Revista Mexicana de
Neurociencia

Academia Mexicana de Neurología, A.C.

Publicación oficial de la Academia Mexicana de Neurología A.C.

VOLUME 22 - NUMBER 2 / March-April 2021 – ISSN: 1665-5044
eISSN: 2604-6180
www.revmexneurociencia.com

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EDITORIAL

SARS-CoV-2 as a future cause of dementia. Is anosmia as "benign" as we think?

SARS-CoV-2 Como causa futura de demencia. ¿Es la anosmia tan "benigna" como pensamos?

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Humans can perceive an immense variety of odors and odor perception greatly contributes to the generation of memories. The prevalence of olfactory impairment in the general population is approximately 3.8-5.8%, which rises up to 13.9% in individuals older than 65 years[1]. Sensory and central processing impairment in the components of olfaction are observed in numerous neurodegenerative conditions[2]. These include Alzheimer's disease, vascular dementia, Parkinson's disease, and frontotemporal dementia. At present, we do not fully understand the mechanisms underlying such associations. What has been recognized is that hyposmia and anosmia are associated with a faster rate of cognitive decline compared to normal olfaction and that impaired olfaction is related to smaller hippocampus, entorhinal, fusiform, and middle temporal cortices volumes[1].

Multiple cross-sectional studies have demonstrated a high incidence of hyposmia and anosmia among COVID-19 patients. Hyposmia has been reported in 20%[3] and olfactory dysfunction in up to 85% of COVID-19 patients[4]. Furthermore, olfactory dysfunction preceded the development of other COVID-19 symptoms in 12% of patients and persisted once other symptoms resolved in 63% of patients[4]. While some of these patients recover olfactory function months after their infection, some patients experience only partial recovery. The fact that olfactory dysfunction in the form of anosmia or hyposmia can occur in isolation in COVID-19 patients suggests that there might be involvement of the olfactory nerve[5]. This is supported by fact that the nasal epithelium is covered by the angiotensin-converting enzyme type 2 receptor. However, the long-term implications of anosmia are currently unknown.

Regardless of the underlying pathology, reduced olfaction is common in COVID-19 patients, where the most intriguing are those who do not regain olfactory function. This abnormality may lead to retrograde degeneration of neurons connected to the hippocampus with unforeseen consequences for patients. Consequently, this potential association merits further investigation. Recognizing patients with olfactory impairment after COVID-19 and following their cognitive status might help us understand if there is a link between olfactory impairment and cognitive decline in these patients. I think a screening during consultation *post-COVID* patients with basic cognitive tests could help us begin to understand if really cognitive

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Date of reception: 07-11-2020
Date of acceptance: 23-12-2020
DOI: 10.24875/RMN.20000113

Available online: 17-03-2021
Rev Mex Neuroci. 2021;22(2):40-41
www.revmexneurociencia.com

decline is something that we will see in these patients, particularly those that had or persist with changes in olfaction.

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ORIGINAL ARTICLE

Diffusion tensor imaging (tractography) in elderly people with dementia type Alzheimer's disease and mixed dementia

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Abstract

Objective: *The objective of the study was to evaluate the characteristics of white matter tracts by diffusion tensor imaging (tractography) in elderly people with dementia type Alzheimer's disease (AD) and mixed dementia through the measurements of fractional anisotropy (FA) and mean diffusivity (MD).* **Methods:** *Eight patients with AD and eight patients with mixed dementia were studied. Clinical, cognitive, and neuroimaging evaluation was carried out. Variables are described using the arithmetic mean, standard deviations, and frequencies. Chi-square and U Mann–Whitney tests were used. Correlation analysis was performed between neuropsychological characteristics and the degree of affection by tractography (FA and MD).* **Results:** *Significant differences were found between the groups of AD versus mixed dementia in FA: right cerebral peduncle 0.5733 versus 0.5557 ($p < 0.05$), left cerebral peduncle 0.5744 versus 0.5476 ($p < 0.01$), right external capsule 0.3619 versus 0.3346 ($p < 0.01$), and left cingulum gyrus 0.4049 versus 0.3756 ($p < 0.05$). MD: right thalamic posterior radiation 0.0016 versus 0.0010 ($p < 0.03$) and left external capsule 0.0015 versus 0.0012 ($p < 0.03$).* **Conclusion:** *Using tractography, it is possible to quantify the extent of damage to the white matter tracts (vascular and neurodegenerative).*

Key words: *Tractography. Fractional anisotropy. Mean diffusivity. Alzheimer's disease. Mixed dementia.*

Imagen de tensor de difusión (tractografía) en personas mayores con demencia tipo enfermedad de Alzheimer y demencia mixta

Resumen

Objetivo: *Evaluar las características de los tractos de sustancia blanca por imagen de tensor de difusión (tractografía) en personas mayores con demencia tipo enfermedad de Alzheimer y demencia mixta, a través de la medición de la fracción de anisotropía y la difusividad media.* **Métodos:** *Se estudiaron 8 pacientes con demencia tipo enfermedad de Alzheimer y 8 pacientes con demencia mixta, se realizó evaluación clínica, cognitiva y neuroimagen. Las variables se describen utilizando media aritmética, desviaciones estándar, Chi-cuadrada y U Mann-Whitney. Se realizó análisis de correlación entre características neuropsicológicas y el grado de afección por tractografía (fracción de anisotropía y difusividad media).* **Resultados:** *Se encontraron diferencias significativas entre los grupos enfermedad de Alzheimer vs. demencia mixta en la fracción de anisotropía: pedúnculo cerebral derecho 0.5733 vs. 0.5557 ($p < 0.05$), pedúnculo cerebral izquierdo 0.5744 vs.*

