were included in the analyses if average Acc≥0.5 (i.e., number of correct responses ≥ number of incorrect responses), in order to guarantee subject's engagement in the task. Following these criteria 31 and 22 participants were included for1-Back and 2-Back analyses, respectively.

2.4. Image acquisition

Acquisitions were performed on a clinically approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Elangen, Germany) scanner in Hospital de Braga, using a 12-channel receive-only Siemens head coil. Two acquisitions were used in the current work: as a structural acquisition a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence with repetition time (TR) = 2730 ms, echo time (TE) = 3.5 ms, flip angle = 7°, field of view (FoV) = 256·256 mm, with isotropic resolution of 1 mm and no slice-gap; the functional acquisition consisted of a T2-weighted Echo Planar Imaging (EPI) sequence, sensible to blood oxygen level dependent (BOLD) contrast, with TR = 2000 ms, TE = 30 ms, FA = 90°, FoV = 224·224 mm, in plane resolution = 3.5 x 3.5 mm, slice thickness = 4 mm, slice-gap = 0.48 mm slice gap, 30 interleaved ascending slices and with 456 volumes for a total of 15 min and 12 s acquisition time. The task was presented using the fully integrated fMRI system IFIS-SA (Invivo Corporation, Orlando, FL, USA) and the same system was used to record the subject key-press responses.

2.5. fMRI and statistical analysis

Pre-processing of functional data was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and consisted of the following steps: slice timing correction, within subject registration of each volume to the first volume of the acquisition in order to correct for head movement, non-linear spatial normalization to MNI standard space, resampling to 2 mm isotropic resolution, spatial smoothing using an 8 mm FWHM Gaussian kernel and high-pass temporal filtering at 128s.

The first-level analysis was performed using the General Linear Model (GLM) framework implemented in SPM. For the included participants, a total of 14 regressors were introduced in the model: 1 regressor modeling the instruction and pause cards; 1 regressor for the rest condition blocks; 3 regressors, one per experimental condition (0-, 1- and 2-Back) modeling the 3 included blocks; 3 regressors, one per experimental condition modeling the block with worst accuracy, excluding it from the analysis; 6 movement regressors. Contrasts of interest were set to create maps of increased or decreased activity in low working memory load (1- >0-Back and 1-<0-Back), high working memory load (2- >0-Back and 2-<0-Back) and high working memory load compared to low working memory load (2->1-Back and 2-<1-Back). In the second-level analysis (group level) one-sample t-tests were performed for the above-mentioned contrasts and areas were considered active for each condition if the corresponding family wise error (FWE) corrected p-values were <0.05 at the voxel level.

Laterality assessment was performed based on the regions of the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) in which active voxels were found and were based on the magnitude signal change as previously described (Jansen et al., 2006). Briefly, mean maximum activation was calculated for each region of interest (ROI) as the mean of the 5% of voxels evidencing the highest T-score. Voxels with signal intensity change higher than 50% of the mean maximum activation were included.

Statistical analyses were performed on Matlab R2009b software (The MathWorks, Inc., Natick, Massachusetts, United States) and images were computed in Prism 6 (GraphPad Software Inc.) and MRIcro (Rorden and Brett, 2000). Independent and paired analyses were performed respectively for population comparisons and assessment of left/right differences in activation. Non-parametric tests were used whenever normality could not be confirmed. For linear regressions,

laterality degree (LD) was calculated as L=AL-AR, where AL = left activation of the specified region on the specified contrast and AR = right activation of the specified region on the specified contrast. P-value <0.05 was considered the threshold for statistical significance and Bonferroni correction was applied whenever multiple comparisons were performed. Data is shown as mean±standard deviation.

3. Results

3.1. Population characterization

Thirty-one subjects showed above-minimum accuracy for inclusion in 1- vs 0-Back analysis respectively and 22 out of these 31 were also included for 2- vs 0-Back and 2- vs 1-Back analyses. Included individuals significantly differed from the remaining population of 29 subjects in terms of gender (32.258 vs 57.143% female), age (60.290±7.708 vs 69.035±7.356 years old; Z=3.861; p<0.001; Cohen's d=1.161) and education (8.484±4.545 vs 5.000±3.162 years of formal education; Z=3.515; p<0.001; Cohen's d=0.890).

3.2. N-Back-associated BOLD response and patterns of asymmetry

One-sample t-tests for contrasts 1-Back>0-Back (Fig. 2, top), 1-Back<0-Back (Fig. 2, bottom), 2-Back>0-Back (Fig. 3, top) and 2-Back<0-Back (Fig. 3, bottom) showed that BOLD response during N-Back performance followed the expected patterns (Owen et al., 2005). No activated/deactivated areas were found in the 2- vs 1-Back contrasts.

A total of 20 ROIs were found to be either activated (1-Back>0-Back) or deactivated (1-Back<0-Back) in the 1-Back condition (Table1, Fig. 2). These comprised the superior and inferior parietal lobules, Crus I of cerebellar hemisphere, orbital part of the middle frontal gyrus, lobules VI and VII of cerebellar hemisphere, middle frontal and precentral gyri, Crus II of cerebellar hemisphere, supplementary motor area, pars triangularis of the inferior frontal gyrus, lobule VIII of cerebellar hemisphere, superior frontal and angular gyri, precuneus, insula, rolandic operculum, medial frontal and superior temporal gyri and medial orbitofrontal cortex.

During the 2-Back part of the task, 14 regions were involved (Table 1, Fig. 3), namely superior and inferior parietal lobules, Crus I of cerebellar hemisphere, orbital part of the middle frontal gyrus, lobule VI of cerebellar hemisphere, precentral gyrus, pars opercularis of the inferior frontal gyrus,

middle frontal gyrus, pars triangularis of the inferior frontal gyrus, superior occipital, pars orbitalis of the inferior frontal gyrus, precuneus, lobules IV and V of cerebellar hemisphere and insula.

Asymmetry of the activated areas was analyzed by comparing left and right BOLD responses. During 1-Back performance (Fig. 4, Sup Table 1), R>L activation was found in the lobule VI of the cerebellum (α=0.016; Z=3.331; Cohen's d=-0.197) and in the angular gyrus (α=0.010; Z=3.469; Cohen's d=-0.497). L>R activation occurred in the precentral gyrus (α<0.001; Z=4.527; Cohen's d=0.655).

During 2-Back trials (Fig. 5, Sup Table 2) R>L activation could be identified in the lobule VI of the cerebellum α=0.028; Z=3.068; Cohen's d=-0.215) while L>R activation was found in the precentral gyrus (α=0.001; Z=3.945; Cohen's d=0.685).

3.3. Lateralized activation in the superior parietal lobule is associated with N-Back performance

The impact of functional laterality on working memory was determined by establishing models with N-Back Acc as the dependent variable and LD as the independent variable. Superior parietal lobule 1-Back LD showed a positive correlation with Acc during this task. More specifically, we found that a leftward asymmetry in the activation of the superior parietal lobe was associated with better performance (α=0.033; β=0.247; R2=0.293 - Fig. 6A, Sup Table 3). Importantly, this association was maintained when age and education were added as co-variables in the regression model (p=0.001; β=0.270; R2=0.416). Simple 1-Back Acc vs left or right BOLD response correlations showed that this association is mainly due to the left hemisphere contribution (Fig. 6B).

No associations between performance and asymmetric activation of brain hemispheres were found in the 2-Back task.

4. Discussion

In the present study we tested the hypothesis that task related BOLD response occurred in a lateralized fashion which could be associated with working memory performance in an older population. We demonstrated that the majority of the activated areas are similarly activated on the left and right hemispheres, although some asymmetries could be observed. Importantly, superior parietal lobule laterality was positively correlated with 1-Back performance.

The classic example of functional laterality is the well-known left lateralization for language (Hugdahl, 2011; Toga and Thompson, 2003), but degree of asymmetry has also been shown to predict performance in cognitive domains such as verbal and visuospatial ability (Gotts et al., 2013). Herein we evaluated functional lateralization during a verbal N-Back task (1- and 2-Back). The N-Back is a common tool for the study of working memory with a well-established pattern of cortical activation, which utilizes 0-Back as a reference task (Cohen et al., 1994; Owen et al., 2005; Yuksel et al., 2018). Our observations were in accordance with the expected pattern and recruited areas included the inferior parietal lobule, lateral cerebellum and prefrontal cortex (Owen et al., 2005). Asymmetrical activity however, was restricted to the lobule VI of the cerebellar hemisphere (R>L, 1- and 2-Back), precentral (L>R, 1- and 2-Back) and angular gyri (R>L, 1-Back).

The Hemispheric Asymmetry Reduction in Older Adults (HAROLD) model (Cabeza, 2002) postulates that high-performing older adults present compensatory bilateral activity. Cabeza and colleagues (2002) have shown that prefrontal cortex PET activation was more symmetrical in aged good performers when compared with young individuals or aged poor performers during a working memory task, suggesting a positive correlation between symmetry and performance. Even though these findings have been widely corroborated (Bergerbest et al., 2009; Cabeza et al., 2004; Piefke et al., 2012), some studies are still finding conflicting evidence (Brambilla et al., 2015; Vermeij et al., 2014) and more recently, the Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) was suggested (Reuter-Lorenz and Cappell, 2008; Schneider-Garces et al., 2010). This model postulates that this age-related symmetry is not necessarily due to homotopic activation, but results from an overall compensatory overactivation that happens not only in older adults, but also in younger subjects performing higher load cognitive tasks. In fact, from the 60 individuals initially included in our assay, only about 50% were able to perform the 2-Back task at an acceptable accuracy, indicating a high difficulty level for this specific population and therefore explaining the low number of lateralized areas.

Nonetheless, regarding the lateralized areas, both the right lateralization of the lobule VI of the cerebellar hemisphere and the precentral gyrus's leftward bias may be associated with motor responses during the task. The latter is a classic motor area whose direction of activation follows the same trend found in the supplementary motor area in the 1->0-Back contrast, strongly suggesting an involvement of motor planning and/or execution. Additionally, left and right activation of the precentral gyrus seem similar in both presented contrasts, which is in accordance with

previous reports of a load independent activation of the left sensorimotor cortex during the N-Back task (Jansma et al., 2000). Similarly, right but not left lobule VI of the cerebellar hemisphere is activated during right finger tapping (Stoodley and Schmahmann, 2009; Stoodley et al., 2010), although an involvement in working memory cannot be discarded as it was also previously reported as independent of motor performance both in an individual study (Stoodley et al., 2010) and in a meta-analysis (Stoodley and Schmahmann, 2009). Regarding the right>left asymmetry of the angular gyrus, this is the first report of functional lateralization in a working memory task. In fact, its activation in this function has been mostly regarded as bilateral (Blokland et al., 2008; Carlson et al., 1998). Nevertheless, this area has consistently shown lateralized activity in multiple other functions such as semantic and number processing, attention or spatial cognition (Seghier, 2013).

Interestingly, leftward asymmetry of the superior parietal lobule was correlated with better performance in the 1-Back task, which was mainly due to an association between left activation and accuracy. This area is known to be involved in working memory (Rottschy et al., 2013) and Koenigs and colleagues (2009) have previously found a lateralized effect, in which right lesions decreased working memory performance involving visuospatial manipulation. The authors were not able to find asymmetry effects in other types of working memory (including N-Back), which could be due to the low number of subjects (9 right vs 4 left lesions). On the other hand, Otsuka and collaborators (2008) have determined that superior parietal lobule left activation was positively correlated with executive function while right activation was associated with short-term storage, further confirming the importance of laterality in this region.

5. Conclusions

In conclusion, herein we generated data confirming the areas activated during the N-Back task (Owen et al., 2005) and the reduced number of functionally lateralized areas, potentially due to age (Cabeza, 2002) or to high cognitive load (Reuter-Lorenz and Cappell, 2008). Importantly, lateralized areas were consistent in the two loads of working memory assessed and we were able to show for the first time a direct correlation between functional lateralization of an area and working memory performance. In fact, superior parietal lobule leftward lateralization was associated with improved accuracy in the 1-Back, highlighting the importance of left/right balance for ideal performance.

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8. Figures

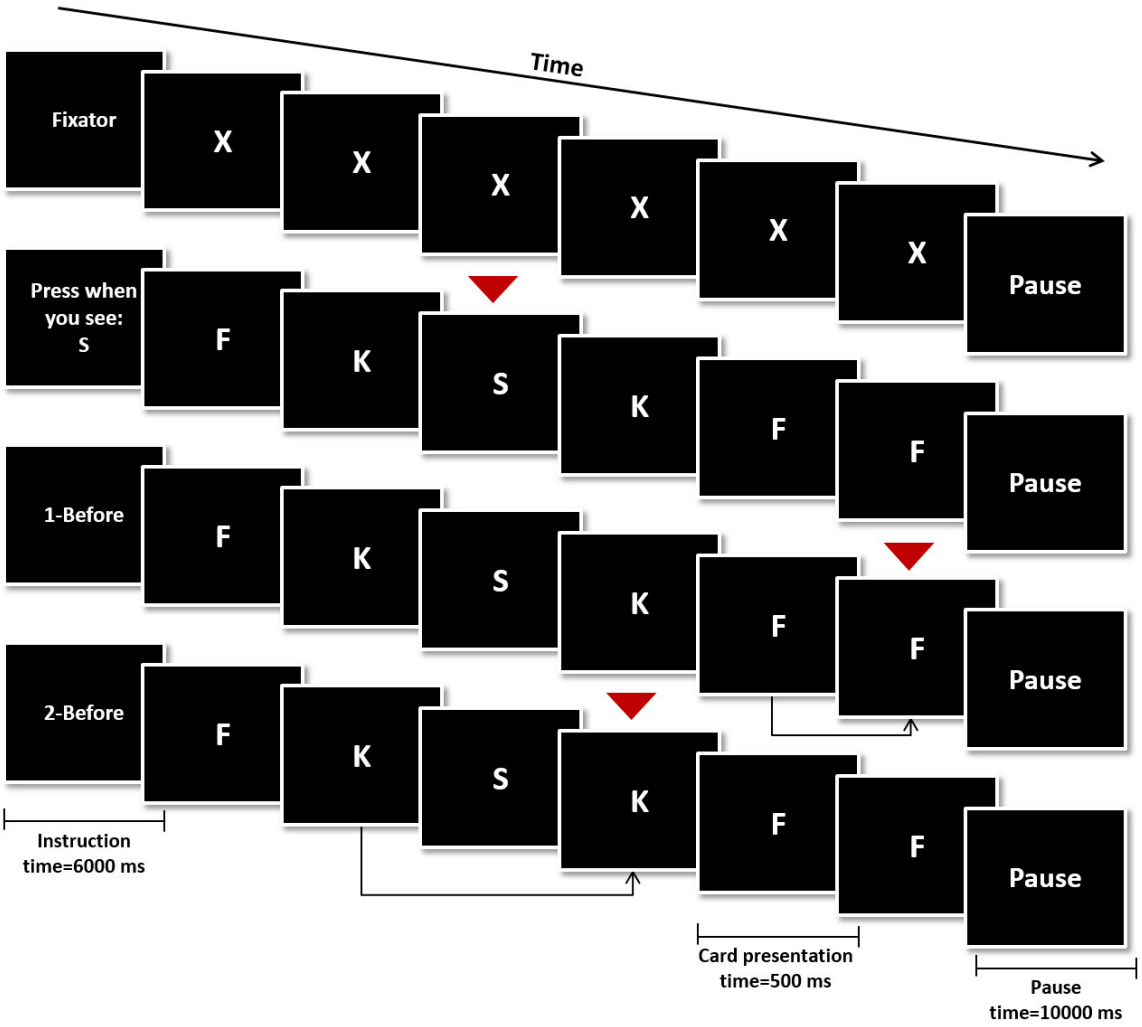

Fig. 1. Schematic representation of the N-Back Task. The four conditions were initiated by an instruction card (6000 ms), followed by the presentation of 16 trial cards (500 ms) and ended with a pause card (10 000 ms). In the rest condition the letter "X" was presented in every trial and none was a target card (top). In the remaining conditions, target cards (red arrows) were a specific letter (0-Back – second row from the top), any card similar to the one shown in the previous trial (1-Back – third row from the top) or any card similar to the one shown two trials before (2-Back – bottom row).

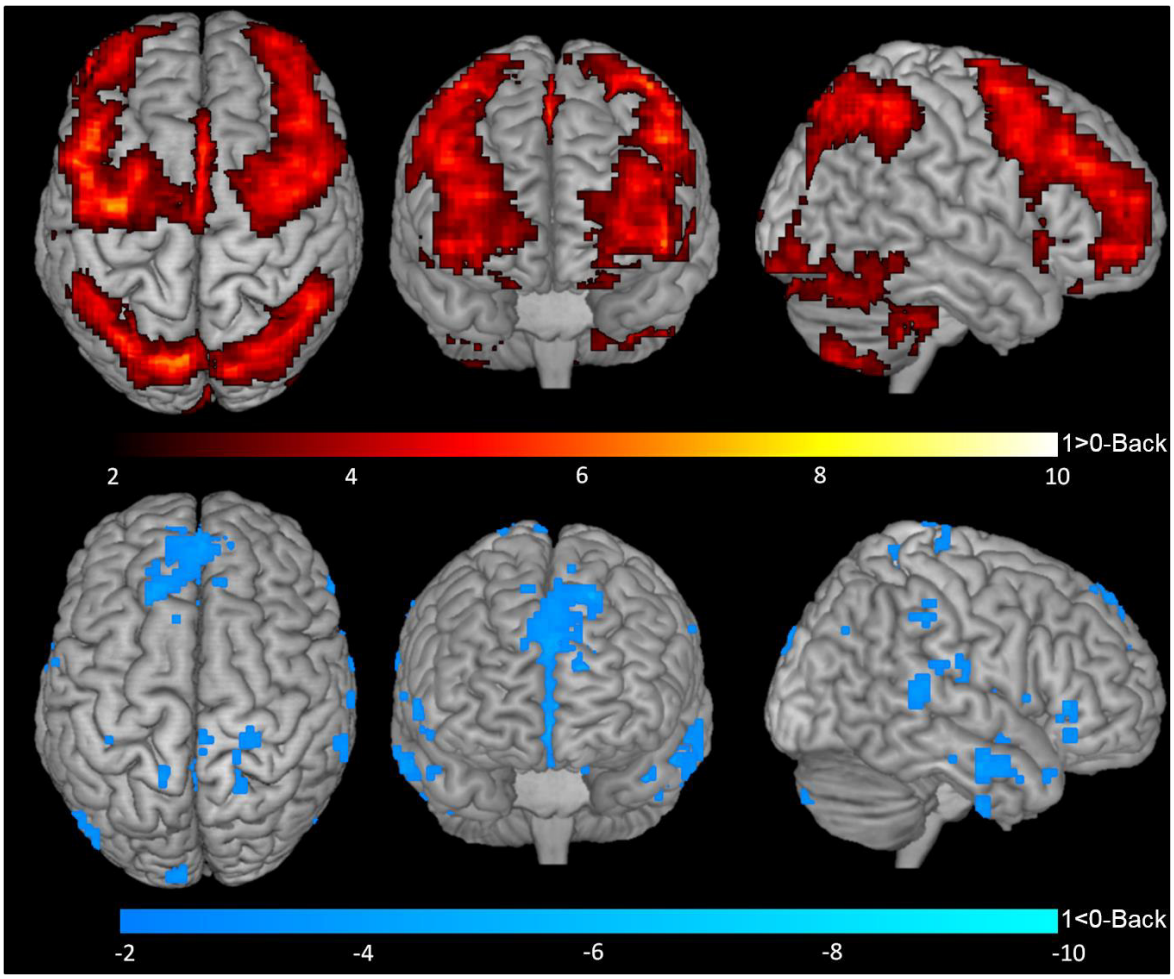

Fig. 2. BOLD response during 1-Back performance. Representation of 1-Back performance functional activation (1-Back>0-Back - top) and deactivation (1-Back<0-Back - bottom) in axial (left), coronal (middle) and sagittal (right) views. Hotter and colder colors represent respectively increased activation and deactivation.

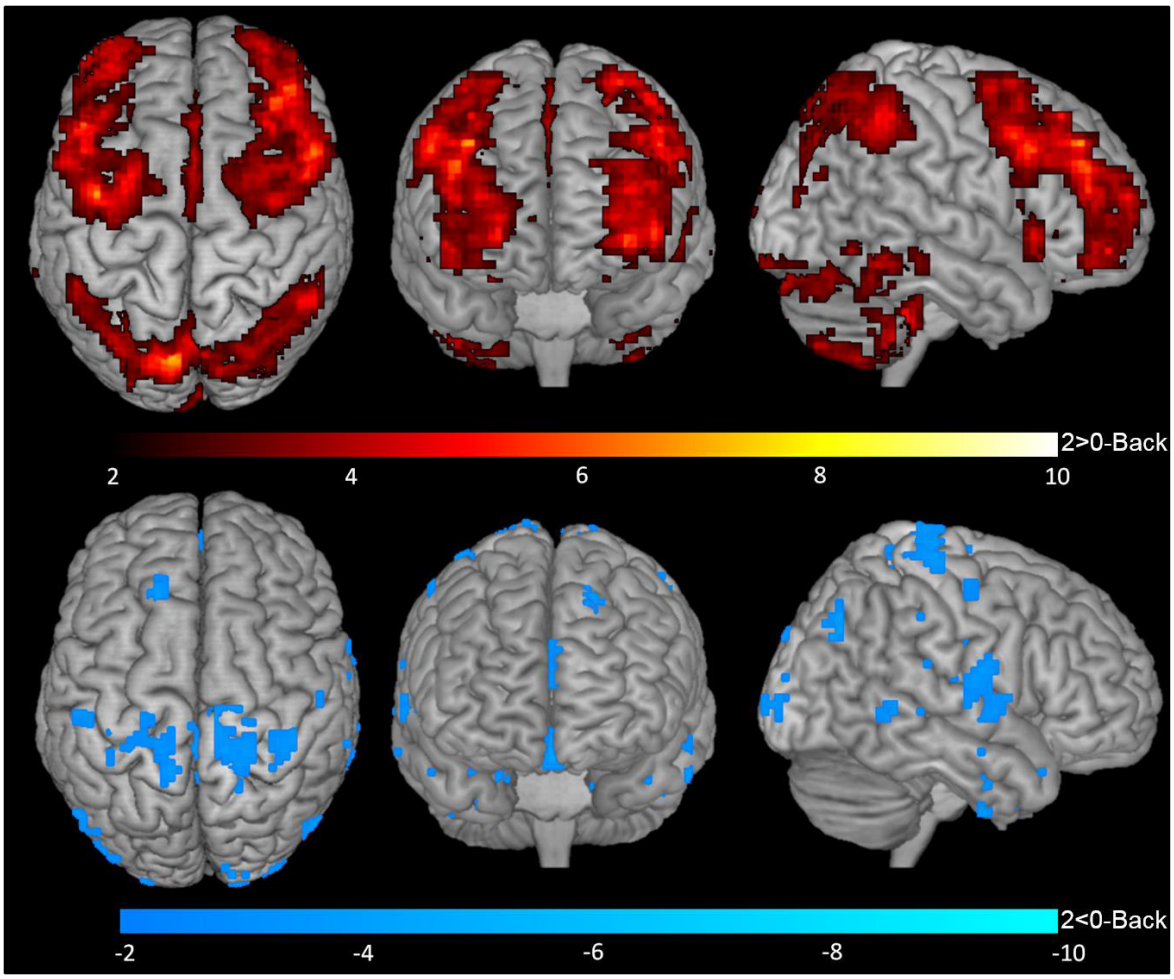

Fig. 3. BOLD response during 2-Back performance. Representation of 2-Back performance functional activation (2-Back>0-Back - top) and deactivation (2-Back<0-Back - bottom) in axial (left), coronal (middle) and sagittal (right) views. Hotter and colder colors represent respectively increased activation and deactivation.

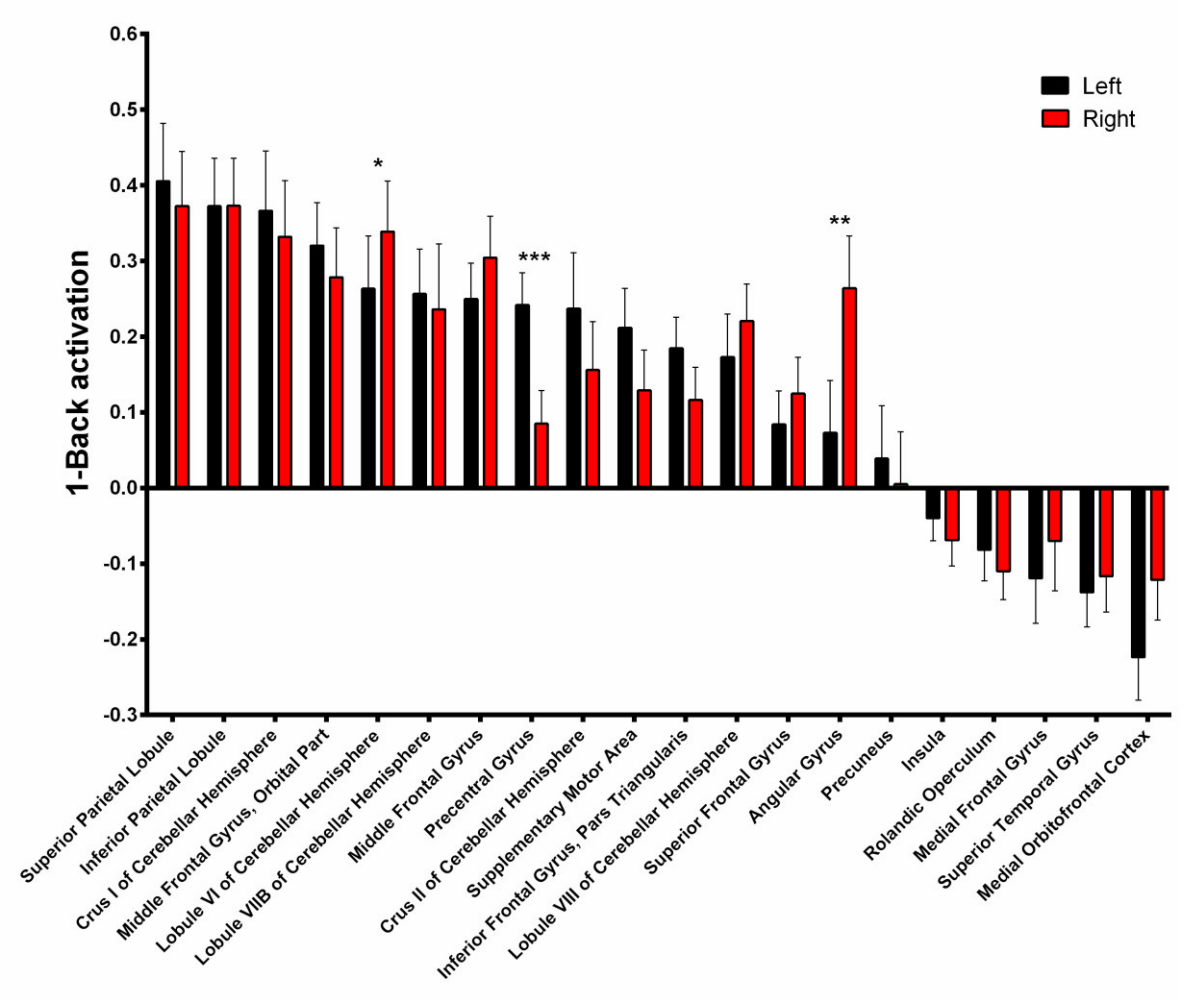

Fig. 4. 1-Back-related BOLD asymmetries. Comparison between left and right activations/deactivations during 1-Back performance. *α <0.05, **α <0.01, ***α<0.001 in Bonferroni-corrected paired comparisons.

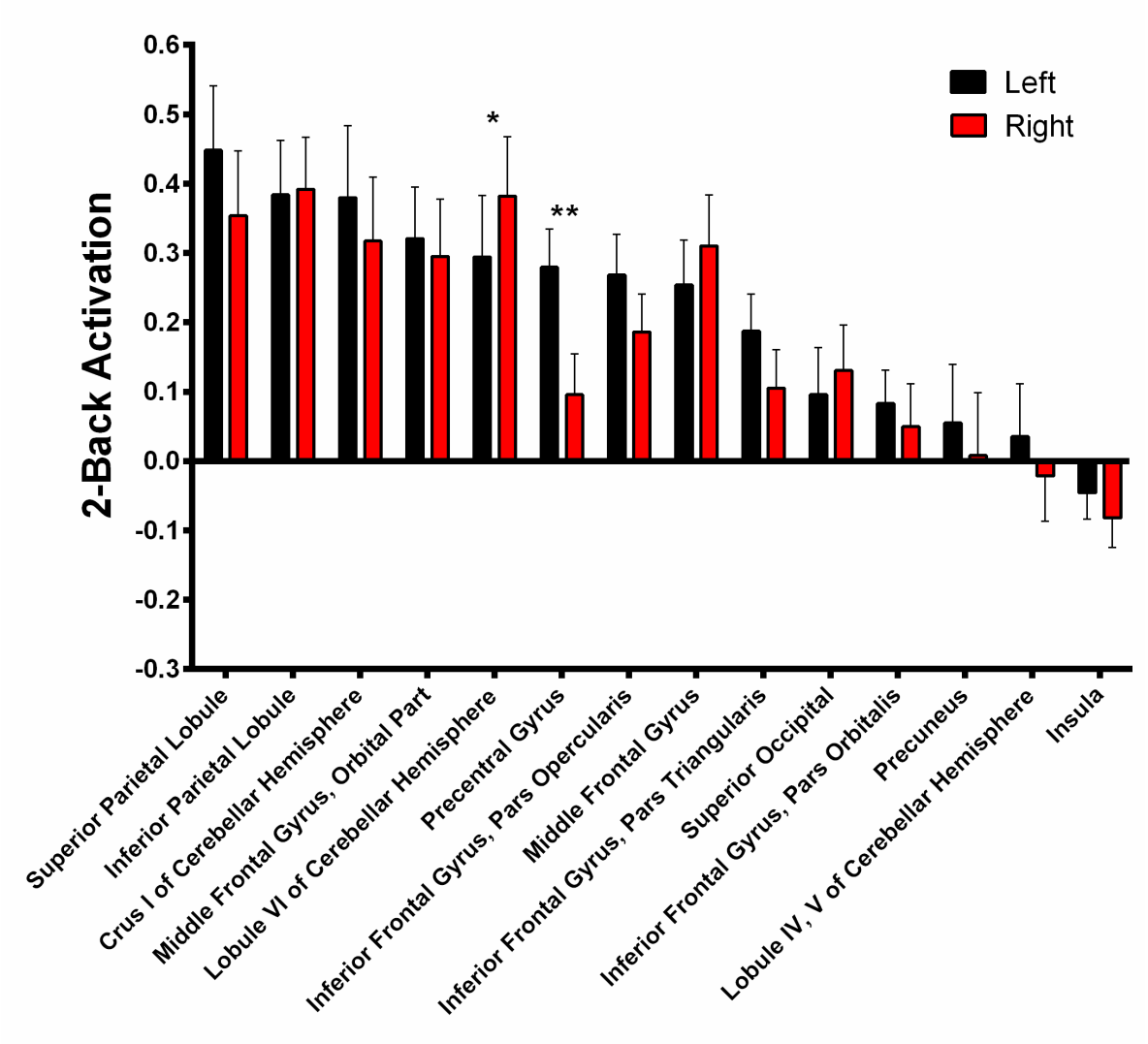

Fig. 5. 2-Back-related BOLD asymmetries. Comparison between left and right activations/deactivations during 2-Back performance. *α <0.05, **α <0.01 in Bonferroni-corrected paired comparisons.

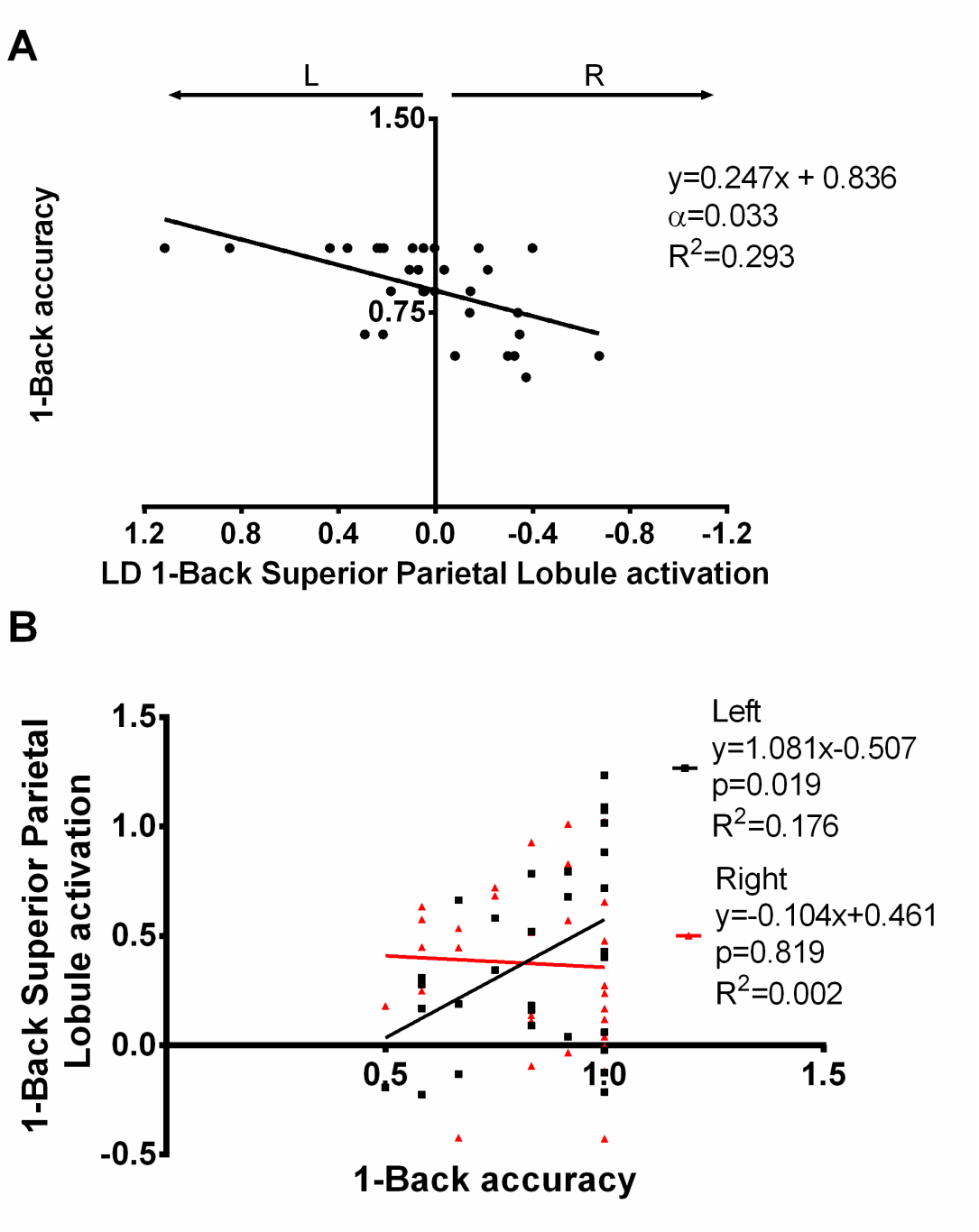

Fig. 6. Superior parietal lobule's laterality degree in association with 1-Back accuracy. (A) Superior parietal lobule's LD was positively correlated with 1-Back accuracy, i.e. increased left to right difference was associated with better performance. (B) The association seen in (A) seems to be mainly due to a positive correlation between left superior parietal lobule activation and task performance. LD=laterality degree; α=Bonferroni corrected p-value.

9. Tables

Cluster		Peak		Coordinates			Hemisphere	AAL Area
α	Size	α	Z	X	Y	Z		
					1->0-Back			
<0.001	191	<0.001	7.033	-30	-66	51	Left	superior parietal lobule
		0.001	5.464	-15	-72	57	Left	superior parietal lobule
		0.002	5.433	-6	-69	57	Left	precuneus
<0.001	59	<0.001	6.416	-30	-3	60	Left	precentral gyrus
		<0.001	5.693	-39	-3	63	Left	precentral gyrus
		0.001	5.592	-24	9	57	Left	middle frontal gyrus
<0.001	174	<0.001	6.354	-48	9	33	Left	precentral gyrus
		<0.001	6.021	-45	24	39	Left	middle frontal gyrus
		<0.001	5.940	-42	3	42	Left	precentral gyrus
<0.001	78	<0.001	5.893	3	15	51	Right	supplementary motor area
		0.004	5.273	-3	0	60	Left	supplementary motor area
<0.001	46	<0.001	5.809	6	-78	-15	N/A	lobule VI of vermis
		0.006	5.180	6	-81	-30	Right	crus II of cerebellar hemisphere
<0.001	60	<0.001	5.671	-42	-45	45	Left	inferior parietal lobule
		0.001	5.546	-51	-42	48	Left	inferior parietal lobule
<0.001	123	<0.001	5.664	39	-60	51	Right	angular gyrus
		0.001	5.521	33	-57	57	Right	superior parietal lobule
		0.002	5.395	51	-45	48	Right	inferior parietal lobule
<0.001	14	<0.001	5.640	-6	-75	-24	Left	crus I of cerebellar hemisphere
<0.001	11	0.001	5.619	-48	48	-3	Left	middle frontal gyrus. orbital part
		0.045	4.793	-45	48	6	Left	inferior frontal gyrus. pars triangularis
<0.001	24	0.001	5.596	30	3	60	Right	superior frontal gyrus
<0.001	26	0.001	5.592	39	15	51	Right	middle frontal gyrus
		0.003	5.318	51	9	36	Right	precentral gyrus
0.001	6	0.001	5.440	36	24	0	Right	insula
<0.001	24	0.002	5.368	42	30	36	Right	middle frontal gyrus
		0.012	5.052	45	39	30	Right	middle frontal gyrus
<0.001	26	0.002	5.360	27	-72	-21	Right	lobule VI of cerebellar hemisphere
<0.001	20	0.004	5.264	12	-75	51	Right	superior parietal lobule
<0.001	29	0.004	5.263	-36	-72	-21	Left	crus I of cerebellar hemisphere
		0.012	5.053	-30	-69	-27	Left	crus I of cerebellar hemisphere
0.007	2	0.011	5.070	-33	-36	-42	Left	lobule VIII of cerebellar hemisphere
0.002	4	0.014	5.016	42	3	63	N/A	undefined
0.015	1	0.028	4.890	33	63	15	N/A	undefined
0.015	1	0.033	4.855	36	60	-9	Right	middle frontal gyrus. orbital part
0.015	1	0.041	4.810	42	3	54	Right	middle frontal gyrus
0.015	1	0.042	4.808	-30	-66	-42	Left	lobule VIIB of cerebellar hemisphere
0.015	1	0.042	4.806	45	51	-6	Right	middle frontal gyrus. orbital part
0.015	1	0.043	4.803	-36	54	18	Left	middle frontal gyrus
					1-<0-Back			
<0.001	49	<0.001	6.474	39	-18	9	Right	insula
		<0.001	6.426	42	-12	15	Right	Rolandic operculum

<0.001	7	0.004	5.281	9	-54	18	Right	precuneus
		0.004	5.248	9	-51	27	Right	precuneus
<0.001	19	0.006	5.172	-6	-54	24	Left	precuneus
		0.009	5.100	-12	-57	18	Left	precuneus
0.015	1	0.017	4.987	42	-12	-3	Right	insula
0.003	3	0.019	4.963	-9	54	12	Left	medial frontal gyrus
0.015	1	0.027	4.895	-42	-33	15	Left	superior temporal gyrus
0.007	2	0.039	4.819	-9	48	3	Left	medial frontal gyrus
0.015	1	0.046	4.788	-3	54	-9	Left	medial orbitofrontal cortex
0.015	1	0.047	4.784	0	45	-9	Left	medial orbitofrontal cortex
2->0-Back								
<0.001	9	<0.001	5.902	-48	9	33	Left	precentral gyrus
<0.001	14	<0.001	5.693	-9	-69	60	Left	precuneus
0.001	4	0.001	5.561	39	42	39	Right	middle frontal gyrus
<0.001	6	0.001	5.438	42	45	27	Right	middle frontal gyrus
<0.001	8	0.002	5.343	33	24	0	Right	insula
<0.001	7	0.003	5.337	-30	24	0	Left	insula
<0.001	8	0.004	5.245	51	9	42	Right	precentral gyrus
0.001	4	0.008	5.129	-42	3	57	Left	precentral gyrus
0.014	1	0.009	5.099	-30	-27	-33	Left	lobule IV. V of cerebellar hemisphere
0.001	4	0.010	5.092	54	21	36	Right	inferior frontal gyrus. pars opercularis
<0.001	7	0.011	5.064	-9	-75	-30	Left	crus I of cerebellar hemisphere
<0.001	10	0.011	5.063	-48	30	30	Left	middle frontal gyrus
0.014	1	0.015	5.008	-30	9	66	N/A	undefined
0.014	1	0.018	4.977	-33	57	-3	Left	middle frontal gyrus. orbital part
0.014	1	0.022	4.937	-24	-69	45	Left	superior parietal lobule
0.005	2	0.023	4.927	-33	-33	-36	Left	lobule VI of cerebellar hemisphere
<0.001	6	0.025	4.910	-27	-66	51	Left	superior parietal lobule
0.014	1	0.026	4.900	36	27	-6	Right	inferior frontal gyrus. pars orbitalis
0.005	2	0.030	4.869	-30	-57	51	Left	inferior parietal lobule
0.014	1	0.036	4.839	-48	24	45	N/A	undefined
0.014	1	0.036	4.838	-24	-72	36	Left	superior occipital
0.014	1	0.036	4.836	42	21	3	Right	inferior frontal gyrus. pars triangularis
0.014	1	0.039	4.821	-42	0	42	Left	precentral gyrus
0.014	1	0.044	4.795	45	45	18	Right	middle frontal gyrus
0.014	1	0.046	4.785	-45	51	-3	Left	middle frontal gyrus. orbital part
2-<0-Back								
<0.001	5	0.018	4.976	-9	-54	24	Left	precuneus

Table 1. Activations found in the N-Back task. Statistically significant BOLD changes found in the 1- and 2-Back (vs 0-Back) contrasts. α=FWE corrected p-values; Z=z-score values of t-statistics; AAL=Automated Anatomical Labeling atlas.

10. Supplementary data

	Left		Right		α	Z	Cohen's d
	mean	SD	mean	SD			
Superior Parietal Lobule	0.406	0.425	0.373	0.401	2.173	0.255	0.080
Inferior Parietal Lobule	0.372	0.352	0.373	0.351	1.598	0.000	-0.001
Crus I of Cerebellar Hemisphere	0.366	0.441	0.332	0.412	3.031	0.666	0.081
Middle Frontal Gyrus, Orbital Part	0.320	0.315	0.278	0.364	2.497	0.353	0.123
Lobule VI of Cerebellar Hemisphere	0.264	0.389	0.339	0.373	0.016	3.331	-0.197
Lobule VIIB of Cerebellar Hemisphere	0.256	0.323	0.236	0.481	3.031	0.627	0.049
Middle Frontal Gyrus	0.250	0.265	0.304	0.304	1.517	1.529	-0.192
Precentral Gyrus	0.242	0.236	0.085	0.243	<0.001	4.527	0.655
Crus II of Cerebellar Hemisphere	0.237	0.412	0.156	0.356	0.623	2.038	0.210
Supplementary Motor Area	0.211	0.290	0.129	0.297	0.299	2.352	0.281
Inferior Frontal Gyrus, Pars Triangularis	0.185	0.229	0.116	0.241	0.218	2.489	0.291
Lobule VIII of Cerebellar Hemisphere	0.173	0.319	0.221	0.273	0.623	2.018	-0.161
Superior Frontal Gyrus	0.084	0.247	0.125	0.266	1.827	1.333	-0.160
Angular Gyrus	0.073	0.385	0.264	0.385	0.010	3.469	-0.497
Precuneus	0.039	0.387	0.005	0.386	2.046	1.137	0.088
Insula	-0.040	0.165	-0.069	0.191	2.019	1.215	0.163
Rolandic Operculum	-0.082	0.229	-0.110	0.208	2.873	0.823	0.130
Medial Frontal Gyrus	-0.119	0.330	-0.070	0.366	1.558	1.470	-0.141
Superior Temporal Gyrus	-0.138	0.256	-0.117	0.263	2.652	0.490	-0.081
Medial Orbitofrontal Cortex	-0.224	0.309	-0.121	0.298	0.613	1.985	-0.338

Sup Table 1. 1-Back laterality statistics. Statistics for left vs right 1-Back BOLD response. SD=standard deviation; α=Bonferroni corrected p-value.

	Left		Right		α	Z	Cohen's d
	mean	SD	mean	SD			
Superior Parietal Lobule	0.448	0.434	0.354	0.437	1.457	1.023	0.216
Inferior Parietal Lobule	0.384	0.369	0.392	0.352	1.370	0.406	-0.022
Crus I of Cerebellar Hemisphere	0.380	0.486	0.317	0.431	1.495	1.055	0.136
Middle Frontal Gyrus, Orbital Part	0.321	0.348	0.295	0.388	1.370	0.373	0.070
Lobule VI of Cerebellar Hemisphere	0.294	0.417	0.382	0.399	0.028	3.068	-0.215
Precentral Gyrus	0.280	0.260	0.096	0.277	0.001	3.945	0.685
Inferior Frontal Gyrus, Pars Opercularis	0.269	0.272	0.186	0.258	0.265	2.289	0.312
Middle Frontal Gyrus	0.254	0.305	0.310	0.347	1.423	1.347	-0.172
Inferior Frontal Gyrus, Pars Triangularis	0.188	0.250	0.105	0.260	0.265	2.256	0.322
Superior Occipital	0.096	0.315	0.131	0.307	1.226	0.893	-0.110
Inferior Frontal Gyrus, Pars Orbitalis	0.083	0.227	0.050	0.290	1.744	1.153	0.127
Precuneus	0.055	0.394	0.008	0.423	1.180	1.510	0.115
Lobule IV, V of Cerebellar Hemisphere	0.036	0.357	-0.021	0.307	0.883	1.704	0.171
Insula	-0.046	0.177	-0.082	0.202	1.744	1.153	0.191

Sup Table 2. 2-Back laterality statistics. Statistics for left vs right 2-Back BOLD response. SD=standard deviation; α=Bonferroni corrected p-value.

	p	α	β	R^2
Superior Parietal Lobule	0.002	0.033	0.247	0.293
Inferior Parietal Lobule	0.015	0.269	0.363	0.188
Crus I of Cerebellar Hemisphere	0.044	0.612	-0.242	0.133
Middle Frontal Gyrus, Orbital Part	0.030	0.518	0.272	0.157
Lobule VI of Cerebellar Hemisphere	0.039	0.625	-0.629	0.139
Lobule VIIB of Cerebellar Hemisphere	0.040	0.625	-0.280	0.142
Middle Frontal Gyrus	0.161	1.770	0.266	0.067
Precentral Gyrus	0.009	0.163	0.536	0.215
Crus II of Cerebellar Hemisphere	0.257	2.316	-0.161	0.044
Supplementary Motor Area	0.708	4.423	0.063	0.005
Inferior Frontal Gyrus, Pars Triangularis	0.835	2.395	-0.041	0.002
Lobule VIII of Cerebellar Hemisphere	0.202	2.017	-0.270	0.056
Superior Frontal Gyrus	0.110	1.434	0.363	0.086
Angular Gyrus	0.632	4.423	-0.061	0.008
Precuneus	0.116	1.434	0.299	0.083
Insula	0.417	3.337	0.234	0.023
Rolandic Operculum	0.798	2.987	0.049	0.002
Medial Frontal Gyrus	0.725	4.248	0.068	0.004
Superior Temporal Gyrus	0.747	3.623	-0.073	0.004
Medial Orbitofrontal Cortex	0.876	1.670	-0.018	0.001

Sup Table 3. 1-Back accuracy correlations with BOLD laterality. Statistics for 1-Back accuracy correlations with laterality degree. p=p-value ; α=Bonferroni corrected p-value.

CHAPTER V

Functional encoding of inhibitory control in rats in the Variable Delay-to-Signal task

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Ongoing work

Title: Functional encoding of inhibitory control in rats in the Variable Delay-to-Signal task

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Abstract:

Impulsivity is an adaptive response allowing fast reaction to stimuli, but its dysregulation is associated with disorders such as substance abuse or attention deficit/hyperactivity disorder (ADHD). Several prefrontal and subcortical areas are known to play a relevant role in response inhibition. However, how these areas interact across the temporal window preceding the execution of timed vs premature actions is yet to be described. We have therefore assessed local field potentials (LFPs) bilaterally in frontal and striatal areas: prelimbic and orbital prefrontal (OPF) cortices, caudate and nucleus accumbens (NAcc) during the performance of a task designed to assess both action impulsivity and delay intolerance, the Variable Delay-to-Signal (VDS). We determined that all these regions play a role in behavioral inhibition in specific time frames up to 3 seconds before the response (timed or premature). Additionally, higher prefrontal-striatal coherence more than 1 second before the response is associated with impaired inhibition, while the prefrontal-prefrontal interaction plays an important role immediately before the response. Regarding delay intolerance, left NAcc theta power before long delays was negatively correlated with the number of premature responses in the same trial. In conclusion, evidence of behavioral (dis)inhibition can be found up to 3 seconds before the actual response, from which time a full network of regions is involved in the determination of the impulsive response.

Keywords: impulsivity, local field potential, left, right, power, coherence

1. Introduction

Impulsivity is defined as the tendency to act prematurely and without foresight. It comprises several forms such as impulsive action, i.e. an inability to stop a motor action, or impulsive choice, the choice of an immediate small reward over a larger but delayed reward. Impulsivity has an important adaptive role, allowing fast reactions to stimuli (Dalley et al., 2011) but dysregulation of this process has been associated with disorders such as substance abuse or attention deficit/hyperactivity disorder (ADHD) (Jentsch et al., 2014; Roberts et al., 2014; Sharma and Couture, 2014).

Functional and structural data point towards an involvement of the prefrontal cortex in close association with subcortical areas in the impulsive decision-making process in both rats (Feja and Koch, 2015) and humans (Hanggi et al., 2016) - see also for review (Dalley et al., 2011; Dalley and Robbins, 2017). In fact, regions in this network can be associated with impulsivity in both direct and indirect ways. The nucleus accumbens (NAcc), for example, plays an important role in motivation (Clithero et al., 2011) and perception of rewarded/unrewarded outcome (Cooper and Knutson, 2008) and its direct stimulation has been shown to increase impulsivity in humans (Luigjes et al., 2011). Similarly, the caudate (Caud) seems to play a role in encoding immediate value (Economides et al., 2015) and its reward-driven activation has been associated with trait impulsivity (Babbs et al., 2013). Regarding frontal regions, the prelimbic (Prl) (Garrison et al., 2013) and orbital pre-frontal (OPFC) (Liu et al., 2011) cortices are also associated with outcome processing and their engagement in the task seem to be crucial for impulsive response inhibition (Antonucci et al., 2006; Bari et al., 2011; Horn et al., 2003).

However, a comprehensive mapping of prefrontal-striatal activity and interactions associated with the impulsive response is yet to be achieved. Although areas involved in impulsive action and choice greatly overlap, they seem to be differentially involved in the process. For example, NAcc core and shell inactivation induces different effects in these two types of impulsivity (Feja et al., 2014; Moreno et al., 2013; Moschak and Mitchell, 2014; Valencia-Torres et al., 2012). Additionally, some data suggests that these processes are lateralized, such as the right>left hemisphere activation in Go/NoGo tasks (Horn et al., 2003) or the correlation between right NAcc-left ventral medial PFC interaction and impulsivity in humans (Diekhof and Gruber, 2010), as well as the negative correlation between impulsivity and left NAcc core gray matter density and the levels of glutamate decarboxylase and dendritic markers (microtubule associated protein 2 and spinophilin) in rats (Caprioli et al., 2014). We have thus taken a systems approach and assessed

local field potentials (LFPs) bilaterally in frontal and striatal areas: prelimbic and OPFC cortices, caudate and NAcc during the performance of an impulsivity task, the Variable Delay-to-Signal (VDS). This task is of simple application and is able to monitor both impulsive action and delay intolerance (Leite-Almeida et al., 2013), allowing determination of the temporal and regional dynamics that lead to an impulsive response.

2. Methods

2.1. Animals

Ten male Wistar-Han rats (Charles-River Laboratories, Barcelona, Spain) 4-5 months old were maintained at 22°C temperature, 12 h light/dark cycle (lights on at 8a.m.) and ad libitum food and water (except during behavior, in which food was restricted to 1h per day). Experiments were conducted according to European Union Directives (2010/63/EU).

2.2. Electrode implantation

Animals were deeply anesthetized using a 2-4% sevoflurane in 100% oxygen mixture and placed in a stereotaxic frame. For each animal, 10 in-house produced single-wire NiChrom electrodes targeted at left and right Prl (anterior-posterior (AP) +3.3, medial-lateral (ML) ±0.8, dorso-ventral (DV) -3.4 relative to bregma) and OPFC (AP +3.3, ML ±1.8, DV -5.6) cortices, NAcc (AP +2.0, ML ±1.4, DV -6.8), Caud (AP +2.0, ML ±1.4, DV -4.6) and claustrum (AP +2.8, ML ±2.5, DV -5.0) were inserted, connected to a Mill-Max connector (Mill-Max Mfg. Corp., USA) and cemented to the skull. Animals were allowed to recover for at least one week before initiating behavioral tests. At the end of the experiment, electrode placement was verified in the sliced brain and channels were excluded based on this analysis (see red marks in Fig. 1A).

2.3. Variable delay-to-signal (VDS)

The VDS task was performed as previously described (Leite-Almeida et al., 2013) in a 35x31cm plexiglas box. One wall contained an orifice 2.5x2.5cm lifted 1.5 cm from the grid floor and was equipped with a nosepoke sensor (infrared beam – sensor 3) and a 3W light (nosepoke orifice). A similar orifice, also equipped with a nosepoke sensor (sensor 6) and a 3W light was present in the opposing wall and was associated with a pellet dispenser (feeding orifice; TSE Systems, Germany). The task was divided in three phases: habituation, training and test.

Four habituation sessions (2 per day, 5 hours apart) were performed. On sessions 1 and 2, animals were allowed to explore the operant box for 15 min with all lights off and 10-12 sugar pellets (45 mg; Bioserv Inc., USA) available at the feeding orifice. On sessions 3 and 4, all lights were on (house light and lights of both orifices), 10-12 and 5-7 pellets were available respectively in the feeding and nosepoke orifices and the animal was allowed to explore for 30 min.

Training occurred in 10-12 sessions (2 per day, 5 hours apart). Each session comprised 100 trials performed in a maximum of 30 min. A session began with the lightning of the house light and the animals learned to respond in the nosepoke orifice after its light was turned on, consequently receiving a sugared pellet in the feeding orifice (timed response). Responses performed in the 3 s delay before the nosepoke orifice light was turned on (delay period) were considered premature, not rewarded and punished with a 3 s timeout (all lights off). The absence of response (no nosepoke within the 60s of timed response time) was also punished with a timeout (see Sup Fig. 1A for detailed organigram of train sessions). A measure of action impulsivity is withdrawn from the prematurity rate at the end of training (Leite-Almeida et al., 2013).

Test was performed in a single session of 120 trials performed in a maximum of one hour. It was delivered in a similar fashion as training except multiple premature responses were allowed (not punished) and delays were variable. The first 25 trials had 3 s delays (3si), the next 70 trials had random 6 s or 12 s delays (6s and 12s respectively) and the final 25 trials returned to 3 s delays (3sf - see Sup Fig. 1A for detailed flowchart of the test session). Delay intolerance is measured by the ratio of prematurity at 3sf relative to 3si (log(3sf/3si; logarithmic transformation guarantees equivalence of positive and negative variations).

2.4. Local field potentials (LFPs) acquisition and analysis

Data was acquired during VDS performance using the dacqUSB system (Axona Ltd., UK) at a sampling rate of 4800 Hz. Signal was bandpass filtered at 3-110 Hz and amplified 4000 times. TTL data regarding light and sensor activation was collected at 25 Hz.

Raw data was imported to Matlab (Mathworks®, USA) and analyzed with custom-written scripts using the Chronux toolbox (http://chronux.org/) (Mitra and Bokil, 2008). In short, data was down-sampled to 600Hz, referenced, cleaned of artifacts in a semi-automatic manner (based on maximum values and distance to average), time-locked with behavior and detrended. In all

analyses, filter was 3-100Hz, time-bandwidth product (TW) was 3 and the number of tapers (K) was 5. Frequency ranges were defined as theta (3-7Hz), beta (7-40Hz), low gamma (40-75Hz) and high gamma (75-100Hz). All statistical analyses were FDR-corrected for multiple comparisons.

All channels were referenced to the left and right claustrum and power data was compared. These two approaches were compared and as no statistically significant differences were found, the analysis proceeded with all channels referenced to the left claustrum.

For training analysis, three bins of one second before and one after the nosepoke were selected and aimed at determining differences between timed and premature responses. Moving window was defined as 0.15s with 66% overlap and frequency resolution was 1.172Hz. For each animal, the spectrogram/coherogram for each trial was calculated and then averaged among all trails. Data zscore allowed averaging of all animals. For analysis in ranges of frequencies, for each time interval, power/coherence within each range was averaged and data was zscored for each animal in the time domain. Statistical analysis was performed for each one-second bin using a two-way repeated measures ANOVA in which factors were time (0.05 s resolution) and type of response (timed vs premature). For assessment of power laterality, a laterality index was established for each trial as (L-R)/L+R), in which L=power in the left region and R=power in right region. All trials of each animal were averaged for group analysis.

For analysis of the test's long delays, average power in the 1 s bin before the first premature response of each 6s and 12s trial was calculated, retrieving a frequency resolution of 0.146Hz. For each animal, spectrums of each trial were zscored, data within each frequency range was averaged, and power of all trials in which the animal showed a given prematurity rate (premature responses per minute) was averaged. Correlations between frequency range-specific power and prematurity rate were performed and similar analyses were done for power laterality and coherence.

3. Results

3.1. Behavior

The majority of the animals reached full performance within 10 training sessions and at this point average omissions were 0.6±1.245 (mean±SD), timed nosepokes were 62.6±14.09 and

premature responses 33.1±19.25 (Fig. 1B). Due to late learning, two animals performed two extra training sessions.

3.2. Functional correlates of premature responding (last training session)

A major power variation in theta, beta and low gamma ranges around the nosepoke is evident in a visual inspection of spectrograms (Fig. 2). An increase in power can be seen immediately before this response in all regions expect left and right OPFC, independently of response type, timed or premature. However, within a second after response power increases in all channels in timed but not in premature responses.

Statistical analyses of differences between timed and premature responses were performed from 3 s before to 1 s after the nosepoke, in 1 s bins. Power analysis (Fig. 2) retrieved effects of response type (resp - timed vs premature) and/or resp vs timepoint (tp) interaction in all time intervals. At 3 to 2 seconds before the response, effects were found in the left Caud (high gamma), left NAcc (low and high gamma), left Prl (low gamma), left OPFC (low and high gamma), and right NAcc (low and high gamma). At 2 to 1 seconds before nosepoke, effects were found in the left Caud (theta and beta), left OPFC (theta) and right NAcc (high gamma). One to 0 second before nosepoke, effects were found in the left Caud (high gamma), right Prl (theta), and right OPFC (theta, beta and high gamma). Finally, within the second after nosepoke, effects were found in almost all regions, including the left Caud (low and high gamma), left NAcc (theta, beta and low gamma), left Prl (theta, beta, low and high gamma), left OPFC (low gamma), right Caud (theta, beta and high gamma), right NAcc (theta, beta, low and high gamma), and right Prl (theta, beta, low and high gamma). Statistics of all power data can be found in Sup Table 1.

On the other hand, effects on laterality analyses were only found in the NAcc (Fig. 3 and Sup Table 2) in the second after the response at low and high gamma ranges.

Coherence analysis revealed resp effects in the low gamma range at 2 to 1 seconds before nosepoke between the left Prl and the left and right NAcc. Within the 1 second before nosepoke, resp x tp interaction effects in the high gamma range were found between left and right Prl and between right Prl and right OPFC. Detailed information can be found in Fig. 4 and Sup Table 3.

3.3. Functional correlates of behavioral inhibition during training

Taking the results presented in the previous section, we aimed to discard eventual interferences that could arise from subtle motor differences between timed and premature responding. Considering the premise that animals should learn the operant association (nosepoke = reward) before learning that this association is only valid when the nosepoke orifice light is turned on (i.e. the difference between a timed and a premature response), the first session at which each animal showed effective learning of operant behavior (i.e. performed 100 trials within the allowed session time of 30 min) was used as control.

Similar differences between timed and premature response power were found in the 3 to 2 s before nosepoke time bin in the left (resp at low gamma - $F_{(1,9)}$=13.398; p=0.005; η^2_p=0.598) and right NAcc (resp at low gamma - $F_{(1,9)}$=7.314; p=0.024; η^2_p=0.448 -, and resp at high gamma – $F_{(1,9)}$=10.634; p=0.010; η^2_p=0.542). At 2 to 1 s before nosepoke, an effect was found in the right NAcc (resp at high gamma – $F_{(1,9)}$=21.987; p=0.001; η^2_p=0.710) and 1 to 0 s before nosepoke, the right Prl showed an interaction effect (tb x resp at theta – $F_{(17,153)}$=1.718; p=0.045; η^2_p=0.160). Finally, during the second after response, effects were found in the left Caud (resp at high gamma - $F_{(1,8)}$=5.558; p=0.046; η^2_p=0.410), left NAcc (resp at theta – $F_{(1,9)}$=6.257; p=0.034; η^2_p=0.410 -, tp x resp at theta – $F_{(17,153)}$=4.055; p<0.001; η^2_p=0.311 -, and tp x resp at beta – $F_{(17,153)}$=3.419; p<0.001; η^2_p=0.275), left Prl (resp at theta – $F_{(1,9)}$=6.812; p=0.028; η^2_p=0.431 -, beta – $F_{(1,9)}$=6.975; p=0.027; η^2_p=0.437 -, and low gamma – $F_{(1,9)}$=12.228; p=0.007; η^2_p=0.576), right Caud (resp at theta – $F_{(1,9)}$=10.793; p=0.009; η^2_p=0.545 -, tp x resp at theta – $F_{(17,153)}$=1.984; p=0.015; η^2_p=0.181 -, and tp x resp at high gamma – $F_{(17,153)}$=2.042; p=0.012; η^2_p=0.185), right NAcc (resp at theta – $F_{(1,9)}$=11.882; p=0.007; η^2_p=0.569) and right Prl (resp at theta – $F_{(1,9)}$=7.184; p=0.025; η^2_p=0.444 -, tp x resp at beta – $F_{(17,153)}$=3.191; p<0.001; η^2_p=0.262 -, resp at low gamma – $F_{(1,9)}$=5.956; p=0.037; η^2_p=0.398 -, and resp at high gamma – $F_{(1,9)}$=6.415; p=0.032; η^2_p=0.416). Similarly, an effect of response was found in low gamma coherence between left Prl and left NAcc in the -2 to -1 time-frame ($F_{(1,9)}$=6.793; p=0.028; η^2_p=0.430).

As the remaining associations were not significant at this early stage, they were considered to represent non-motor correlates of the timed vs premature response and are represented in Fig. 5. Thus, 3 to 2 s before a nosepoke, left Prl low gamma power is higher when the future response is premature rather than timed. Similarly, power at low gamma in the left OPFC and high gamma in

the left Caud, left and right NAcc, and left OPFC generally showed an increase when the future response was premature and a decrease when it was timed, starting at approximately -2.5 s. In the -2 to -1 s interval, premature responses were associated with an oscillation of power in the theta and beta range in the left Caud, while power before a timed response remained relatively stable. In the left OPFC, theta power associated with a premature nosepoke suffered an abrupt increase at approximately -1.6 s, followed by a slow decrease, which was also not seen before a timed response. Coherence between left Prl and right NAcc was relatively stable during this time frame, but was consistently higher before a premature, rather than a timed response. Within the second before responding, high gamma power in the left Caud and right OPFC showed a steep decline at approximately 0.7 s before a premature, inverting the type of response showing highest power (i.e. from 1 to 0.5-0.6 s before the response, power is higher for premature and after that, power is higher for timed responses). In the right OPFC, both response types were associated with stable theta and beta power throughout most of the interval, increasing immediately before the nosepoke (-0.2 to -0.1 s), although premature responses showed a steep increase at approximately -1 to -0.8 s. High gamma coherence between the left and right Prl showed a relatively stable increase/decline in this time interval before timed/premature responses, respectively. High gamma right Prl vs ROPFC coherence for timed/premature responses seemed to vary in different directions throughout this time-frame, seeming to peak/bottom, respectively, at -0.5 and 0 s.

In the second after nosepoke, all simple effects of resp, namely beta power in left NAcc, right Caud, right NAcc and right Prl, low gamma in left and right NAcc and high gamma in left Prl and right Caud were due to overall higher power after timed than premature responses. In the left Caud, low gamma power slowly decreased in the 0.4 s after both types of response, but this was followed by abrupt variations after timed responses that could not be found after premature nosepokes. On the other hand, high gamma in the same region showed a steady increase after a timed nosepoke, while a premature response induced an oscillation in power that peaked and bottomed at approximately 0.2 and 0.5 s, respectively. Low gamma power in the left OPFC varied similarly up to 0.5 s after either response, at which point a small decrease/increase in power after timed/premature responses respectively, inverts the pattern of higher power for timed responses. High gamma power in the right NAcc shows a general increase throughout time after a timed response, which cannot be found after a premature nosepoke. In the right Prl, high gamma power shows a small peak immediately after a premature, but not a timed response, followed by a decrease/increase up to 0.7 s after a premature/timed response, respectively, and a stabilization

for both, although at different levels. NAcc power laterality also showed significant effects at this time interval. In the low gamma range, a peak (i.e. L>R power) was seen immediately after (0.2 s) a timed, but not a premature response. In the high gamma range, variations mostly seem to happen in opposite directions, which is more obvious in the first and last 0.2 s of the intervals, in which a timed nosepoke is associated with L>R and R>L power, respectively, while premature nosepokes show respectively R>L and R=L power.

3.4. Functional correlates of delay intolerance in the test session

Premature responding accumulates throughout delay time (Fig. 6A). Aiming to determine the effects of the long delays (6s and 12s), spectrum and coherence analysis in the second before the first premature of each of these delays was compared to the number of premature responses in the same trial (normalized for available time, i.e. premature responses per min). Theta power in the left NAcc was determined to be negatively correlated with subsequent prematurity rate (R^2=0.141; cor p=0.016; Sup Table 4, Fig. 6B). No effects were found regarding power laterality or coherence.

4. Discussion

In this study we have analyzed functional correlates of impulsivity during execution of the VDS task. We took a network approach that allowed us to identify the temporal progression of prefrontal and striatal involvement from 3 s before to 1 s after the actual response. Additionally, we showed that left NAcc power prior to trial initiation has predictive value for premature responding.

Behavioral results are consistent with published data (Carvalho et al., 2017; Cunha et al., 2017; Leite-Almeida et al., 2012; Leite-Almeida et al., 2013; Melo et al., 2016), with all animals effectively learning the task (virtually 0 omissions by the end of training) and showing expected prematurity rates (approximately 30% by the end of training and increasing during the long delays in the test session). A power increase in the theta, beta and low gamma ranges preceding both timed and premature nosepokes in all regions, except the OPFC, can be recognized in the spectograms. Additionally, a clear difference between timed and premature responses after the nosepoke can be seen in all channels, reflecting differences between reward delivery in the former but not in the latter. To date, only one work has assessed oscillatory activity around impulsive action, within comparable, although discrete ranges of theta (7.5-9.5 Hz) and gamma (55-60 Hz) frequencies

(Donnelly et al., 2014). Results in theta are very similar between these two works, showing prefrontal and striatal power increases before any type of response, followed by a post-response increase/decrease for correct/premature nosepokes, respectively. These authors also show a decline in gamma immediately before any type of nosepoke, immediately followed by an increase in power. This is not as clear in our data, although such may be associated with relatively smaller overall variations in power in this range of frequencies (in our data, zscored lower frequencies may vary from -0.5 to +2.0, while 55-60 Hz gamma varies between -0.5 and +0.5).

Aiming to determine the temporal progression of different regions' involvement in behavioral inhibition, 4 bins 1 s-wide were defined. Reward collection happens within a minimum of 3 and 6 s before a premature and a timed response, respectively. Time points of analysis should therefore present no interferences associated with reward delivery. Similarly, the analysis of 1 s after the nosepoke was chosen, as this is the average time that an animal takes to reach the food orifice after a timed response.

However, potential motor differences intrinsically associated with timed or premature responses, or even due to distinct lags between reward collection and the first bin of analysis (see Sup Fig. 1) cannot be entirely disregarded. We therefore excluded any findings regarding timed vs premature differences that were also present in a training session at which the animal had just learned the operant component of the task (i.e. nosepoke=reward) and therefore had not yet learnt the difference between a timed and a premature response. The rationale behind this restriction is that there cannot be behavioral inhibition when the animal is still at an early learning stage. This is a conservative approximation that at the limit will increase type II (false negative), rather than type I (false positive) errors.

The Caud, and particularly the left Caud, showed an involvement in behavioral inhibition in all stages of analysis, in which timed- and premature-associated power seems to vary differently across time both before and after the nosepoke. This is, to our knowledge, the first study in which such a temporal association has been established. However, in human literature, this region's response to immediate value of rewards (Economides et al., 2015) has been previously associated with impulsivity (Babbs et al., 2013), as well as its dopamine receptor availability (Ghahremani et al., 2012; Kim et al., 2014), showing that this area plays a role in the process.

Left and right NAcc showed an unsurprising timed vs premature difference after the response, timed responses inducing an increase in power. As reward/punishment is immediately signaled (i.e. pellet dispensing or lights out, respectively), such should be associated with perception of outcome (Cooper and Knutson, 2008; Liu et al., 2011; Stenner et al., 2015) or reward prediction error (Garrison et al., 2013; Glascher et al., 2010; Rodriguez et al., 2006; Spicer et al., 2007). Interestingly, and although both hemispheres showed some degree of involvement, these effects seem to be lateralized. In fact, after a timed nosepoke, an immediate shift of NAcc power to the left was seen, while premature responses were associated with the opposite trend. Additionally, 2 to 2.5 seconds before a premature response, power was higher than before a timed response in both hemispheres. Such may reflect increased motivation (Clithero et al., 2011) and appetitive drive (Kroemer et al., 2014), consequently limiting ability for response inhibition.

On the other hand, left (but not right) NAcc power at a much lower frequency range (theta) was found to be negatively correlated with prematurity in the long delays of the test. It should be noted that there are important timepoint differences between the two assessments. While training prematurity prediction occurs at approximately 2 seconds before the response, test prematurity is being predicted within the second before the first premature response, but up to 12 s away from the last. Additionally, as described in the original paper (Leite-Almeida et al., 2013), the training and test phases of the VDS are mostly associated with action impulsivity and delay tolerance, respectively, and, by LFP signal definition, i.e. oscillatory waves are able to travel through relatively small distances, it is possible that, not only the core, but also the shell of the NAcc is contributing to the recorded signal. It is thus possible that these power associations with higher and lower impulsivity in the two VDS phases reflect the two types of impulsivity here assessed, or that the differential core/shell role is here being captured. In fact, although it is known that the NAcc is involved in impulsivity (Dalley et al., 2011), there are marked core/shell differences in the mediating effect of noradrenergic (Economidou et al., 2012) and dopaminergic (Besson et al., 2010; Economidou et al., 2012; Moreno et al., 2013) transmission in these processes, effects which may also depend on the type of impulsivity assessed. For instance, while NAcc shell inactivation increases impulsive choice in a delay-based T-maze task and motor impulsivity in the 5-CSRTT, NAcc core inactivation has been described to decrease delay tolerance in the T-maze (Feja et al., 2014) and delay discounting (Valencia-Torres et al., 2012) (although this last result has been refuted (Moschak and Mitchell, 2014)), but to affect only performance in the 5-CSRTT (Feja et al., 2014). On the other hand, deep brain stimulation in the NAcc of OCD patients has also

increased impulsivity (Luigjes et al., 2011) and data in rats shows that core/shell stimulation respectively decreases/increases motor impulsivity (Sesia et al., 2008).

The PrI presented a role very similar to NAcc during training. Both left and right PrI showed higher activation after a timed than a premature response, which may also reflect the role of the medial prefrontal cortex in the computing of reward prediction error (Garrison et al., 2013; Knutson and Wimmer, 2007). Additionally, 2 to 3 s before premature nosepoking, left PrI power was higher than before a timed response and, interestingly, a decrease in coherence between left and right PrI immediately before the response was associated with a premature, rather than a timed response. In fact, in a stop-signal task, inhibition of this region has been shown to lead to an increase in reaction time to a stop signal (Bari et al., 2011) and an increase in motor responses (Jonkman et al., 2009). Such role in behavioral inhibition seems to rely on associations with striatal structures. The medial PFC has shown a mediating effect in NAcc dopamine response's association with impulsivity (Weiland et al., 2014) and intra-hemispheric ventromedial PFC disconnection with NAcc shell increases action impulsivity (Feja and Koch, 2015). Accordingly, our results show that higher coherence between the left PrI and the right NAcc leads to impaired behavioral inhibition (i.e. premature response). Such is in strong accordance with human reports that left higher anteroventral PFC vs right NAcc functional interaction is strongly associated with higher impulsivity in a delay discounting task (Diekhof and Gruber, 2010).

The OPFC presented side-specific involvement in this task. While the left region presented timed vs premature differences at all time-bins except immediately before the response, the right hemisphere only seemed to be involved at that stage. On the other hand, both left and right OPFC presented steeper variations in power before a premature, than before a timed response, which was reversed after the response, possibly reflecting outcome perception (Liu et al., 2011; Spicer et al., 2007). This region has previously been reported as involved impulsivity, and manipulations, namely inactivation or lesion increases impulsivity in motor (Bari et al., 2011) and choice (Kheramin et al., 2004) impulsivity, respectively. Also in humans, some degree of involvement has been shown both at structural – higher volumes associated with higher motor impulsivity (Antonucci et al., 2006; Gansler et al., 2011) –, and functional – higher fMRI activation in stop vs go trials in a stop-signal task (Ghahremani et al., 2012) – levels. It has also been reported that human behavioral inhibition is linked to more prominent activation of the right than left OPFC (Horn

et al., 2003), which is line with our data of side-dependent OPFC activation and specific involvement of right frontal connectivity in the premature response.

5. Conclusions

In conclusion, the involvement of the prefrontal-striatal network in the premature response shifts throughout the window that precedes it. In fact, more than 2 seconds before a timed or a premature response, there seems to be a major effect of the left hemisphere network, which is followed by a left Prl-right hemisphere association, determining the outcome. Additionally, left NAcc activity showed predictive value for immediate prematurity associated with delay intolerance.

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8. Figures

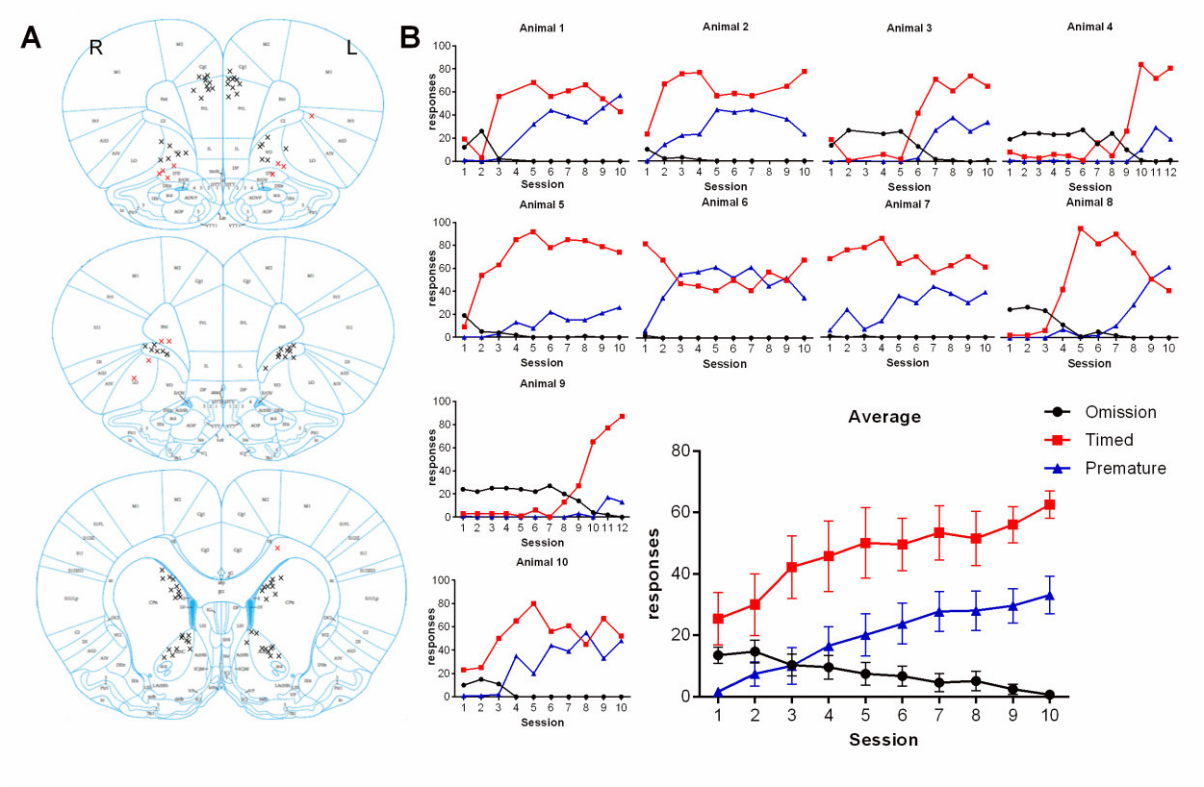


Fig. 1. Electrode placement and task learning. (A) Electrode placement in the prelimbic, orbital frontal cortex (top), claustrum (middle), caudate and nucleus accumbens (bottom). Black and red markings show respectively included and excluded electrodes, considering their post-mortem-identified location. Image adapted with permission from *The rat brain in stereotaxic coordinates*, Paxinos and Watson, pp 50,52,62, Copyright Elsevier, 2007. **(B)** Behavior during training, evidencing progression throughout the sessions individually and as average and standard error of the mean (SEM - right) for the number of omissions (black), timed nosepokes (red) and premature nosepokes (blue).

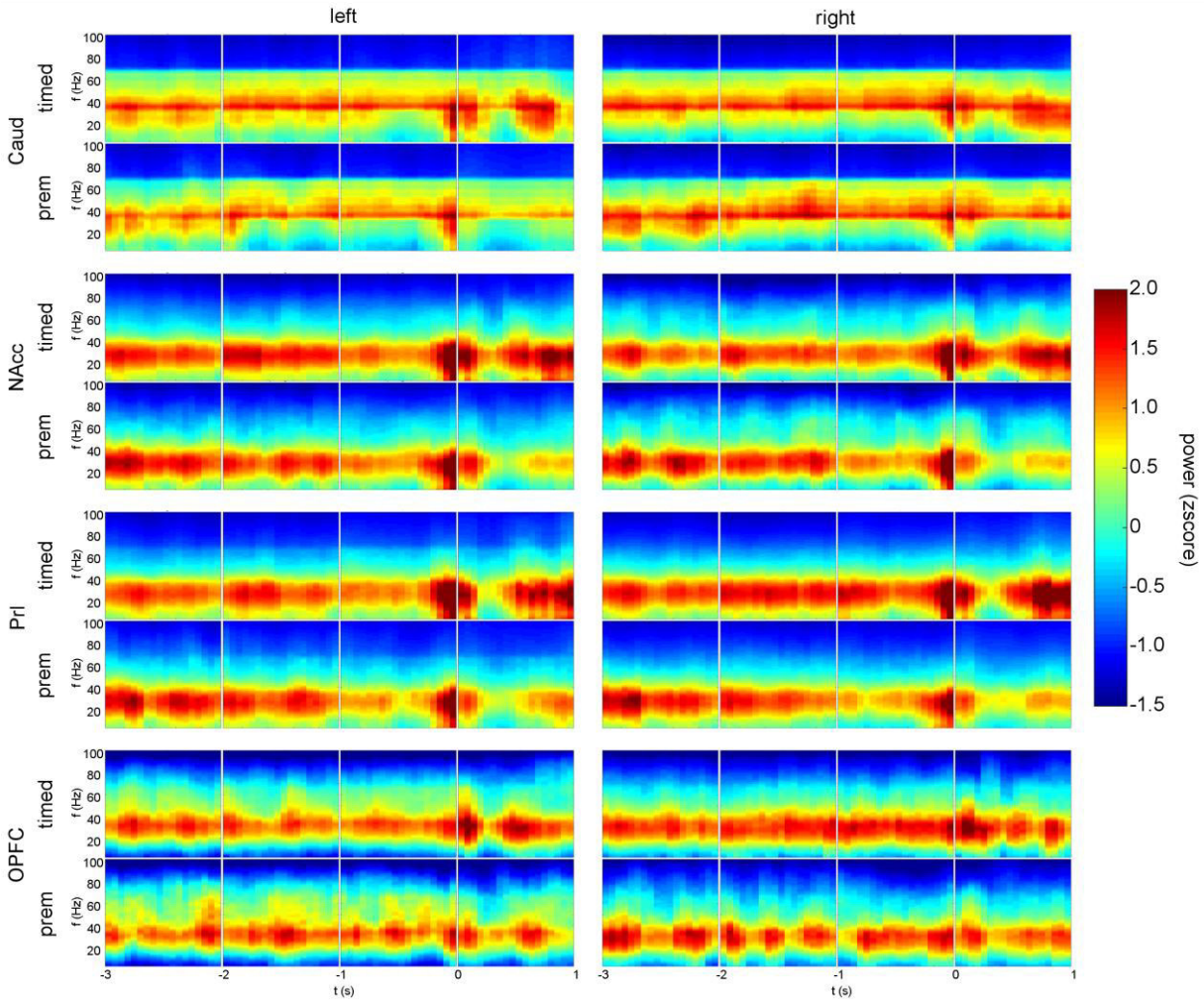


Fig. 2. Average spectrograms for timed and premature responses. Average spectrograms for timed and premature responses on the last training session are depicted. Frequencies of 3-100Hz in 1 s time-bins around time of nosepoke are shown for left (left) and right (right) channels (from top to bottom – Caud, NAcc, Prl and OPFC). Caud=Caudate; NAcc=nucleus accumbens; Prl=prelimbic cortex; OPFC=orbital prefrontal cortex; timed=timed response; prem=premature response; f=frequency; t=time.

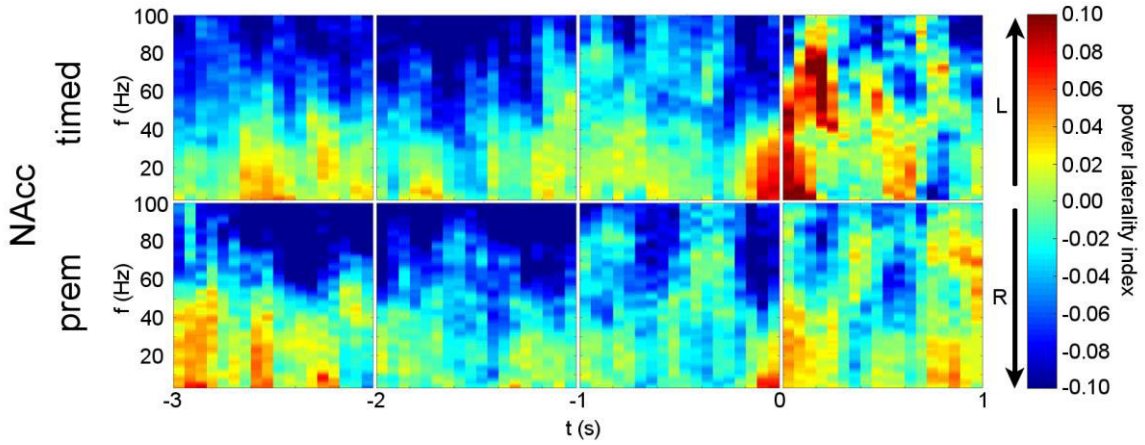


Fig. 3. Average spectrograms of NAcc laterality index for timed and premature responses. Average NAcc power laterality index is depicted for timed (top) and premature (bottom) responses in the last training session. Frequencies of 3-100Hz in 1 s time-bins around time of nosepoke are shown. L=left; R=right; NAcc=nucleus accumbens; timed=timed response; prem=premature response; f=frequency; t=time.

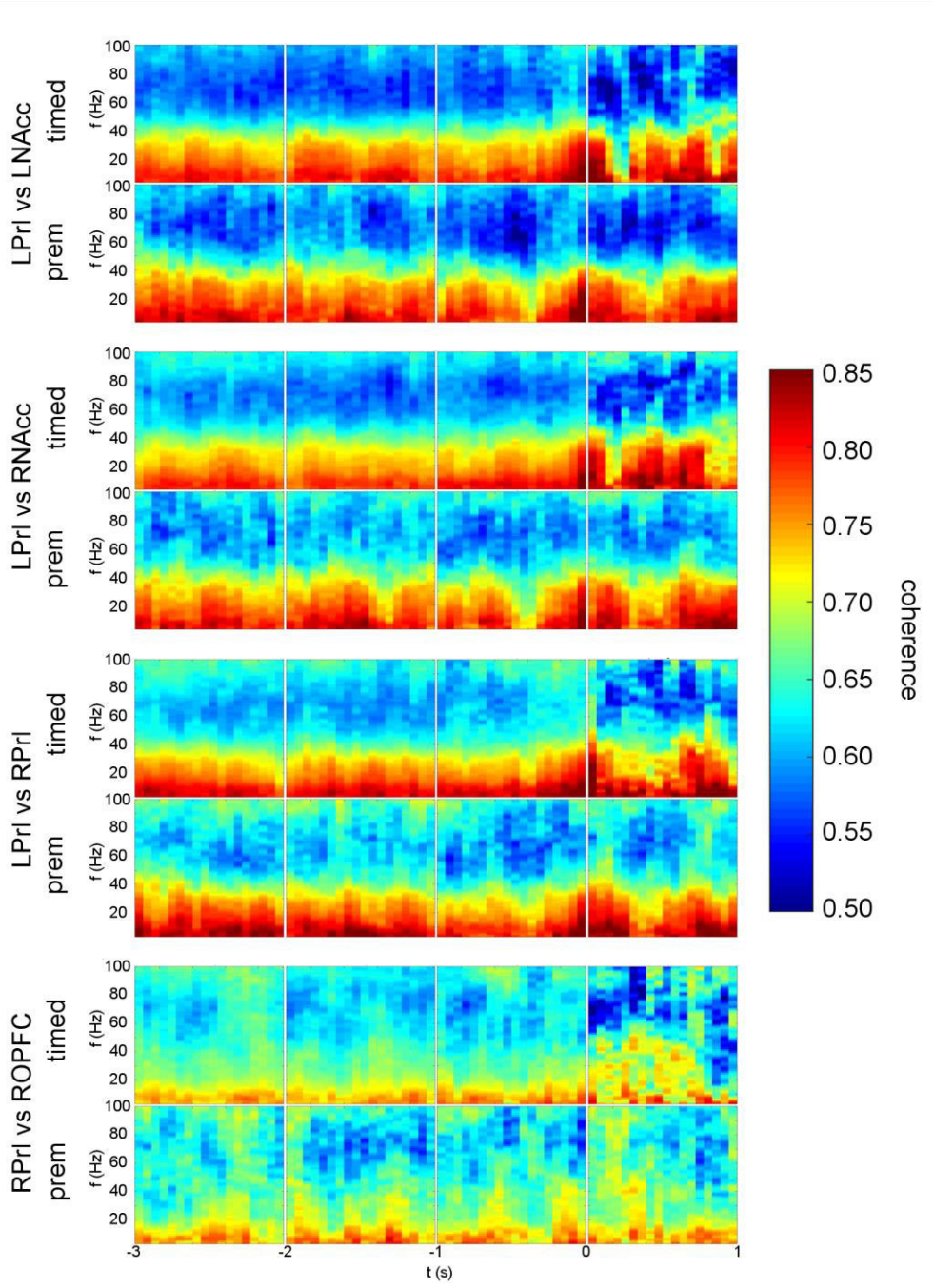


Fig. 4. Significant average coherograms for timed and premature responses. Depiction of average coherograms on the last training session for which significant timed vs premature differences were observed. Frequencies of 3-100Hz in 1 s time-bins around time of nosepoke are shown for left (left) and right (right) channels (from top to bottom – left Prl vs left NAcc, left Prl vs right NAcc, left Prl vs right Prl and right Prl vs right OPFC). L=left; R=right; Caud=Caudate; NAcc=nucleus accumbens; Prl=prelimbic cortex; OPFC=orbital prefrontal cortex; timed=timed response; prem=premature response; f=frequency; t=time.

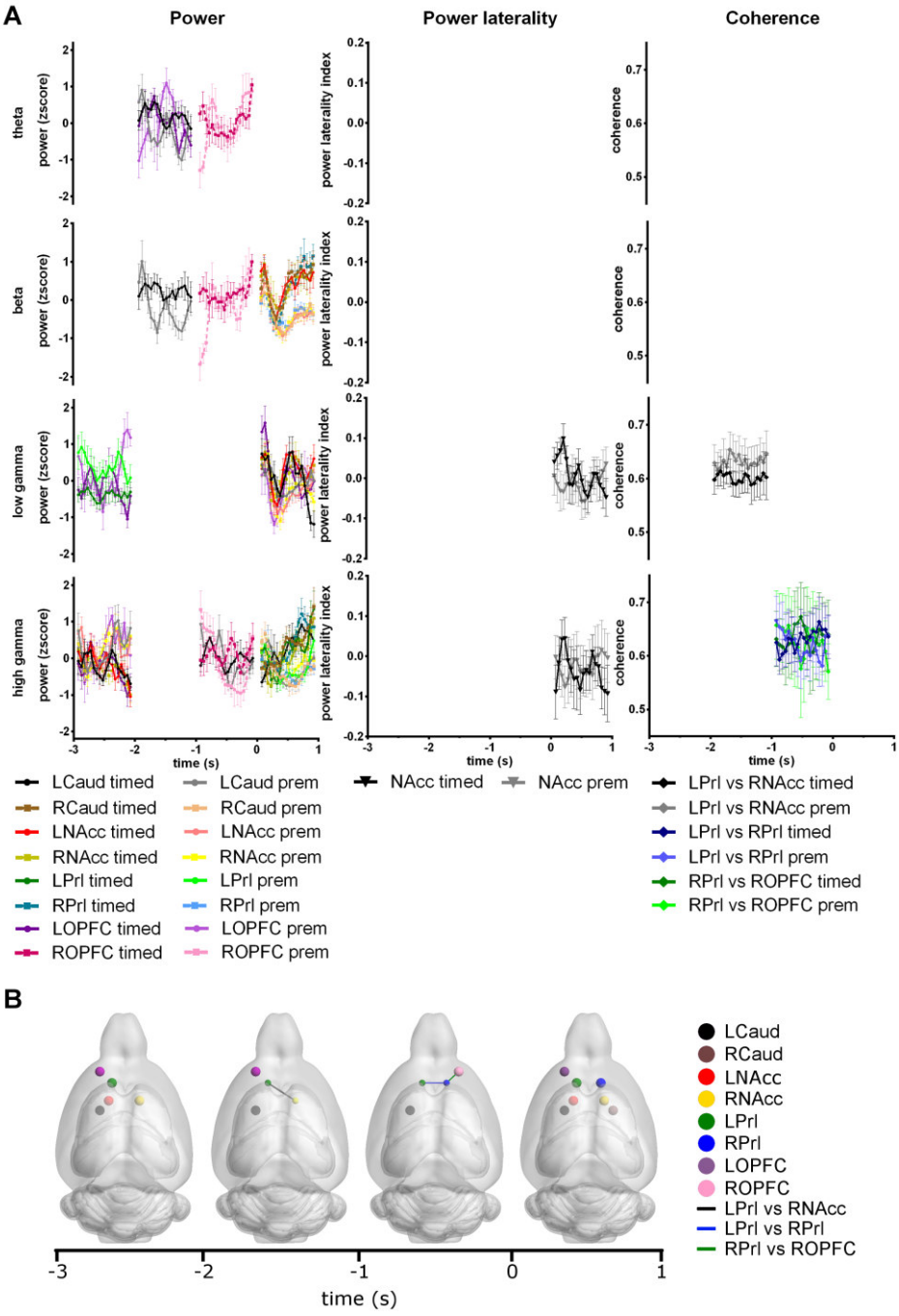


Fig. 5. Power, laterality and coherence significant data. Depiction of data that maintained significance after exclusion of effects found at the learning stage. **(A)** Power (left), power laterality (middle) and coherence (right) are shown separately for all analyzed ranges of frequency (from top to bottom – theta, beta, low gamma and high gamma) for 1 s time-bins around the time of nosepoke. **(B)** Pictographic depiction of regions that showed effects in the 4 time bins in terms of power (large circles) or coherence (small interconnected circles). L=left; R=right; Caud=Caudate; NAcc=nucleus accumbens; Prl=prelimbic cortex; OPFC=orbital prefrontal cortex; timed=timed response; prem=premature response.

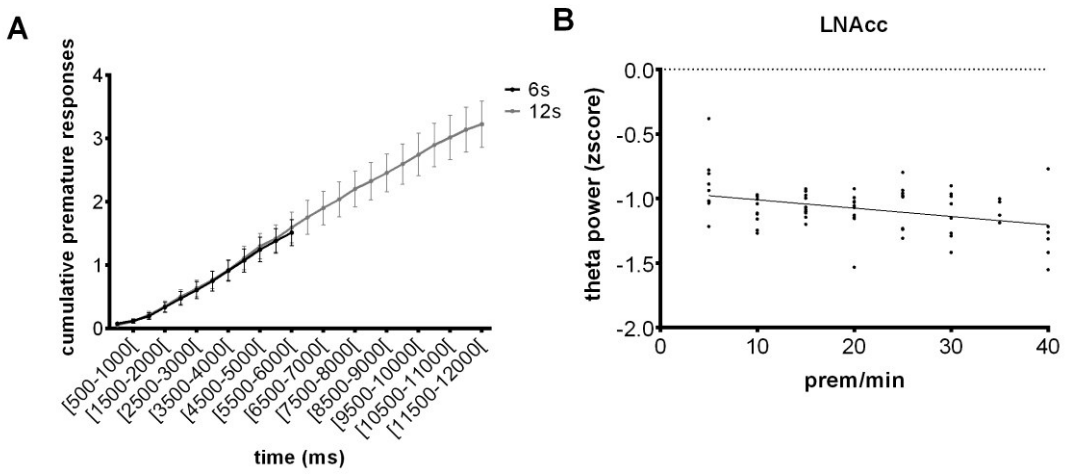
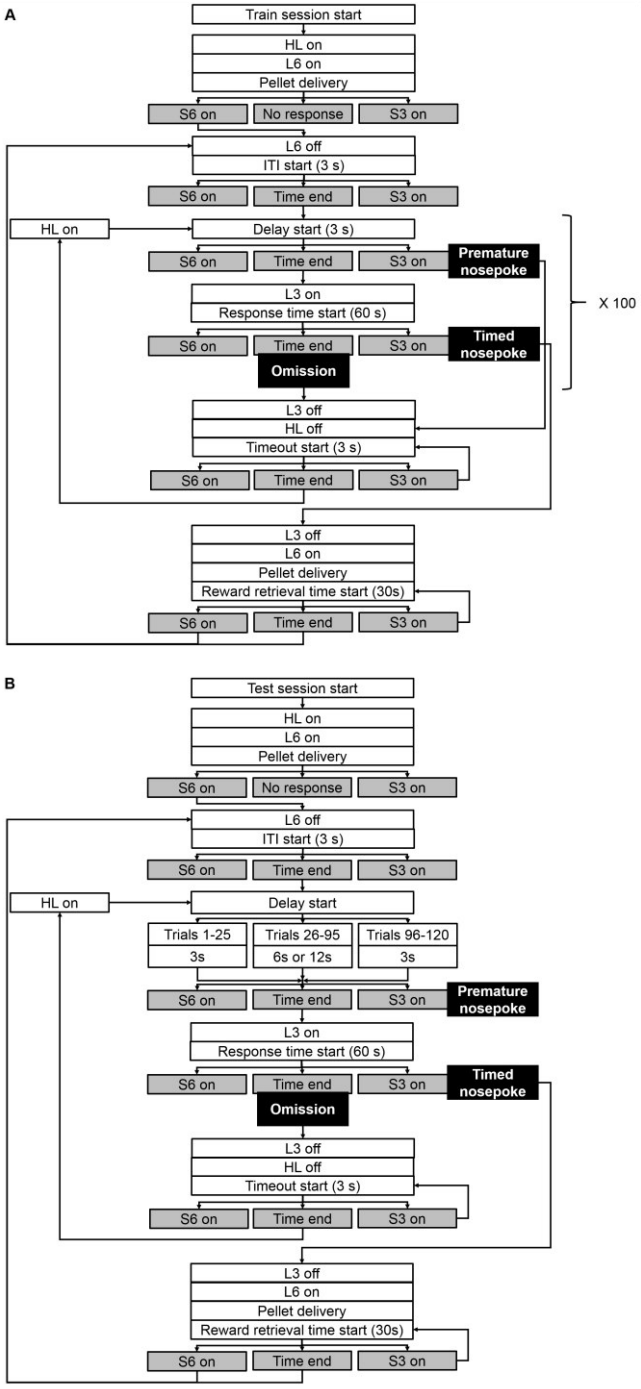


Fig. 6. Behavioral and electrophysiological correlates of delay intolerance. (A) Cumulative premature responses throughout the long delays (6s and 12s). **(B)** Linear correlation of LNAcc theta power in the second before the first premature response of each long delay trial (6s and 12s) and the prematurity rate of the same trial. LNAcc=left nucleus accumbens, prem/min=prematurity rate.

9. Supplementary data



Sup Fig. 1. Flowchart of the VDS task. Schematic representation of VDS train **(A)** and test **(B)**. All system actions are shown in white background (i.e. time counts, light control and reward dispensing), all possible animal responses are shown in grey background (i.e. nosepokes, reward retrievals and absence of response), and all responses utilized in the analyses are shown in black background (i.e. omissions, timed and premature responses). HL=house light; L=light; S=sensor; ITI=inter-trial interval; 3=nosepoke orifice; 6=feeding orifice.

			left												right											
			Caud			Nacc			Prl			OPFC			Caud			Nacc			Prl			OPFC		
			tp	res	tpxresp	tp	res	tpxresp	tp	Res	tpxresp	tp	res	tpxresp	tp	res	tpxresp	tp	res	tpxresp	tp	res	tpxresp	tp	res	tpxresp
-3 to -2 s	theta	FDR p	0.987	0.885	0.999	0.021	0.947	0.999	0.783	0.947	0.999	0.987	0.885	0.528	0.085	0.885	0.999	0.025	0.885	0.999	0.021	0.947	0.999	0.648	0.947	0.528
		η^2_p	0.046	0.076	0.074	0.199	0.001	0.054	0.090	0.003	0.074	0.099	0.256	0.239	0.161	0.227	0.100	0.189	0.067	0.044	0.199	0.001	0.026	0.175	0.001	0.225
	beta	FDR p	0.916	0.658	0.988	0.002	0.658	0.988	0.027	0.658	0.964	0.999	0.658	0.344	0.036	0.658	0.649	<0.001	0.658	0.988	<0.001	0.658	0.988	0.408	0.981	0.964
		η^2_p	0.080	0.077	0.082	0.232	0.047	0.064	0.183	0.089	0.098	0.047	0.131	0.263	0.174	0.149	0.132	0.297	0.184	0.061	0.295	0.036	0.041	0.190	<0.001	0.173
	low gamma	FDR p	0.513	0.847	0.887	0.793	0.014	0.948	0.793	0.050	0.948	0.793	0.563	0.001	0.900	0.563	0.076	0.417	0.050	0.948	0.793	0.563	0.948	0.900	0.849	0.887
		η^2_p	0.152	0.014	0.113	0.107	0.680	0.071	0.089	0.476	0.053	0.168	0.164	0.408	0.070	0.073	0.177	0.157	0.500	0.070	0.096	0.119	0.064	0.103	0.008	0.174
	high gamma	FDR p	0.299	0.240	0.044	0.582	0.217	0.003	0.018	0.061	0.856	0.582	0.319	0.008	0.962	0.061	0.097	0.864	0.040	0.044	0.853	0.132	0.856	0.916	0.245	0.630
		η^2_p	0.166	0.213	0.196	0.115	0.230	0.238	0.213	0.455	0.076	0.203	0.197	0.342	0.050	0.485	0.154	0.085	0.602	0.174	0.094	0.327	0.066	0.122	0.288	0.166
-2 to -1 s	theta	FDR p	0.251	0.432	0.008	0.601	0.432	0.951	0.451	0.734	0.998	0.251	0.959	0.016	0.251	0.432	0.951	0.698	0.959	0.951	0.837	0.432	0.998	0.601	0.959	0.951
		η^2_p	0.169	0.184	0.247	0.101	0.179	0.087	0.123	0.062	0.045	0.238	0.002	0.327	0.157	0.275	0.083	0.088	0.009	0.080	0.068	0.201	0.030	0.169	0.001	0.169
	beta	FDR p	0.456	0.906	0.032	0.456	0.906	0.851	0.575	0.944	0.851	0.604	0.944	0.851	0.604	0.944	0.851	0.738	0.944	0.851	0.575	0.906	0.851	0.824	0.906	0.851
		η^2_p	0.155	0.224	0.224	0.154	0.072	0.077	0.124	0.001	0.119	0.178	0.014	0.147	0.101	0.002	0.083	0.085	0.016	0.075	0.116	0.064	0.067	0.118	0.248	0.171
	low gamma	FDR p	0.022	0.834	0.935	0.055	0.846	0.935	0.974	0.315	0.935	0.216	0.834	0.845	<0.001	0.446	0.069	0.645	0.179	0.535	1.000	0.315	0.935	0.974	0.179	0.535
		η^2_p	0.219	0.016	0.095	0.175	0.004	0.059	0.067	0.226	0.062	0.233	0.033	0.173	0.358	0.129	0.191	0.105	0.439	0.136	0.022	0.209	0.055	0.118	0.587	0.209
	high gamma	FDR p	0.611	0.329	0.276	0.974	0.391	0.736	0.974	0.102	0.476	0.974	0.508	0.988	0.974	0.225	0.276	0.974	0.023	0.736	0.974	0.067	0.280	0.974	0.375	0.988
		η^2_p	0.165	0.192	0.173	0.102	0.101	0.099	0.093	0.395	0.121	0.131	0.092	0.108	0.072	0.256	0.151	0.057	0.645	0.093	0.046	0.489	0.142	0.183	0.226	0.069
-1 to 0 s	theta	FDR p	<0.001	0.355	1.000	<0.001	0.698	1.000	<0.001	0.355	1.000	0.008	0.355	0.113	<0.001	0.444	1.000	<0.001	0.532	1.000	<0.001	0.064	0.014	0.014	0.863	0.025
		η^2_p	0.647	0.243	0.100	0.706	0.030	0.054	0.643	0.276	0.054	0.314	0.330	0.264	0.649	0.129	0.024	0.736	0.080	0.074	0.686	0.561	0.216	0.295	0.007	0.316
	beta	FDR p	<0.001	0.815	0.946	<0.001	0.815	1.000	<0.001	0.577	1.000	0.013	0.815	1.000	<0.001	0.815	1.000	<0.001	0.815	1.000	<0.001	0.254	0.330	0.057	0.599	0.041
		η^2_p	0.539	0.043	0.121	0.606	0.008	0.049	0.589	0.221	0.030	0.301	0.034	0.157	0.555	0.043	0.027	0.714	0.006	0.024	0.610	0.417	0.147	0.255	0.277	0.321
	low gamma	FDR p	0.741	0.752	0.982	0.010	0.650	0.982	<0.001	0.598	0.982	0.779	0.752	0.982	0.187	0.752	0.982	<0.001	0.752	0.982	0.001	0.456	0.982	0.307	0.598	0.982
		η^2_p	0.095	0.013	0.078	0.199	0.107	0.062	0.339	0.186	0.043	0.125	0.068	0.114	0.139	0.021	0.070	0.372	0.048	0.108	0.244	0.346	0.051	0.203	0.278	0.157

	high gamma	FDR p	0.036	0.930	0.042	0.088	0.930	0.359	<0.001	0.930	0.359	0.271	0.930	0.359	0.018	0.930	0.081	0.003	0.930	0.359	0.358	0.930	0.850	<0.001	0.930	<0.001
		η^2_p	0.192	0.001	0.207	0.152	0.040	0.118	0.282	0.003	0.114	0.202	0.063	0.188	0.190	0.008	0.168	0.225	0.027	0.127	0.109	0.046	0.067	0.438	0.039	0.415
0 to 1 s	theta	FDR p	<0.001	0.102	0.958	0.001	0.024	0.008	<0.001	0.010	0.889	0.110	0.331	0.285	<0.001	0.003	0.052	<0.001	0.002	0.773	<0.001	0.003	0.144	0.110	0.102	0.958
		η^2_p	0.315	0.318	0.064	0.231	0.501	0.224	0.408	0.602	0.084	0.232	0.188	0.223	0.302	0.702	0.184	0.251	0.792	0.098	0.296	0.732	0.157	0.233	0.489	0.087
	beta	FDR p	0.002	0.172	0.644	0.001	0.019	0.034	<0.001	0.019	0.644	0.020	0.310	0.379	0.002	0.005	0.034	0.002	0.002	0.892	<0.001	0.002	0.034	0.318	0.160	0.644
		η^2_p	0.241	0.240	0.109	0.246	0.525	0.184	0.358	0.546	0.095	0.289	0.204	0.211	0.224	0.673	0.188	0.215	0.800	0.062	0.294	0.746	0.184	0.188	0.412	0.154
	low gamma	FDR p	0.001	0.745	<0.001	<0.001	0.028	0.336	0.003	0.028	0.212	<0.001	0.745	0.036	<0.001	0.063	0.212	<0.001	0.018	0.103	0.007	0.018	0.212	0.311	0.556	0.962
		η^2_p	0.260	0.014	0.291	0.322	0.515	0.115	0.214	0.506	0.138	0.408	0.043	0.307	0.291	0.391	0.132	0.258	0.612	0.163	0.196	0.650	0.135	0.189	0.135	0.085
	high gamma	FDR p	0.426	0.044	0.001	0.201	0.506	0.323	<0.001	0.044	0.077	0.283	0.177	0.652	0.201	0.038	<0.001	0.426	0.492	0.030	0.331	0.027	<0.001	0.331	0.674	0.652
		η^2_p	0.114	0.523	0.267	0.149	0.067	0.121	0.284	0.458	0.159	0.223	0.429	0.143	0.153	0.544	0.336	0.107	0.090	0.181	0.120	0.633	0.387	0.200	0.038	0.152

Sup Table 1. Statistics of power analysis on the last training session. Results (corrected p-value and effect size) for two-way ANOVA analysis in each region, frequency range and nosepoke-relative time interval. Caud=caudate; NAcc=nucleus accumbens; Prl=prelimbic cortex; OPFC=orbital prefrontal cortex; tp=timepoint (0.05 s resolution); resp=response (i.e. timed or premature nosepoke); FDR p=p-value corrected for multiple comparisons; η^2_p=partial eta squared.

			Nacc		
			tp	res	tpxresp
-3 to -2 s	theta	FDR p	0.565	0.937	0.571
		η^2_p	0.092	0.001	0.116
	beta	FDR p	0.597	0.826	0.872
		η^2_p	0.089	0.052	0.102
	low gamma	FDR p	0.859	0.538	0.988
		η^2_p	0.106	0.134	0.111
	high gamma	FDR p	0.009	0.121	0.706
		η^2_p	0.207	0.423	0.101
-2 to -1 s	theta	FDR p	0.937	0.813	0.974
		η^2_p	0.055	0.042	0.046
	beta	FDR p	0.977	0.775	0.972
		η^2_p	0.057	0.010	0.047
	low gamma	FDR p	0.906	0.939	0.789
		η^2_p	0.065	0.001	0.126
	high gamma	FDR p	0.957	0.885	0.102
		η^2_p	0.051	0.009	0.171
-1 to 0 s	theta	FDR p	0.013	0.668	0.998
		η^2_p	0.195	0.104	0.030
	beta	FDR p	0.596	0.323	0.988
		η^2_p	0.112	0.205	0.040
	low gamma	FDR p	0.225	0.942	0.570
		η^2_p	0.156	0.001	0.093
	high gamma	FDR p	0.293	0.974	0.896
		η^2_p	0.135	<0.001	0.070
0 to 1 s	theta	FDR p	0.531	0.750	0.452
		η^2_p	0.105	0.013	0.112
	beta	FDR p	0.470	0.987	0.481
		η^2_p	0.110	<0.001	0.109
	low gamma	FDR p	0.077	0.436	0.010
		η^2_p	0.187	0.162	0.233
	high gamma	FDR p	0.967	0.746	0.015
		η^2_p	0.054	0.037	0.226

Sup Table 2. Statistics of power laterality analysis on the last training session. Results (corrected p-value and effect size) for two-way ANOVA analysis in the region in which significant results were found, all frequency ranges and nosepoke-relative time intervals. NAcc=nucleus accumbens; tp=timepoint (0.05 s resolution); resp=response (i.e. timed or premature nosepoke); FDR p=p-value corrected for multiple comparisons; η^2_p=partial eta squared.

			LPrl vs LNAcc			LPrl vs RNAcc			LPrl vs RPrl			RPrl vs ROPFC		
			tp	res	tpxresp	tp	res	tpxresp	tp	res	tpxresp	tp	res	tpxresp
-3 to -2 s	theta	FDR p	0.581	0.617	0.996	0.653	0.659	0.895	0.581	0.611	0.895	0.581	0.948	0.895
		η^2_p	0.102	0.120	0.044	0.096	0.066	0.093	0.106	0.209	0.104	0.171	0.001	0.176
	beta	FDR p	0.258	0.424	0.985	0.443	0.424	0.985	0.052	0.424	0.931	0.819	0.500	0.931
		η^2_p	0.141	0.149	0.068	0.121	0.166	0.085	0.186	0.222	0.094	0.138	0.182	0.164
	low gamma	FDR p	0.326	0.793	1.000	0.826	0.793	1.000	0.237	0.907	1.000	0.237	0.951	1.000
		η^2_p	0.140	0.218	0.086	0.079	0.138	0.074	0.151	0.076	0.063	0.251	0.029	0.151
	high gamma	FDR p	0.994	0.982	0.993	0.994	0.982	0.993	0.994	0.982	0.993	0.211	0.982	0.993
		η^2_p	0.101	0.003	0.071	0.080	0.081	0.089	0.040	0.035	0.064	0.311	0.004	0.204
-2 to -1 s	theta	FDR p	0.840	0.896	0.438	0.481	0.430	0.837	0.332	0.585	0.944	0.332	0.772	0.686
		η^2_p	0.087	0.021	0.128	0.125	0.470	0.092	0.167	0.224	0.060	0.241	0.137	0.180
	beta	FDR p	0.976	0.889	0.905	0.754	0.771	0.905	0.976	0.889	0.919	0.483	0.944	0.905
		η^2_p	0.052	0.122	0.121	0.141	0.434	0.113	0.070	0.082	0.084	0.258	0.035	0.148
	low gamma	FDR p	0.986	0.015	0.983	0.999	0.015	0.983	0.999	0.363	0.983	0.986	0.987	0.221
		η^2_p	0.118	0.714	0.064	0.044	0.744	0.069	0.031	0.283	0.070	0.182	0.002	0.281
	high gamma	FDR p	0.998	0.772	0.318	0.893	0.772	0.960	0.246	0.772	0.991	0.827	0.772	0.960
		η^2_p	0.047	0.177	0.167	0.091	0.208	0.094	0.190	0.210	0.049	0.197	0.181	0.167
-1 to 0 s	theta	FDR p	<0.001	0.676	0.975	<0.001	0.107	0.975	<0.001	0.177	0.994	0.291	0.734	0.975
		η^2_p	0.385	0.067	0.123	0.298	0.498	0.133	0.287	0.361	0.066	0.210	0.076	0.155
	beta	FDR p	<0.001	0.798	0.944	<0.001	0.798	0.353	<0.001	0.795	1.000	0.888	0.795	0.944
		η^2_p	0.377	0.026	0.139	0.311	0.038	0.171	0.310	0.120	0.051	0.123	0.283	0.188
	low gamma	FDR p	0.001	0.878	0.581	0.802	0.878	0.977	0.246	0.391	0.838	0.927	0.878	0.949
		η^2_p	0.277	0.112	0.143	0.104	0.014	0.052	0.157	0.432	0.113	0.125	0.345	0.141
	high gamma	FDR p	0.999	0.986	0.680	0.999	0.986	0.973	0.999	0.986	0.034	0.999	0.986	0.034
		η^2_p	0.098	0.034	0.115	0.071	0.062	0.056	0.063	0.047	0.216	0.097	0.173	0.338
0 to 1 s	theta	FDR p	0.028	0.940	0.901	0.902	0.988	0.901	0.441	0.940	0.987	0.902	0.988	0.987
		η^2_p	0.234	0.049	0.137	0.081	0.001	0.109	0.148	0.053	0.047	0.120	<0.001	0.076
	beta	FDR p	0.016	0.913	0.337	0.673	0.994	0.299	0.041	0.974	1.000	0.773	0.913	1.000
		η^2_p	0.234	0.006	0.167	0.116	<0.001	0.179	0.214	0.001	0.062	0.156	0.020	0.104
	low gamma	FDR p	0.646	0.804	0.952	0.915	0.729	0.952	0.350	0.775	0.952	0.777	0.729	0.952
		η^2_p	0.142	0.040	0.105	0.081	0.104	0.098	0.175	0.084	0.121	0.186	0.313	0.140
	high gamma	FDR p	0.973	0.647	0.960	0.973	0.180	0.995	0.394	0.175	0.995	0.973	0.066	0.995
		η^2_p	0.091	0.111	0.150	0.085	0.413	0.123	0.170	0.437	0.096	0.160	0.752	0.158

Sup Table 3. Statistics of coherence analysis on the last training session. Results (corrected p-value and effect size) for two-way ANOVA analysis in the pairs of regions in which significant results were found, all frequency ranges and nosepoke-relative time intervals. L=left; R=right; NAcc=nucleus accumbens; Prl=prelimbic cortex; OPFC=orbital prefrontal cortex;

tp=timepoint (0.05 s resolution); resp=response (i.e. timed or premature nosepoke); FDR p=p-value corrected for multiple comparisons; η^2_p=partial eta squared.

		left				right			
		Caud	Nacc	Prl	OPFC	Caud	Nacc	Prl	OPFC
theta	R^2	0.012	0.141	0.057	0.001	0.019	0.002	0.093	0.009
	FDR p	0.691	0.016	0.206	0.892	0.623	0.841	0.061	0.799
beta	R^2	0.070	0.051	0.015	<0.001	0.001	0.013	0.033	0.006
	FDR p	0.287	0.287	0.655	0.989	0.895	0.655	0.415	0.880
low gamma	R^2	0.055	0.033	0.009	0.001	0.018	0.008	0.032	<0.001
	FDR p	0.436	0.436	0.686	0.961	0.641	0.686	0.436	0.963
high gamma	R^2	0.094	0.028	0.038	0.004	0.002	0.006	<0.001	0.041
	FDR p	0.219	0.490	0.490	0.838	0.838	0.838	0.934	0.513

Sup Table 4. Statistics of correlation between power and number of premature responses in the long delays of the test session. Results (corrected p-value and R^2) for correlations between power within 1 second before the first premature response in the long delays (6s and 12s) and premature responses per minute in the same trial, in four ranges of frequency. Caud=caudate; NAcc=nucleus accumbens; Prl=prelimbic cortex; OPFC=orbital prefrontal cortex; FDR p=p-value corrected for multiple comparisons.

CHAPTER VI

General discussion, conclusions and future perspectives

General discussion

The field of laterality, particularly of human laterality, remains to the present day markedly influenced by language asymmetries (Bishop, 2013; Tzourio-Mazoyer, 2016). its enduring influence in the field may be greatly explained by historical context - it was the first lateralized function to be described – its undeniable scientific relevance associated with the central role of communication in human societies, as well as the strong morphofuctional association. However, the importance of lateralization in other functional domains such as memory, attention or emotional processing, remains largely unknown. We therefore aimed to explore the laterality-cognition interplay at multiple levels. We have assessed structural and functional asymmetries and took advantage of two complimentary models - humans and rats. Additionally, considering the limited descriptions of laterality-cognition associations and the consequent absence of an established link, we opted for a brain-wide approach.

We started by analyzing a large cohort extensively characterized at the cognitive level (**CHAPTER II**). Using a region of interest (ROI) approach, which was validated against the more commonly used voxel-based morphometry (VBM), we have determined that regional structural asymmetry of the brain is ubiquitous. Additionally, no organizational patterns were evident, i.e. left>right or left<right areas were not clustered. Such result was expected as clusters of regions lateralized towards the same direction would be associated with poor usage of space in the opposing hemisphere, while dispersion of this lateralization may facilitate this balance. This result is therefore in accordance with theories that attribute the development of laterality to a necessity of efficient distribution of cognitive load and maximization of available space for neural tissue (Denenberg, 1981; Vallortigara et al., 2011).

We have uncovered a number of associations between asymmetry and specific parameters of a large batery of cognitive tasks, such as memory, vocabulary, or depressive mood (**CHAPTER II**). These correlations are complex and vary according to region, cognitive/emotional domain, sex and education. In fact, educational level was found to mediate associations of structural asymmetry with memory and vocabulary, domains known to benefit from increased formal education (Verhaeghen, 2003; Yuan et al., 2018). On the other hand, the insula asymmetry-mood association was not dependent on education or sex, even though afections of this emotional domain are more common in females (Altemus et al., 2014), while correlations with memory and selective attention/response inhibition, were mediated by sex. Considering that this population is aged,

presents low educational level and lives in a rural area, it is possible that these results reflect traditional gender roles. Indeed, the population from the same cohort to whom the the N-Back task was applied (**CHAPTER IV**) included 57% women, but amongst those able to achieve minimum performance only 32% were female. It is therefore possible that, not only education, but also sex differences here described, are related with cognitive reserve. In fact, this measure has been suggested to play a role in brain asymmetry, with ex-iliterate subjects displaying reduced lateralization in a language task (Nunes et al., 2009). In this cohort, the connectome in function of cognitive reserve has been established (Marques et al., 2016) and its analysis in terms of laterality may bring further insight into this perspective.

It results from the above that brain asymmetries are plastic. In fact, lateralization of music processing in regions that display an auditory role has been suggested to depend on musical training (Ellis et al., 2013), and animal models have shown that neo-natal environmental enrichment modulates behavioral (Tang and Reeb, 2004; Tang et al., 2003; Tang and Verstynen, 2002), and hippocampal volume (Verstynen et al., 2001) asymmetries. On the other hand, aging has also been associated with changes in asymmetries at structural (Guadalupe et al., 2016) and functional (Cabeza et al., 2002; Reuter-Lorenz and Cappell, 2008) levels. Functionally, some data suggests an age-dependent frontal asymmetry reduction associated with a compensation phenomenon and better performance (Cabeza, 2002). In fact, in our cohort, few regions showed asymmetric recruitment during performance of a working memory task (**CHAPTER IV**). On the other hand, we also showed for the first time, a linear association between asymmetrical recruitment of a region (superior parietal lobule) and improved execution of that task, suggesting that, also functionally, the (a)symmetry-performance association is region-dependent.

Structurally, laterality was stable in the overall population within an 18-month time-frame (**CHAPTER III**). This suggests that age-dependent atrophy, and, consequently, age-dependent asymmetry changes, occur at a slow pace. However, we did detect higher variability in asymmetry variation in subcortical regions, which may be associated with a higher rate of atrophy in these areas (Fjell et al., 2009). Importantly, we determined that higher asymmetry changes were not due to left or right epiphenomena, but rather to concomitant changes in left and right volume that occurred in an asymmetrical fashion. These variations co-evolved with cognitive measures, reinforcing the associations found in the transversal approach. This particular population is currently undergoing a third assessment, and analyses of changes occurring within 36 months

(moment 1 to moment 3) may bring new insight into longer-term plasticity and associated cognitive changes. Additionally, it would be of interest to determine the effects of cognitive training in asymmetrical changes. Alzheimer's patients, for example, have been shown to present asymmetry reductions in several regions including the inferior parietal lobule (Kim et al., 2012) and the hippocampus (Shi et al., 2009), as well as a striking increase in leftward asymmetry of the lateral ventricle (Apostolova et al., 2012; Fjell et al., 2009). It has also been demonstrated that cognitive training provides benefits for these patients (Gates and Sachdev, 2014) and we may therefore speculate that such effect is associated with a reduction in asymmetrical atrophy of associated brain regions.

Despite the bulk of information, human data on laterality have been essentially inferential. Rodent studies have therefore been instrumental to establish causal relations and were here utilized as a complementary approach. At high temporal and spatial resolution, we were able to show a time-specific involvement of a cortico mesolimbic network associated with impulsive decision-making in a side-dependent manner. Indeed, time-dependent regional involvement starting up to 3 seconds before a response includes a left-lateralized network, which is followed by left-right hemisphere interaction, seeming to lead to a specific outcome (i.e. timed or premature response) (**CHAPTER V**). This animal model is of great value as it can bring mechanistic information, allowing direct manipulation of brain laterality through lesion, drugs or optogenetics and concomitant assessment of cognitive displays. Lateralized ventromedial prefrontal cortex lesion, for example, has been shown to induce a sex-dependent effect, with right, but not left lesions reducing stress response (Sullivan and Gratton, 1999) and inducing anxiety-like behavior (Sullivan et al., 2014) in male animals. Similar anxiety-like behavior was attained in females after left, but not right lesion (Sullivan et al., 2014). Specifically in our data, it would be of extraordinary value to optogenetically manipulate a specific region on the timepoint at which this region showed to be of interest. Concomitant analysis of the behavioral response and electrophysiological activity in the remaining regions would provide important information on network function.

Similarly, these models allow determination of the asymmetrical consequences of environment, training or peripheral manipulations. Lateralized neuropathic pain, for example, has been shown to induce side-specific changes in behavior (Leite-Almeida et al., 2012), associated with lateralized brain activations (Leite-Almeida et al., 2014). Importantly, current techniques now allow

assessment of brain structure and function in the living animal (Magalhaes et al., 2017), creating oportunities for longitudinal assessments.

Conclusions and future perspectives

We have here shown that, despite an apparent symmetry, both the human and the rat brain present ubiquitous lateralization with functional relevance. These cognition-laterality associations are complex and mediated by multiple factors, showing region-, function-, age- and even sex-specificity. In fact, while functions like language benefit from structural and functional lateralization of the peri-Sylvian region, others display age-dependent changes, with compensatory frontal contralateral activation (and consequent decreased asymmetry) in older individuals leading to improved memory performance. However, it is particularly relevant that our data shows the importance of asymmetry in healthy subjects, reflecting hemispheric specialization in basal conditions and potentially indicating an evolutionary advantage.

This thesis has taken steps towards what we believe to be the necessary future of the field, namely the determination of laterality-cognition associations and the utilization of techniques that are able to, or at least are an approximation for, causality inference. Assessment of the importance of brain laterality requires large-scale longitudinal assessments, as well as region-specific manipulations that can only be achieved in deep-brain stimulation (DBS) patients or in laboratory animals. Large databases specifically built for the study of laterality, such as BIL&GIN (Mazoyer et al., 2015) or ENIGMA (ENIGMA-consortium) are available, but do not seem to include longitudinal data and/or a full neuropsychological profile. Also, access to patients undergoing DBS is extremely scarce and limited to very specific regions. Hemisphere-specific manipulations using transcranial magnetic stimulation are also commonly performed, but region specificity is relatively low and its effectiveness has recently been put in question (Vöröslakos et al., 2018). On the other hand, technologies for rodent application are becoming more specific, more dynamic and less expensive.

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APPENDIX A

Age and sex as determinants of rats' trait impulsivity in the Variable Delay-to-Signal task

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Title: Age and sex as determinants of rats' trait impulsivity in the Variable Delay-to-Signal task

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Abstract:

Impulsivity is a naturally occurring behavior that can assume pathological characteristics in many neuropsychiatric disorders. However, conflicting findings have been reported regarding the role of factors as age and sex in the expression of trait impulsivity. We have therefore analyzed the effect of sex, age, strain and estrous cycle in impulsive behavior in the variable delay-to-signal (VDS) task, using a large population of 188 rats. This cohort included (i) control animals from previous experiments; (ii) animals specifically raised for this study; and (iii) animals previously used for breeding purposes. Aging was associated with a general decrease in action impulsivity and an increase in delay tolerance. Females generally performed more impulsive actions than males but no differences were observed regarding delay intolerance. In terms of estrous cycle, no differences in impulsive behavior were observed, and regarding strain, Wistar Han animals were, in general, more impulsive than Sprague-Dawley. While confirming well-established associations (decrease in impulsivity with aging), we demonstrated strain and sex influences that modulate different aspects of impulsive behavior manifestations.

Keywords: variable delay-to-signal; impulsive action; delay tolerance; sex; age; strain

1. Introduction

Impulsivity is a naturally occurring behavior involving a complex spectrum of poorly conceived and prematurely expressed actions and choices that often result in undesirable results (Daruna and Barnes, 1993; Evenden, 1999). It is a complex and multidimensional construct encompassing action restraint and/or action cancelation - impulsive action - as well as impulsive decision-making that is related with non–deliberated decisions – impulsive choice – and is characterized by a tendency for smaller but immediate gratification over larger but delayed ones (Bari and Robbins, 2013; Dalley et al., 2011; Dalley and Roiser, 2012; Evenden, 1999).

Despite being considered an adaptive behavior, studies on impulsive behavior have mostly been performed in the context of neuropsychiatric diseases like addiction, compulsive eating and aggressive behavior (Bari and Robbins, 2013; Fawcett et al., 2012). Nevertheless, evidence suggests a bidirectional relationship between this so-called trait impulsivity and increased propensity for the development of maladaptive behavior (Anker et al., 2009; Diergaarde et al., 2008; Kayir et al., 2014; Mendez et al., 2010; Paine et al., 2003; Perry et al., 2005; Saunders et al., 2008) – see for review (Dawe and Loxton, 2004; de Wit, 2009; Pattij and De Vries, 2013). Importantly, numerous neurotransmitters and drugs influence impulsive behavior (Abbas et al., 2017; Carvalho et al., 2017; Melo et al., 2016).

It has been recognized that age and sex are, among others, determinant factors for the manifestation of trait impulsivity. Age influence in impulsive behavior is relatively consistent across human (Eppinger et al., 2012; Green et al., 1994; Green et al., 1996; Green et al., 1999; Williams et al., 1999) – see for review (Drobetz et al., 2012; Mather, 2016). Likewise, most studies using rodents, report an age-associated decrease in impulse behavior (Adriani and Laviola, 2003; Burton and Fletcher, 2012; Doremus-Fitzwater et al., 2012; Lukkes et al., 2016; Muir et al., 1999; Pinkston and Lamb, 2011; Simon et al., 2010; Sonntag et al., 2014) – see for review (Hunt et al., 2016). Nonetheless, in some studies this trend was associated with other factors such as strain (Pinkston and Lamb, 2011) or sex (Lukkes et al., 2016), while other studies did not observe this age-impulsivity relation (Breton et al., 2015) or observed the inverse association – i.e. young adults presenting higher impulsivity than adolescent rats (Sturman et al., 2010). Regarding sex, results on impulsive action and choice are often conflicting and even contradictory – for instance confront (Bayless et al., 2012; Burton and Fletcher, 2012) and (Bayless et al., 2013; Doremus-Fitzwater et

al., 2012; Eubig et al., 2014; Koot et al., 2009; Lukkes et al., 2016; Van Haaren et al., 1988) for action and choice impulsivity, respectively.

To clarify this complex relation between biological parameters such as age and sex in action and choice impulsivity we collected and analyzed variable delay-to-signal (VDS) data from a large population of 188 rats. The VDS is a validated paradigm that provides a fast assessment of both impulsive action and delay tolerance (associated with choice impulsivity), which makes it particularly adequate for the characterization of large samples (Leite-Almeida et al., 2013).

2. Methods

2.1. Subjects and experimental conditions

A total of 188 rats were used in all experiments (see below for details). Animals were kept in a room with controlled temperature (22°C ± 1°C), humidity (50-60%) and light cycle (12 hours; lights on at 8 a.m.) and were housed in groups of 2–3 in standard plastic cages with food and water available ad libitum. 2–3 days prior to the initiation of the VDS protocol food availability was restricted to 1h per day. Animals' weight was controlled thourghout the protocol to prevent drops below 15% of baseline values. All procedures involving animals adhered to the guidelines of the European Communities Council Directive 2010/63/EU.

2.2. Data collection

A database of VDS records obtained in our institute was compiled. These were obtained from 3 different sources: i. animals that performed the VDS as controls in the context of other experiments (mostly males); rats that underwent any type of drug treatment or whose experimental records were incomplete were excluded; ii. animals that were specifically raised for this study (mostly females); and iii. aged animals mostly used for breeding purposes prior to inclusion in this experiment. The final database contained a total of 188 entries. Groups were assembled by age – 1-2, 2-6, 6-12 and 12-18 months-old (m.o.) – sex and strain – Sprague-Dawley (SD) and Wistar Han (WH) – (Fig. 1A). Additionally, a group of young adult females were classified according to the estrous cycle phase (see below).

2.3. Variable delay-to-signal (VDS) task

VDS was performed as previously described (Leite-Almeida et al., 2013). Briefly, the protocol was performed in 5-hole operant chambers (OC; 25x25 cm; TSE Systems, Germany) placed inside ventilated, sound attenuating boxes. One of the OC walls contains five square apertures (#1-#5; 2.5 cm), elevated 2 cm from the grid floor. The opposing wall contains a similar aperture (#6) connected to a pellet dispenser. Each aperture contains a 3W lamp bulb and an infrared beam system that detects the activity of the animals.

2.3.1. Habituation

Animals were habituated to the OB in 2 daily sessions (a.m./p.m.; 4 hours apart), for 2 consecutive days (Fig. 1B). In the first 2 sessions (habituation day 1) animals were left to explore the OB for 15 minutes; all lights were off and 10-15 sugared pellets (45 mg, Bioserv Inc., New Jersey,USA) were available on aperture #6 while apertures #1 to #5 were blocked with metallic caps. In sessions 3 and 4 (habituation day 2), animals were left to explore the OB for 30 minutes. Apertures #3 and #6 were accessible and contained 3-5 and 10-15 pellets, respectively. During these sessions house light and apertures' light #3 and #6 were on.

2.3.2. Training

Training comprises 2 daily sessions (a.m./p.m.; 4 hours apart) of 100 trials (or 30 minutes) each, for 5 consecutive days (Fig. 1B). In these sessions, the animals were trained to wait for 3 seconds (delay period), after which aperture #3's light was turned on (response period) up to a maximum of 60 seconds. Nose pokes in the response period (correct responses) were rewarded with the delivery of a sugared pellet at #6. Responses in the delay period (premature responses, PR) and omissions (absence of response) were punished with a timeout period (3s) in complete darkness and no reward was delivered (Fig. 1C top). House light was always on except during timeout periods. In the training phase action impulsivity is measured in a manner akin to that of the 5-choice serial reaction time task (Bari et al., 2008; Carli et al., 1983), i.e. by assessing the percentage of PRs.

2.3.3. Test

The VDS test consists in a single session encompassing 120 trials (Fig.1B). These are similar to the training phase, except that nose pokes are allowed (i.e., not punished) (Fig. 1C bottom) and the delays vary throughout the test. It starts with an initial block of 25 trials at 3 second delay (3si), followed by 70 trials of randomly distributed 6 or 12 second delays (6s and 12s) and again a final block of 25 trials at 3 second delays (3sf). Impulsive behavior in the VDS test manifests in two moments: in the 1st second of the delay – action impulsivity – and prematurity rate (PR rate) during the delays - delay tolerance. Particularly relevant in this context is the PR rate increase observed in 3sf after exposure to the longer intervals (6s and 12s) that correlates with delay-discounting (Leite-Almeida et al., 2013).

PR rate is defined as the amount of PR per minute of total delay, PR/min

$$PR/min = \frac{PR_i}{N_i \times T_i} \times 60$$

where PRi is the amount of premature responses, Ni is the number of trials and Tí is the delay time for i = 3si, 6s, 12s or 3sf.

2.4. Estrous cycle assessment

To determine the stage of the estrous cycle, the vaginal cytology method was used. The vaginal smear was performed after the VDS test. Cells from the smear were transferred to a dry glass slide and were air dried and stained with the Papanicolaou staining technique. The four stages: proestrus, estrus, metestrus and diestrus were classified based on the presence or absence of nucleated epithelial cells, cornified epithelial cells and leukocytes, according to (Caligioni, 2009); see also (Cora et al., 2015).

2.5. Statistical analysis

Statistical analyses were done in IBM SPSS Statistics 22 (IBM software, Inc., New York, USA). The evolution of training was performed by mixed-design ANOVA analysis with session as within-subjects' and sex and age as between-subjects' effects. The sphericity assumption was statistically assessed with the Mauchly's test. Comparisons between groups with one level were done applying one-way ANOVA and between groups with more than one level were done using two-way ANOVA.

Bonferroni post-hoc correction was performed for multiple comparisons. Statistical significance was considered if $p<0.05$. All results are presented as mean ± SEM. Strain and estrous cycle comparisons were performed using sub-samples of the total database as described in the results section.

3. Results

3.1. Task acquisition

The analysis of Mauchly's test revealed that the assumption of sphericity was not met ($\chi^2_{(44)}=3515.1$, $p<0.001$). As such, degrees of freedom (df) were corrected according to the Huynh-Feldt correction ($\varepsilon=0.236$). There was a significant decrease in the percentage of omissions across the 10 training session ($F_{(2.2,309.8)}=109.40$, $p<0.001$, $\eta_p^2=0.423$). Significant between-subjects effects were observed for age ($F_{(3,149)}=31.58$, $p<0.001$, $\eta_p^2=0.389$), sex ($F_{(1,149)}=26.13$, $p<0.001$, $\eta_p^2=0.149$) and age*sex interactions ($F_{(3,149)}=8.73$, $p<0.001$, $\eta_p^2=0.150$). In addition, statistically significant age*session ($F_{(6.6,329.0)}=17.33$, $p<0.001$, $\eta_p^2=0.259$), sex*session ($F_{(2.2,329.0)}=12.12$, $p<0.001$, $\eta_p^2=0.075$) and age*sex*session ($F_{(6.6,329.0)}=5.53$, $p<0.001$, $\eta_p^2=0.100$) interactions were observed (Table 1 and Sup Table 1). As can be observed through the analysis of longitudinal trajectories, there was a parametric effect of age on the baseline number of omissions (older ages were proportionally associated with an increased number of omissions). Additionally, younger animals (1-2, 2-6 m.o.) effectively reached full performance (0 omissions) in 2-4 sessions while older animals (6-12 and 12-18 m.o.) took about 6 sessions to reach the same performance (Fig. 2A). Regarding sex, it was noted that male animals displayed an increased mean number of omissions, which was particularly evident at the baseline (Fig. 2B). Finally, when decomposing the effects of the age*sex*session effects (Fig. 2C-F), it was observed that, in the older groups of animals (6-12 and 12-18 m.o.), there was a significant sex difference, with males presenting higher number of omission in the first 4-5 sessions.

3.2. Impulsive action

Similarly to the percentage of omissions across training sessions, the results from the mixed-design ANOVA revealed a violation of the assumption of sphericity ($\chi^2_{(44)}=3312.8$, $p<0.001$) and consequently df were adjusted in accordance with a Huynh-Feldt correction ($\varepsilon=0.245$). Significant increases (within-subjects effects) were observed for PR across training sessions ($F_{(5.3,814.7)}=70.72$, $p<0.001$, $\eta_p^2=0.313$). There were main between-subjects effects of age ($F_{(3,155)}=39.56$, $p<0.001$,

η_p^2=0.434) and sex ($F_{(1,155)}$=33.73, p<0.001, η_p^2=.179), but no significant interaction term between these variables ($F_{(3,155)}$=1.59, p=0.193, η_p^2=.434). Post-hoc analysis revealed that the mean PRs across sessions were significantly higher for the younger animals (i.e., 1-2 and 2-6 m.o.) in comparison to older animals (i.e., 6-12 and 12-18 m.o.); furthermore, females performed significantly more PRs (M=35.49%, SE=1.24) than males (M=25.26%, SE=1.24). With respect to within-between effects, significant findings were observed for (i) age*session ($F_{(15.8,814.7)}$=4.01, p<.001, η_p^2=.072), (ii) sex*session ($F_{(5.2,814.7)}$=4.92, p<0.001, η_p^2=0.031), and (iii) age*sex*session interactions ($F_{(15.8,814.7)}$=2.13, p=0.006, η_p^2=0.040) (Table 1 and Sup Table 1). The assessment of the longitudinal trajectories for different groups indicated that (i) whereas the older animals display a constant, progressive increase in the number of PRs across sessions, younger animals display a large increase in the number of PRs during the first three sessions (Fig. 3A); (ii) despite both sexes displaying a similar number of PRs at the baseline, females display a substantially larger increase in the number of these responses until the third session (Fig. 3B); (iii) decomposing the age*sex*session interaction (Fig. 3C-F), differences were only found in the 12-18 m.o. group, with males presenting higher levels of impulsivity ($F_{(4.2,96.5)}$=5.36, P<0.001; Fig. 3F) in comparison with the opposite sex across training sessions.

Analysis of 3si block early PRs (first second) in the VDS test provides an additional readout of action impulsivity (Fig. 4 and Table 1). Although, as in training, PR rate tended to decrease in an age-dependent manner (Fig. 4A). Results from the univariate analysis of variance failed to suppress the threshold for statistical significance ($F_{(3,178)}$= 2.09, p=0.103, η_p^2=0.034). On the other hand, there were statistically significant effects of sex on this parameter ($F_{(1,178)}$=10.55, p=0.001, η_p^2=0.056; Fig. 4B). No age*sex interaction was found in this measure ($F_{(3,178)}$=0.54, p=0.659, η_p^2=0.009).

3.3. Delay intolerance

Regarding delay intolerance, age affected PR rate in all intervals (Table 2 and Sup Table 2) – 3si $F_{(3,176)}$=8.30, p<0.001 η_p^2=0.124; 6s $F_{(3,176)}$=13.14, p<0.001, η_p^2=0.183; 12s $F_{(3,176)}$=9.60, p<0.001, η_p^2=0.141; 3sf $F_{(3,176)}$=18.92, p<0.001, η_p^2=0.244 (Fig. 5A) – and the 3sf ratio to baseline 3si ($F_{(3,179)}$=7.61, p<0.001; Fig. 5C). 12-18 m.o. animals systematically presented the lowest PR rate in all intervals (Fig. 5A), as well as a decrease in PR rate in the 3sf interval in comparison to baseline (log(3sf/3si); Fig. 5C). On the other hand, 2-6 m.o. animals mostly presented the highest PR rate (Fig. 5A) and showed an increase in PR rate at 3sf (Fig. 5C).

Sex effects (Table 2) were observed in the initial block, 3si ($F_{(1,176)}$=10.85, p=0.001, η_p^2=0.058; Fig. 5B) reflecting the same pattern observed in the training sessions, i.e. females showed higher PR rates. However, no other statistically significant sex differences or age vs sex interactions were observed in the intervals (Fig. 5B) or in the 3sf/3si comparison (Fig. 5D). See also Sup Fig. 1 for cumulative PRs throughout all intervals divided by both age and sex.

3.4. Latency to feed

Overall latency to feed (Table 3) was affected by age ($F_{(3,179)}$=7.25, p<0.001, η_p^2=0.108; Fig. 6A), older animals taking on average 244.6 and 143.9 ms more to recover the reward than 2-6 m.o. and 1-2 m.o. animals, respectively. In a similar fashion, there were significant effects of sex on latency to feed ($F_{(1,179)}$=4.00, p=0.047, η_p^2=0.022; Fig. 6B), whereas no age*sex interaction significant effects ($F_{(3,179)}$=0.569, p=0.636, η_p^2=0.009) were observed.

3.5. Sub-sampling analyses

To further explore the effect of strain and estrous cycle in impulsive behavior the analysis was restricted to specific ages in order to obtain a homogenous group where variables were equally represented.

3.6. Strain

Analysis of strain effects strain*sex interaction in impulsive behavior was restricted to 12-18 m.o. males and females (SD: N=18 (8M, 10F); WH: N=17 (12M, 5F); Tables 4 and 5). Results from the mixed-design ANOVA (Huynh-Feldt corrected df: ε=0.360), revealed that there were no significant main effects of neither strain ($F_{(1,20)}$<0.01, p=0.984, η_p^2=0.148) nor strain*session ($F_{(3.2,64.9)}$=0.197, p=0.910, η_p^2<0.001 on the percentage of omissions (Fig. 7A). For PR during training (Huynh-Feldt corrected df: ε=0.820), although there were no significant main effects of strain ($F_{(1,21)}$=3.63, p=0.071, η_p^2=0.147), statistically significant strain*session interaction effects were observed ($F_{(7.4,154.9)}$=5.60, p<0.001, η_p^2<0.001; Fig. 7B). Also 1st second PR rate ($F_{(1,33)}$=6.22, p=0.018, η_p^2=0.172; Fig. 7C) revealed that WH rats have higher premature actions than SD animals. No effects of sex, or sex*strain interaction were seen in this group (see Table 4 section for detailed statistics).

Similarly, delay tolerance parameters show that WH animals present higher PR rate in all intervals except 3si (Fig. 7D, Table 5) – 3si $F_{(1,33)}$=3.52, P=0.071, η_p^2=0.105; 6s $F_{(1,33)}$=10.00, p=0.004, η_p^2=0.250; 12s $F_{(1,33)}$=8.45, p=0.007, η_p^2=0.220; and 3sf $F_{(1,33)}$=4.55, p=0.042, η_p^2=0.231. No statistically significant differences between strains were observed in the log(3sf/3si) analysis and there were no effects of sex or sex*strain interaction.

3.7. Estrous cycle

Analysis of possible relations between estrous cycle (diestrus/proestrus) and impulsive behavior was restricted to 2-6 m.o. females. Estrous cycle assessment was performed immediately after the VDS test (Diestrus: n=9; Proestrus: n=6) and only this phase was analyzed (Sup Fig. 2 and Sup Table 3). No differences were observed in impulsive actions as evaluated using the 1st second of the 3si interval ($F_{(1,14)}$=0.75, p=0.402, η_p^2=0.055; Sup Fig. 2A). Furthermore estrous cycle phase had no influence in any of the VDS blocks (Sup Fig. 2B) – 3si $F_{(1,12)}$=0.93, p=0.355, η_p^2=0.072; 6s $F_{(1,13)}$=1.92, p=0.189 η_p^2=.129; 12s $F_{(1,12)}$=3.50, p=.086, η_p^2=.226; and 3sf $F_{(1,12)}$=1.14, p=0.307, η_p^2=0.087 - nor in the variation to baseline PR rate ($F_{(1,14)}$<0.01, P=0.997; Sup Fig. 2C). Nonetheless, females in the proestrous phase tended to present higher levels of impulsivity in all referred analyses.

4. Discussion

In the present study we characterized action impulsivity and delay-tolerance in a group of 188 rats in the VDS. The entire protocol lasts for 8 days, but the VDS itself takes place in a single session making it adequate to characterize short-lived phenomena or life stages (e.g. adolescence; estrous cycle phases, etc.) and simultaneously capturing action impulsivity and delay tolerance. Task acquisition, reflected in the evolution of omission trials until complete fading, was achieved within 2-6 sessions; older animals required on average more sessions. Importantly, all animals were able to learn it and from training sessions 7 to 10, virtually no omissions were recorded. Similar age effects have been previously reported in other operant behavior protocols (Ohta et al., 1993; Port et al., 1996; Roesch et al., 2012; Roux et al., 1994).

Premature responses in the training phase reflect impulsive action. Indeed, the behavioral construct is similar to that of the 5-choice serial reaction time task (5-csrtt) (Bari et al., 2008; Carli et al., 1983). Additionally, this type of impulsive behavior can also be captured in the early

moments of the 3si delays (Leite-Almeida et al., 2013). In both cases, impulsive action decreased with aging. Similarly, a previous study in a 2-csrtt showed that 1 m.o. rats were more impulsive than >3 m.o. animals (Burton and Fletcher, 2012). However, the opposite was observed in a single instrumental nose poke task using animals of similar ages (Sturman et al., 2010). Interestingly, this last task bears some resemblances with the VDS test and results are probably more akin to delay intolerance rather than behavioral inhibition (see below). Regarding aged animals, Muir and colleagues observed that 10-11 m.o. rats were more impulsive than 23-24 m.o. rats, though this observation was restricted to a specific condition (longer delays) (Muir et al., 1999).

Sex was also associated with distinct action impulsivity behavior, with females performing more premature responses than males. Similar findings were reported in 3 m.o. rats in a 2-csrtt task, although these differences manifested specifically in longer delays (Burton and Fletcher, 2012). In fact, sex-related differences are frequently associated with specific delay conditions – see for instance (Bayless et al., 2012; Burton and Fletcher, 2012; Jentsch and Taylor, 2003). Interestingly, we also report that this sex effect is age-specific, which was previously indicated by Burton and Fletcher (2012) in a smaller age range.

The VDS test has also a delay (in)tolerance component that manifests in an increment of impulsive response rate upon exposure to large delays to signal/reward that correlates with delay discounting (DD) behavior (Bari et al., 2008; Carli et al., 1983). In our population, 2-6 m.o. animals presented an increase in PR rate in the 3sf block compared to baseline rate in 3si, while the remaining groups maintained (1-2 and 6-12 m.o.) or even slightly decreased (12-18 m.o.) their response rate. In line with our observations, it has been shown in DD protocols that 1 m.o. rats were less impulsive than 2 m.o. animals (McClure et al., 2014; Sturman et al., 2010) and 25 m.o. rats were less impulsive than 6 m.o. (Simon et al., 2010). Lukkes and colleagues, also observed that early adolescent female (but not male) rats were less impulsive in a DD task than young adult/adult females (Lukkes et al., 2016). However, DD findings are often contradictory and difficult to systematize. For instance, in a spatial DD task with adjusting delays, no age-related differences in discounting rates were found between 5, 9 and >27 m.o. (Breton et al., 2015), while Doremus-Fitzwater and colleagues, observed that 1 m.o. rats presented higher impulsive choices than 2 m.o. (Doremus-Fitzwater et al., 2012). Similarly, in a spatial (T-maze) DD 1 m.o. animals were shown to be more impulsive than 3 m.o. but only in 1 specific condition (10 and 15 seconds delay) (Sonntag et al., 2014). A major difference between DD and VDS paradigms is that in the former delay and reward-

size effects cannot be isolated. When the amount of reward is controlled and the indifference point calculated over an option between an adjusted delay and a variable (random) delay, adolescent animals demonstrated to be less impulsive than young adult animals (McClure et al., 2014) as observed in the VDS – see also (Sturman et al., 2010). In this context, this aspect is of relevance as reward-driven behavior in adolescents is more directed by the exogenous stimulus as opposing adults that is more goal-directed (Hammerslag and Gulley, 2014) – see also (Ernst et al., 2011). Indeed, adolescent and adult rats differ in their reward evoked activities of the dorsal striatum and orbitofrontal cortex (Sturman and Moghaddam, 2011, 2012) – see also for review (Simon and Moghaddam, 2015). Also, procedural differences – adjusting vs increasing delay (Craig et al., 2014), ascending vs descending delay (Tanno et al., 2014) and reinforcement magnitude (Orduna et al., 2013) – might explain some divergence in the published data.

Female and male rats presented similar premature responding rates in all blocks of the VDS paradigm, except in the 3si where females presented higher PR/min probably reflecting differences in action impulsivity already observed in the training phase. In line with our findings a number of previous DD studies also found no sex-associated differences (Eubig et al., 2014; Smethells et al., 2016). However, other studies reported small differences in particular experimental conditions (Koot et al., 2009) or even opposing findings – males>females (Bayless et al., 2013) and females>males (Van Haaren et al., 1988). In females, some variability might also derive from the estrous cycle phase. Indeed, our results show a tendency for an increased delay tolerance in the diestrus in comparison to the proestrus phase. In humans too, it has been shown that women were less impulsive in the mid phase of the menstrual cycle (Diekhof, 2015; Smith et al., 2014). Mechanisms underlying these cyclic alterations in impulsivity are probably related to fluctuations on sex steroids, as these hormones are known to affect dopaminergic tone (Almey et al., 2015; Sun et al., 2016; Zheng, 2009) – which is critically associated with impulsive behavior (Dalley et al., 2011; Dalley and Roiser, 2012; Winstanley, 2011).

An additional potential source of inter-study variability is the strain. For instance, it has been systematically shown that male Lewis rats discount faster than Fisher 344 (Anderson and Diller, 2010; Anderson and Woolverton, 2005; Garcia-Lecumberri et al., 2011; Huskinson et al., 2012; Madden et al., 2008; Stein et al., 2012) though differences can be attenuated/eliminated with repeated assessment as an effect of learning (and/or aging) (Aparicio et al., 2015). Other two studies using wider sets of rat strains (Richards et al., 2013; Wilhelm and Mitchell, 2009), support

inter-strain differences in rats. In our study, when restricting the analysis to the 12-18 m.o. group (aiming to achieve balanced strain groups), WH animals presented increase PR in comparison with SD animals both during training and test, revealing strain differences in both action and delay intolerance components of impulsivity.

5. Conclusions

In conclusion, we confirmed a general age-dependent decrease of action impulsivity and delay tolerance. Importantly, delay tolerance was maximal in early adulthood, and not in adolescence, contrary to a prevailing idea arising mainly from human studies (Eppinger et al., 2012; Gleich et al., 2015; Green et al., 1994; Green et al., 1996; Green et al., 1999; Williams et al., 1999) – see also for review (Drobetz et al., 2012; Mather, 2016). Also, sex-specific differences became clear in our analysis for action impulsivity, females performing significantly more premature responses than males, while no sex effect was found in delay tolerance. Finally, WH animals were, in general, more impulsive than SD.

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8. Figures

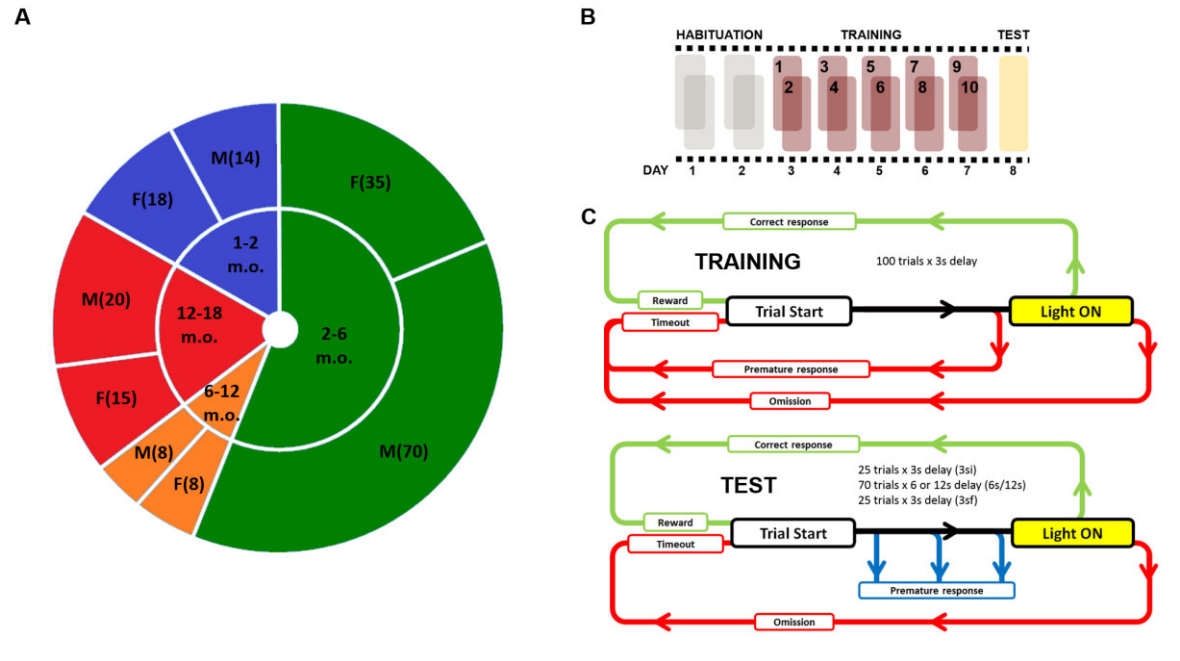


Fig. 1. Experimental organization. (A) Organization of the animals assessed in this work (N=188) by age and sex. (B) The VDS protocol includes 3 phases: habituation, training and test. Habituation and training are performed in 4 and 10 sessions respectively, 2 sessions a day. The test session is performed on a single session on the last day. (C) Each training session (top) comprises 100 trials in a maximum of 30 minutes. In this phase, the animal learns to nosepoke after a light signal and correct responses are reward with a sugared pellet. If the animal does not respond to the light (omission) or responds before the light turns on (i.e., within the 3s delay; premature response), no reward is delivered and the animal is punished with a timeout in complete darkness. The test session (bottom) comprises 120 trials performed in a maximum of 60 minutes. This session is similar to training except that premature responses are allowed (i.e., not punished) and the delays are variable: 25 initial trials with 3s delays (3si), 70 trials with randomized 6s or 12 s delays (6s and 12s respectively) and 25 final trials with 3s delays (3sf). F - female; M - male.

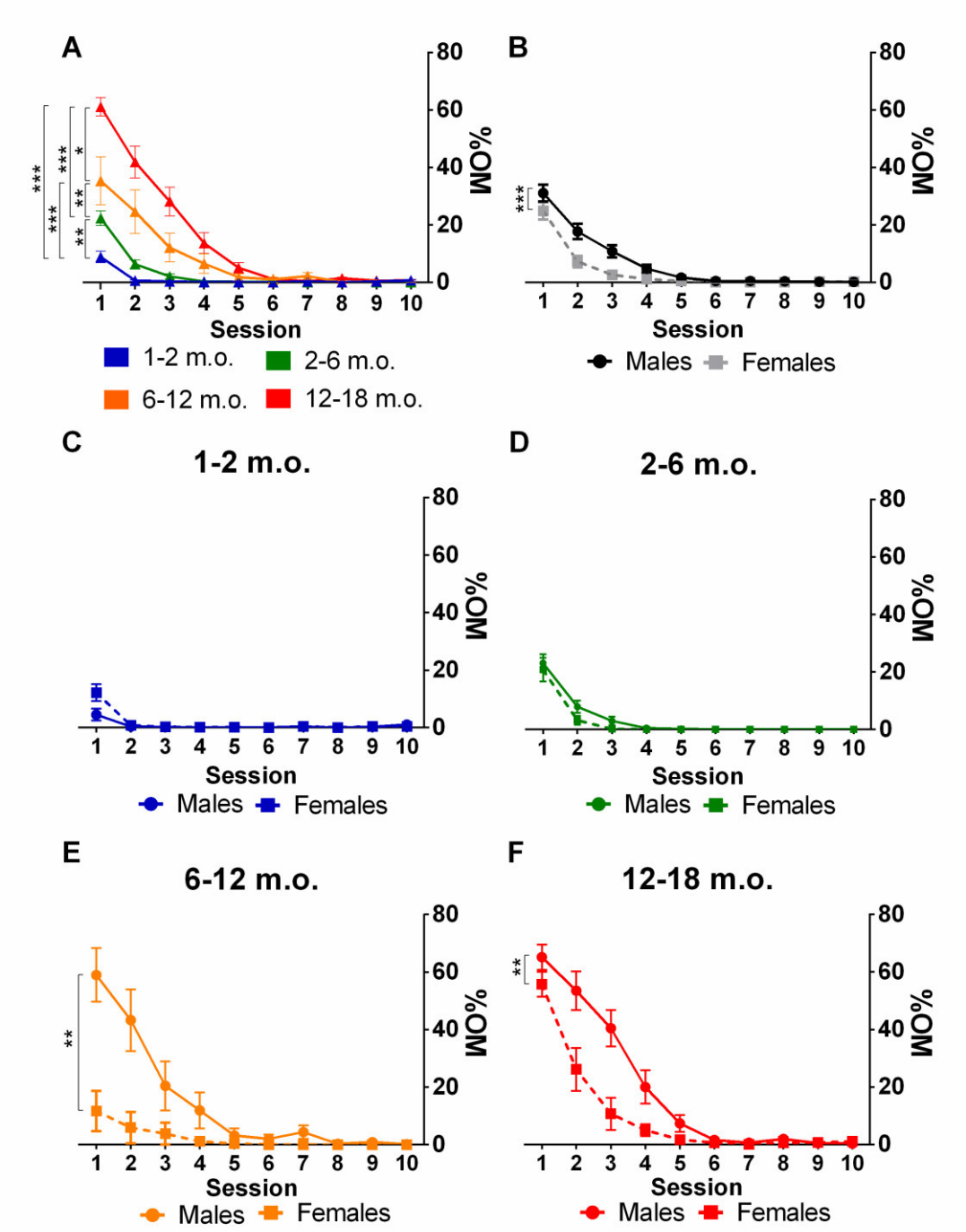


Fig. 2. VDS task acquisition. Evolution of omissions across sessions by age and sex. (A) Older animals and (B) males require on average more sessions to reach a steady performance close to zero omissions. This sex effect was particularly evident in (E) 6-12 and (F) 12-18 m.o. animals. Data is presented as mean ± SEM and statistically significant comparisons between groups are marked with *; * - P<0.05; ** - P<0.01; *** - P<0.001; m.o. - months old; %OM - percentage of omissions.

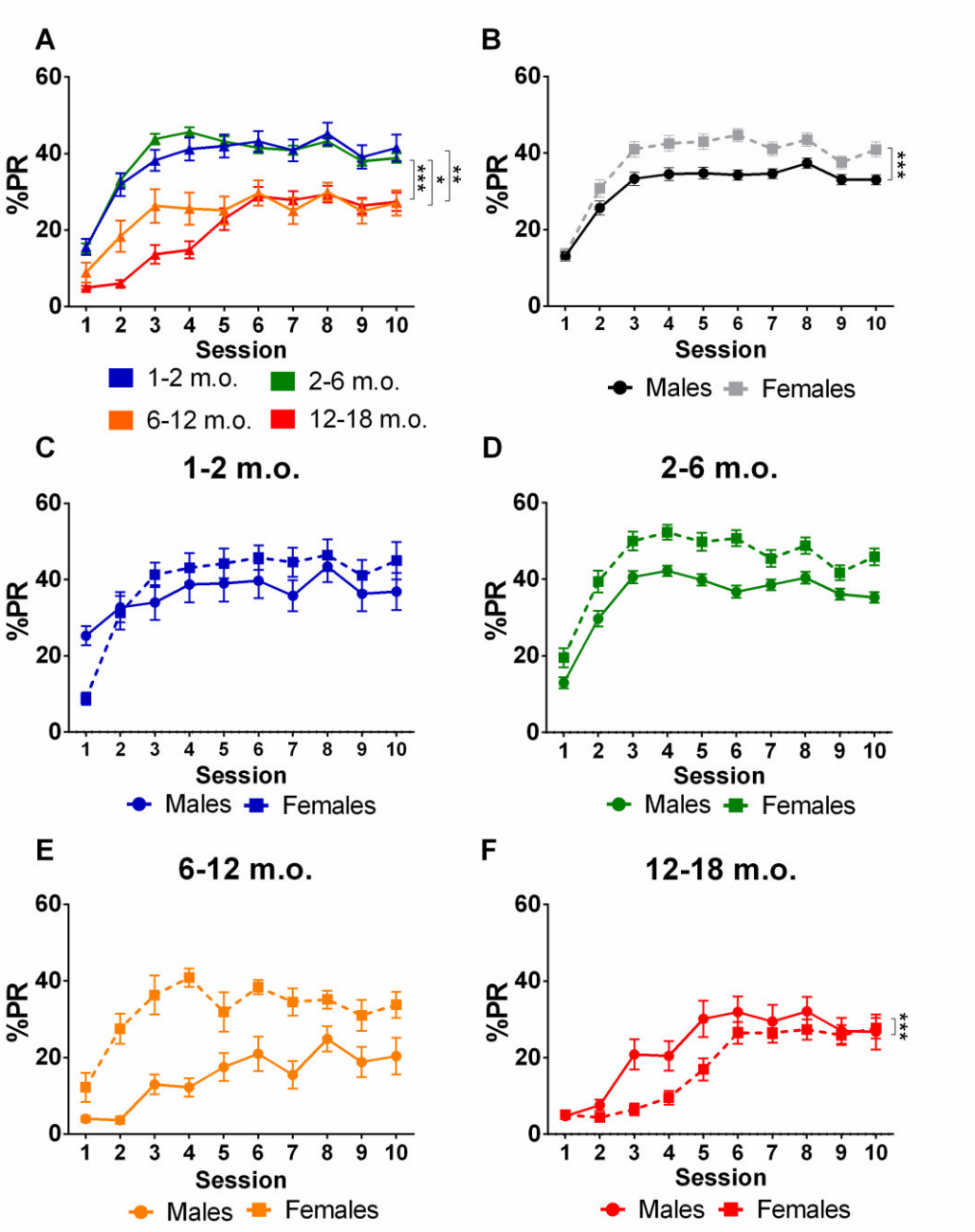


Fig. 3. Action impulsivity (training phase). Evolution of premature responses by age and sex in the training phase. (A) Older animals and (B) males presented reduced action impulsivity during training. When analyzing sex effects for each age group, it was only evident in (F) 12-18 m.o. animals. Data is presented as mean ± SEM and statistically significant comparisons between groups are marked with *; * - P<0.05; ** - P<0.01; *** - P<0.001; m.o. - months old; %PR - percentage of premature responses.

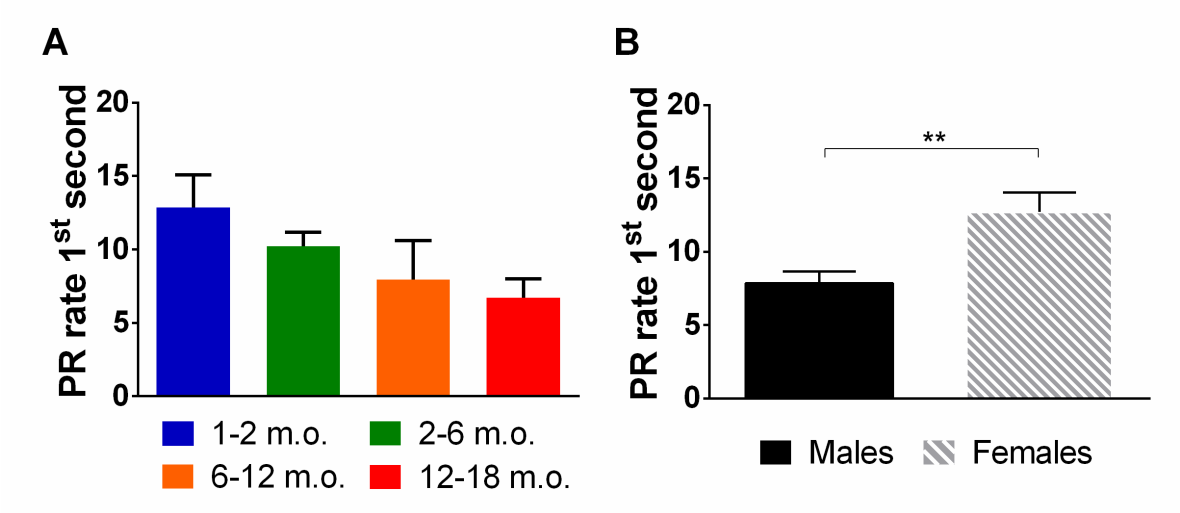


Fig. 4. Action impulsivity (VDS). PR rate in the 1st second of the 3si block of the test session by age and sex. (A) No age-dependent statistically significant differences were found. (B) males presented reduced action impulsivity in comparison with females. Sex differences for individual age groups are not shown because no age vs sex interaction was found. Data is presented as mean ± SEM and statistically significant comparisons between groups are marked with *; ** - P<0.01; m.o. - months old; %PR rate - rate of premature responses per minute.

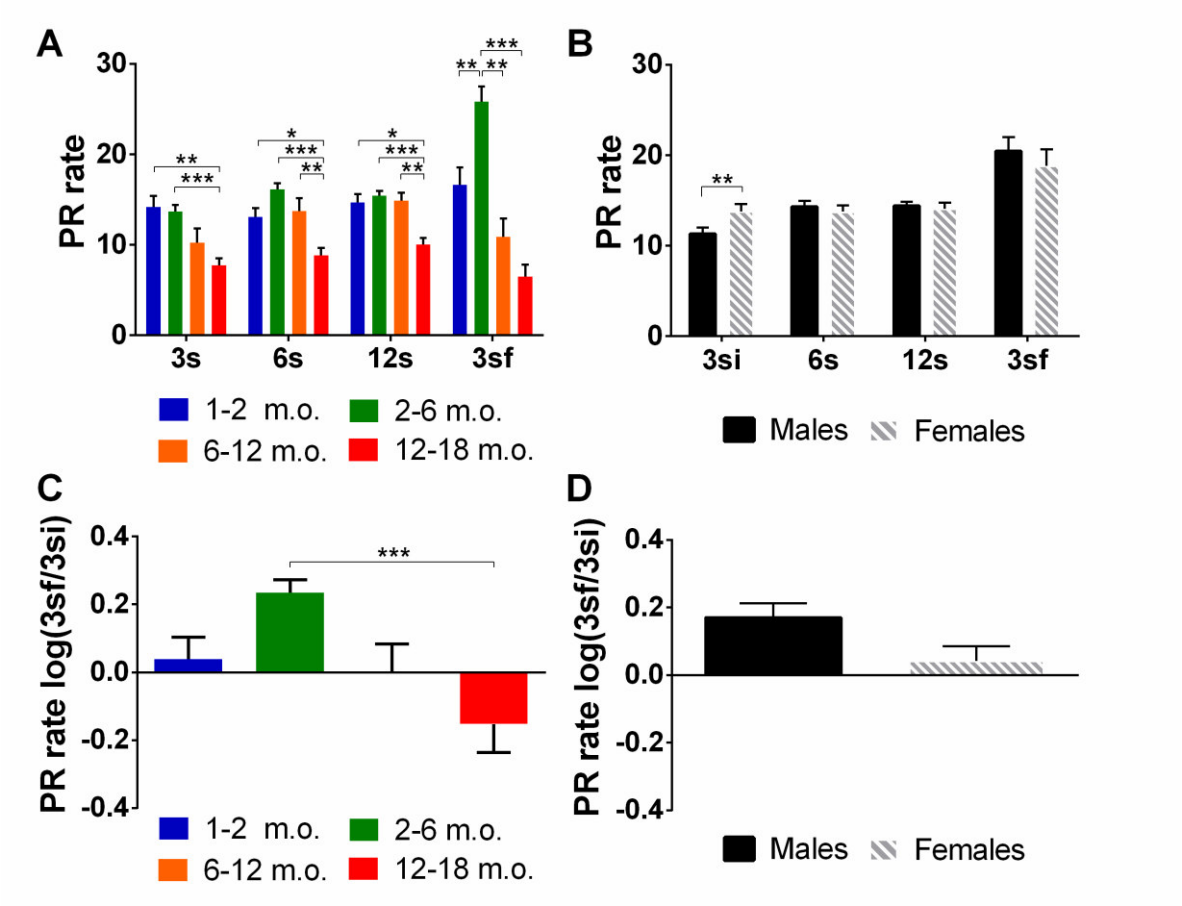


Fig. 5. Delay tolerance. Rate of PRs is shown in each phase of the test by age and sex. (A) The rate of premature responses decreased with age in all blocks, while (B) sex differences were only shown in the 3s interval. The variation of PR rate in the 3sf block in comparison to baseline 3si shows the same trend regarding both age (C) and sex (D). Sex differences for individual age groups are not shown because no age vs sex interaction were not found in the 3sf block, nor in the 3sf/3si. Data is presented as mean ± SEM and statistically significant comparisons between groups are marked with *; ** * - P<0.05; ** - P<0.01; *** - P<0.001; m.o. - months old; PR rate - rate of premature responses per minute.

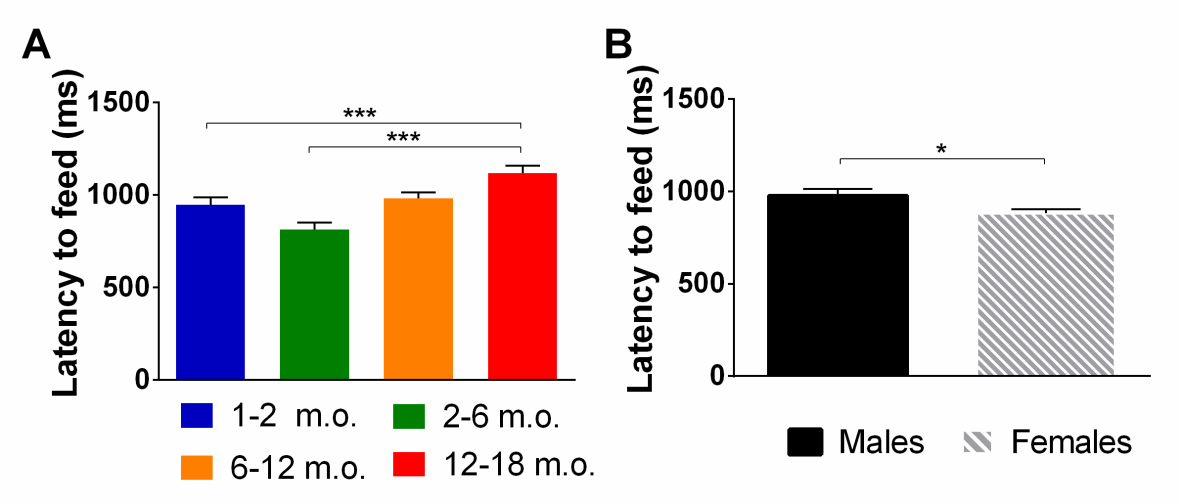


Fig. 6. Latency to feed during the VDS test. Overall latency to feed by age and sex. (A) Latency to feed was influenced by age, with older animals (12-18 m.o.) showing increased time to retrieve the reward than younger (1-2 and 2-6 m.o. animals). (B) No sex effect was found. Sex differences for individual age groups are not shown because no age vs sex interaction was found. Data is presented as mean ± SEM and statistically significant comparisons between groups are marked with *; *** - P<0.001.

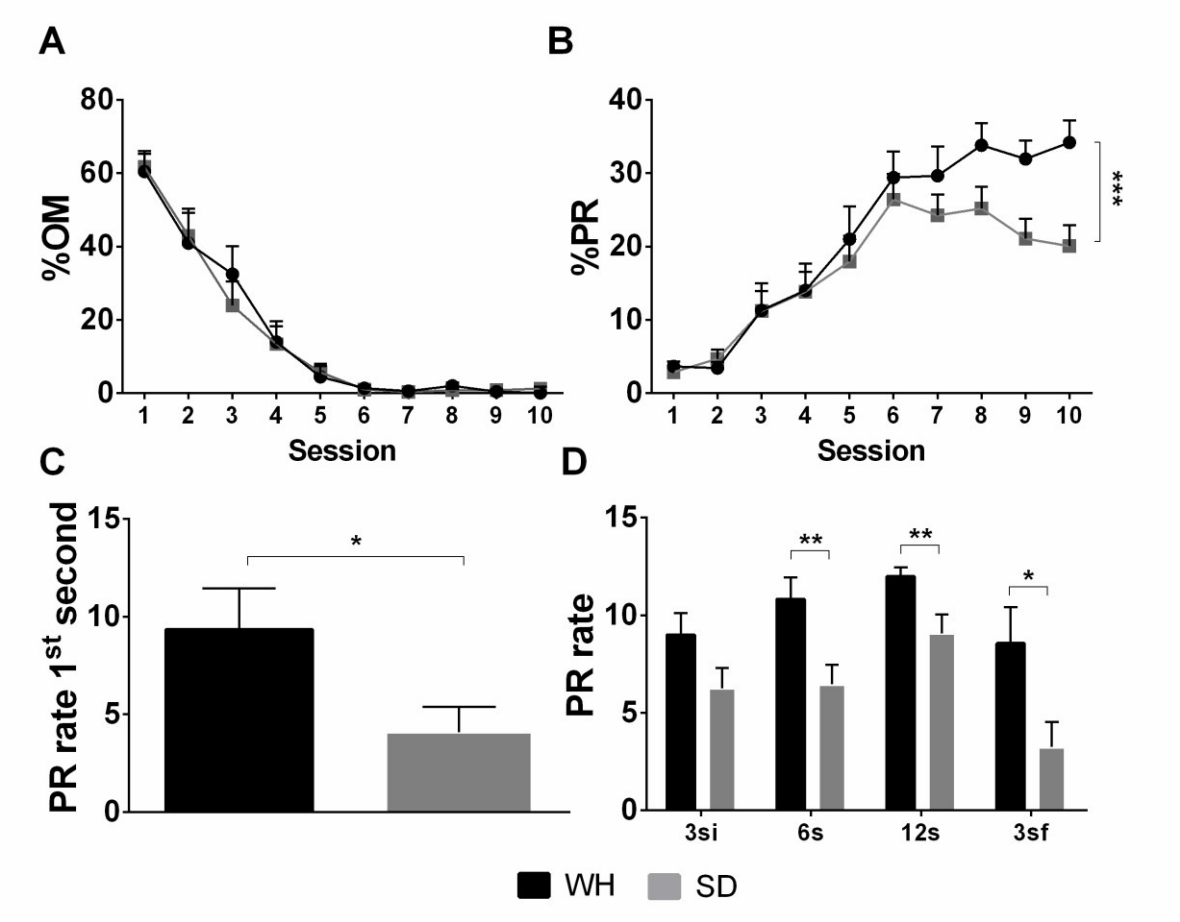


Fig. 7. Influence of strain in choice impulsivity and delay intolerance. The analysis of the effect of the strain in impulsive behavior was restricted to 12-18 m.o. males and females. (A) Both strains learned the task equally, progressively reducing %OM to 0. WH presented higher action impulsivity in both (B) training and (C) PR rate on the early initial block of the test. (D) During the test, WH animals consistently showed higher PR rate in 6s, 12s and 3sf blocks. Data is presented as mean ± SEM and statistically significant comparisons between groups are marked with *; * - P<0.05; ** - P<0.01; *** - P<0.001; %OM - percentage of omission responses; %PR - percentage of premature responses; %PR rate - rate of premature responses per minute; WH - Wistar Han; SD - Sprague-Dawley.

9. Tables

	Training				Test
	%OM effects		%PR effects		PR rate 1st sec effects
	Group	Group*Session	Group	Group*Session	Group
Age	$F_{(3,149)}$=31.584; p<0.001	$F_{(6.2,309.8)}$=17.331; p<0.001	$F_{(3,155)}$=39.556; p<0.001	$F_{(14.6,752.33)}$=4.012; p<0.001	$F_{(3,178)}$=2.094; p=0.103
Sex	$F_{(1,149)}$=26.132; p<0.001	$F_{(2.1,309.8)}$=12.118; p<0.001	$F_{(1,155)}$=33.726; p<0.001	$F_{(4.9,752.3)}$=4.918; p<0.001	$F_{(1,178)}$=10.554; p=0.001
Sex* Age	$F_{(1,149)}$=8.733; p<0.001	$F_{(6.6,309.8)}$=5.526; p<0.001	$F_{(3,155)}$=1.594; p=0.193	$F_{(14.6,752.3)}$=2.133; p=0.008	$F_{(3,178)}$=0.535; p=0.659

Table 1. General effects of sex and age on learning and action impulsivity. Effects on task learning were assessed through the percentage of omissions during training (%OM) while effects on action impulsivity were evaluated in the percentage of premature responses during training (%PR) and prematurity rate on the first second of the test. Main effects of group (between factor) and session/group (within/between factors), in which groups are divided by age and sex, are shown. P<0.05 was considered the threshold for statistical significance and age is measured in months.

	Test				
	PR rate effects				
	3si	6s	12s	3sf	log(3sf/3si)
Age	$F_{(3,176)}$=8.298; p<0.001	$F_{(3,176)}$=13.138; p<0.001	$F_{(3,176)}$=9.602; p<0.001	$F_{(3,176)}$=18.919; p<0.001	$F_{(3,179)}$=7.607; p<0.001
Sex	$F_{(1,176)}$=10.845; p=0.001	$F_{(1,176)}$=0.987; p=0.322	$F_{(1,176)}$=0.051; p=0.821	$F_{(1,176)}$=0.400; p=0.528	$F_{(1,179)}$=0.499; p=0.504
Sex* Age	$F_{(3,176)}$=1.478; P=0.222	$F_{(3,176)}$=2.518; p=0.060	$F_{(3,176)}$=2.481; p=0.063	$F_{(3,176)}$=0.813; p=0.488	$F_{(3,179)}$=0.574; p=0.632

Table 2. General effects of sex and age on delay intolerance. Effects were assessed based on the prematurity rate (PR rate) during the test phases (3si, 6s, 12s and 3sf) and in the 3sf normalized to baseline (log(3sf/3si)). Main effects of group (age and sex) are shown. P<0.05 was considered the threshold for statistical significance and age is measured in months.

	Test
	Group
Age	$F_{(3,179)}$=7.249; p<0.001
Sex	$F_{(1,179)}$=3.997; p=0.047
Sex*Age	$F_{(1,149)}$=0.569; p=0.636

Table 3. Effects of sex and age on latency to feed. Effects were assessed based on the latency to feed during the VDS test. Main effects of group (age and sex) are shown. P<0.05 was considered the threshold for statistical significance and age is measured in months.

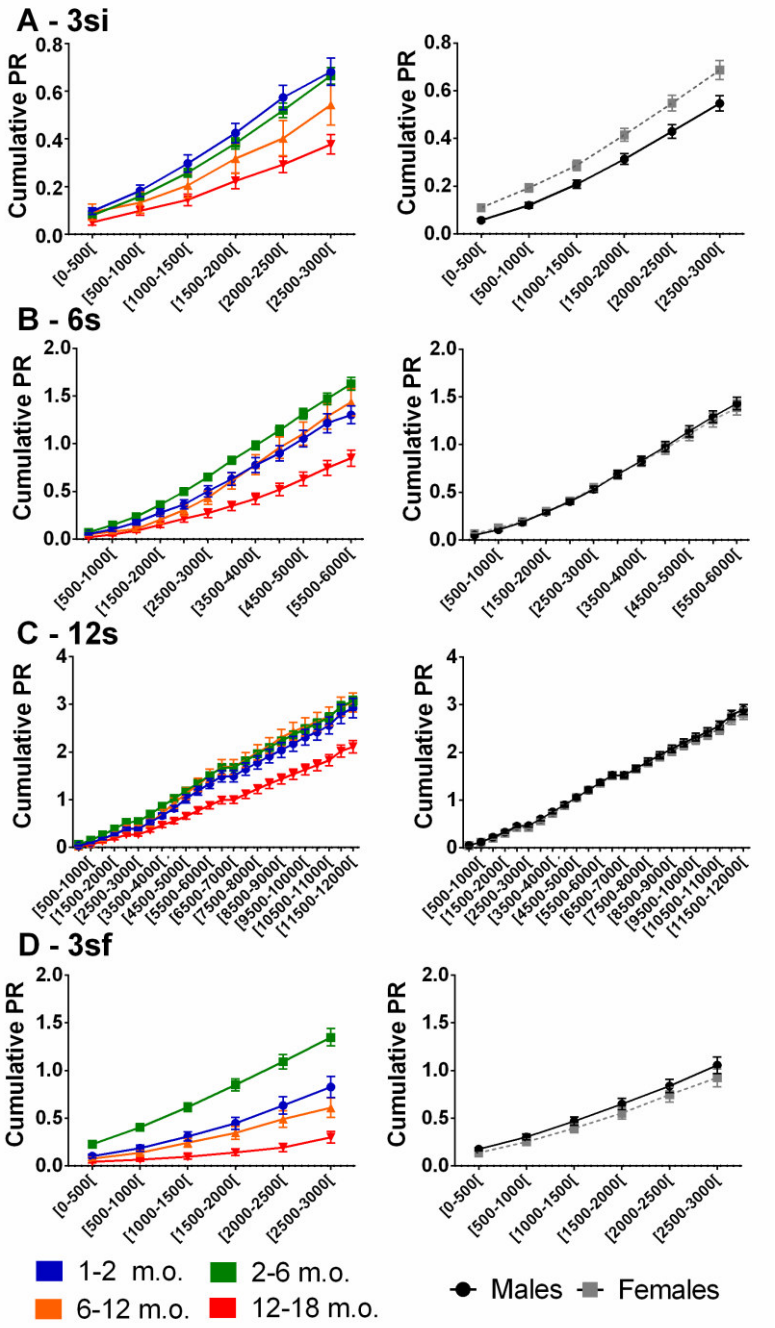
	Training				Test
	%OM effects		%PR effects		PR rate 1st sec effects
	Group	Group*Session	Group	Group*Session	Group
Strain	$F_{(1,20)}$<0.001; p=0.984	$F_{(3.2,64.9)}$=0.197; p=0.910	$F_{(1,21)}$=3.625; p=0.071	$F_{(7.4,154.92)}$=5.596; p<0.001	$F_{(1,33)}$=6.224; p=0.018
Sex	$F_{(1,20)}$=5.066; p=0.036	$F_{(3.2,64.9)}$=4.295; p=0.007	$F_{(1,21)}$=11.556; p=0.003	$F_{(7.4,154.92)}$=4.760; p<0.001	$F_{(1,33)}$=3.011; p=0.093
Strain* Sex	$F_{(1,20)}$=2.488; p=0.130	$F_{(3.2,64.9)}$=1.518; p=0.216	$F_{(1,21)}$=2.111; p=0.161	$F_{(7.4,154.92)}$=1.089; p=0.371	$F_{(1,33)}$=0.072; p=0.790

Table 4. General effects of strain and sex on learning and action impulsivity. Effects on task learning were assessed through the percentage of omissions during training (%OM) while effects on action impulsivity were evaluated in the percentage of premature responses during training (%PR) and prematurity rate on the first second of the test. Main effects of group (between factor) and session/group (within/between factors), in which groups are divided by strain and sex, are shown. P<0.05 was considered the threshold for statistical significance.

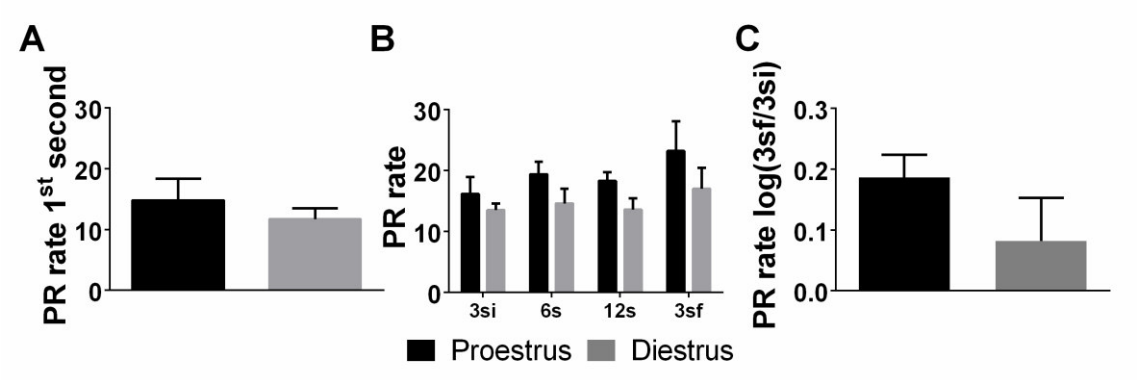
	Test				
	PR rate effects				
	3si	6s	12s	3sf	log(3sf/3si)
Strain	$F_{(1,33)}$=3.515; p=0.071	$F_{(1,33)}$=10.002; p=0.004	$F_{(1,33)}$=8.446; p=0.007	$F_{(1,33)}$=4.552; p=0.042	$F_{(1,33)}$=0.484; p=0.492
Sex	$F_{(1,33)}$=1.095; p=0.304	$F_{(1,33)}$=0.126; p=0.725	$F_{(1,33)}$=0.788; p=0.382	$F_{(1,33)}$=0.353; p=0.557	$F_{(1,33)}$=1.321; p=0.259
Strain* Sex	$F_{(1,33)}$=0.418; p=0.523	$F_{(1,33)}$=4.992; p=0.033	$F_{(1,33)}$=7.881; p=0.009	$F_{(1,33)}$=0.474; p=0.496	$F_{(1,33)}$=0.081; p=0.778

Table 5. General effects of strain and sex on delay intolerance. Effects were assessed based on the prematurity rate (PR rate) during the test phases (3si, 6s, 12s and 3sf) and in the 3sf normalized to baseline (log(3sf/3si)). Main effects of group (strain and sex) are shown. P<0.05 was considered the threshold for statistical significance.

10. Supplementary data



Sup Fig. 1. Representation of cumulative PRs in all blocks of the test. PRs for each interval within each block - (A) 3si, (B) 6s, (C) 12s and (D) 3sf - are shown, separated by age (left) and sex (right). The xx axis shows intervals (in miliseconds) within each block. PR - premature response; m.o - months old.



Sup Fig. 2. Influence of estrous cycle in choice and delay intolerance impulsivity. The analysis of the effect of the estrous cycle in impulsive behavior was restricted to 2-6 m.o. females in the diestrus or proestrus phase of the cycle. Estrous cycle did not affect (A) impulsive actions in the first second of the 3si block of the test, (B) PR rate in any block or (C) PR rate 3sf/3si comparison. Data is presented as mean ± SEM. PR rate - rate of premature responses per minute.

			%OM Effects				
			session/age				session/sex
			1-2	2-6	6-12	12-18	
%PR Effects	session/age	1-2		<0.010	<0.001	<0.001	0.376
		2-6	0.469		<0.010	<0.001	0.418
		6-12	0.396	<0.050		<0.050	<0.01
		12-18	<0.010	<0.001	0.109		<0.01
	session*sex		0.058	0.110	0.126	<0.001	

Sup Table 1. Effects found in the comparison of the different age groups on learning and action impulsivity. Effects on task learning were assessed through the percentage of omissions (%OM - white area) while effects on action impulsivity were evaluated in the percentage of premature responses (%PR - grey area) during training. Main session/group (within/between) effects are shown for comparison between two age groups and comparison between males and females within the same age group. P<0.05 was considered the threshold for statistical significance and age is measured in months.

			age			
			1-2	2-6	6-12	12-18
			3si			
age	1-2	6s		1.000	0.698	<0.010
	2-6		0.146		0.799	<0.001
	6-12		1.000	1.000		0.619
	12-18		<0.050	<0.001	0.007	
			12s			
age	1-2	3sf		0.148	1.000	<0.050
	2-6		<0.010		1.000	<0.001
	6-12		1.000	<0.010		<0.010
	12-18		0.115	<0.001	0.808	
			log(3sf/3si)			
age	1-2			0.070	1.000	0.582
	2-6				0.129	<0.001
	6-12					1.000
	12-18					

Sup Table 2. Effects found in the comparison of the different age groups on delay intolerance. Effects were assessed based on the prematurity rate (PR rate) during the test phases (3si - top white area, 6s - top grey area, 12s - middle white area and 3sf - middle grey area) and in the 3sf normalized to baseline (log(3sf/3si) - bottom white area). Main effects of group (age interval), are shown. P<0.05 was considered the threshold for statistical significance and age is measured in months.

	Test					
	PR rate 1st sec effects	PR rate effects				
		3si	6s	12s	3sf	log(3sf/3si)
Estrous Cycle	$F_{(1,14)}$=0.751; p=0.402	$F_{(1,13)}$=0.925; p=0.355	$F_{(1,13)}$=1.92; p=0.189	$F_{(1,13)}$=3.50; p=0.086	$F_{(1,13)}$=1.138; p=0.307	$F_{(1,14)}$=0.000; p=0.997

Sup Table 3. General effects of estrous cycle on action impulsivity and delay intolerance. Effects were assessed based on the prematurity rate (PR rate) during the first second of the test, during the several test phases (3si, 6s, 12s and 3sf) and in the 3sf normalized to baseline (log(3sf/3si)). Main effects of group (estrous cycle) is shown. P<0.05 was considered the threshold for statistical significance.

APPENDIX B

Effects of stress on brain asymmetry and cognition: a longitudinal study in medical students

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Work performed in the context of Inês Vaz's master thesis in medicine

Abstract:

Structural asymmetries have been widely described, including gross distortions such as counterclockwise Yakovlevian torque and area-specific biases such as the left>right planum temporale. These lateralized aspects are thought to be influenced by multiple factors, including sex, age or stress. In this work, we have evaluated a population of medical students (23.94±2.26 years old; 26 male and 38 female) in terms of structural asymmetries (using MRI and FreeSurfer segmentation), stress (Perceived Stress Scale – PSS and salivary cortisol) and cognition (Stroop interference score). Twenty-five individuals were re-assessed in a longitudinal perspective. We have determined that, similarly to what has been described in an older population, most regions present some degree of asymmetry. PSS was associated with the laterality index of the medial orbitofrontal cortex in a sex-mediated way (i.e. higher PSS was associated with leftward asymmetry in women, while the opposite trend was found in men) in both transversal and longitudinal analyses. Additionally, the transversal analysis of Stroop interference associations with laterality showed negative (i.e rightward) and positive (leftward) correlations in the nucleus accumbens and lateral orbitofrontal cortex, respectively. In conclusion, perceived stress is associated with medial orbitofrontal cortex asymmetry, showing different effects in male and female subjects. Additionally, Stroop interference is correlated with both frontal (lateral orbitofrontal cortex) and subcortical (nucleus accumbens) structural asymmetries, independently of stress.