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Date of reception: 29-09-2020
Date of acceptance: 09-12-2020
DOI: 10.24875/RMN.20000106

Available online: 17-03-2021
Rev Mex Neuroci. 2021;22(2):42-50
www.revmexneurociencia.com

0.5476 (p < 0.01), cápsula externa derecha 0.3619 vs 0.3346 (p < 0.01) y giro del cíngulo izquierdo 0.4049 vs 0.3756 (p < 0.05). Difusividad media: radiación posterior talámica derecha 0.0016 vs. 0.0010 (p < 0.03) y cápsula externa izquierda 0.0015 vs. 0.0012 (p < 0.03). **Conclusión:** *Mediante la tractografía es posible cuantificar la magnitud de daño de los tractos de la sustancia blanca (vascular y neurodegenerativo).*

Palabras clave: *Tractografía. Fracción de anisotropía. Difusividad media. Enfermedad de Alzheimer. Demencia mixta.*

Introduction

Tractography is used to correlate the integrity of white matter tracts with cognitive function in cognitively healthy people and with dementia[1]. It is a special technique of simple magnetic resonance imaging (MRI), which consists of non-invasive diffusion tensor imaging (DTI), sensitive to the diffusion of water molecules[2], which allows a live three-dimensional reconstruction of the tracts within the central nervous system[1,3]. The fibers of white matter are classified into three categories: (a) association fibers: those that interconnect cortical areas of the same hemisphere, (b) commissural fibers: interconnect areas between both hemispheres, and (c) projection fibers, which connect the cortex with formations of lower levels[4].

The interruption of the tracts can be detected as a decrease in fractional anisotropy (FA) (diffusion orientation and directionality) and an increase in mean diffusivity (MD) (degree of water diffusion)[1,4,5,6]. An anisotropy of "0" corresponds to a perfect sphere, while 1 would be an ideal linear diffusion. Well-defined tracts generally have an FA greater than 0.2[1,6,7]. Preliminary studies show a decrease in white-matter connections in patients with Mild cognitive impairment and Alzheimer's disease (AD). Especially noticeable is the increased MD and decreased FA in these patients compared to normal controls. These changes were found in the posterior cingulate fasciculus, the uncinate fasciculus, or both at once[1].

AD is the most common neurodegenerative disorder that causes dementia in elderly individuals. The cause of AD is unknown in most cases. The most powerful risk factor for developing AD is age, with AD affecting as many as 40-50% of individuals older than 85 years[8]. Mixed dementia (MixD) represents the third leading cause of dementia, only behind AD and vascular dementia (VaD). The prevalence range of MixD varies between 20% and 40%[9,10]. The Vascular Impairment of Cognition Classification Consensus Study (VICCCS) proposes in its classification of vascular cognitive impairment (VCI) the definition of MixD, which includes phenotypes that represent the combination of vascular and neurodegenerative diseases, that is, VCI-AD, in addition to other possible combinations[11]. From a clinical viewpoint, loss of memory (especially episodic and semantic) is considered a typical feature of AD, whereas executive dysfunction has traditionally been associated with MixD. Several MRI analysis methods can track structural atrophy in AD and the presence of ischemic lesions on computed tomography or MRI is key in the diagnosis of individuals with MixD[9].

Populations with high cardiovascular risk, patterns are more frequently observed cognitive skills of mixed type and the pure forms of dementia or sole are not the first diagnostic option in older subjects with memory complaints. This aspect is important because the adequate identification of these potentially risk factors modifiable could help intervene in an entity still without a definitive therapeutic route[9]. Accurate diagnosis of AD and MixD is of crucial significance for epidemiological purposes and for preventive and therapeutic strategies[12].

The objective of our study was to evaluate the characteristics of white matter tracts by DTI (tractography) in elderly people with dementia type AD and MixD through the measurements of FA and MD.

Methods

Type of study and patients

Cross-sectional study carried out in a memory disorder clinic of a third-level hospital in Mexico City. All the patients were recruited in a period from March 2019 to December 2019. They all signed a consent form and underwent clinical and cognitive evaluations by a specialist in neurology and/or geriatrics. From the comprehensive geriatric evaluation, sociodemographic variables were obtained such as sex, age, education, Katz Index[13], Lawton and Brody Scale[14], and Yesavage Geriatric Depression Scale (GDS)[15]. The neuropsychological evaluation consisted of applying the NEUROPSI test[16], Mini-Mental State Examination (MMSE)[17], verbal fluency test[18], clock-drawing test[19], frontal assessment battery (FAB)[20], and the Clinical Dementia Rating (CDR) Scale[21].

Patients with visual and auditory acuity deficits that would make it impossible to apply neuropsychological tests were excluded, as well as patients with dysthyroidism (hyper or hypothyroidism) without treatment, hypertension, dyslipidemia and/or uncontrolled diabetes mellitus, and those with glycosylated hemoglobin levels higher than 9%, patients with severe hypoglycemia, other causes of dementia (vascular, frontotemporal, Lewy bodies), other uncontrolled medical conditions (cardiovascular, renal, or advanced lung disease), inflammatory diseases, active smokers, patients with significant depressive symptoms (GDS score > 5/15), CDR > 1, and the presence of metallic objects, devices, or conditions that would make MRI impossible.

Clinical diagnosis of dementia

The patients were classified into two groups: AD n = 8 and MixD n = 8, according to their performance in neuropsychological evaluation and current clinical criteria. For the diagnosis of dementia type AD, the criteria of the Diagnostic and Statistical Manual of Mental Disorders version 5 (American Psychiatric Association DSM-5)[22] and the criteria of the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[23] were used. For the diagnosis of MixD, the VICCCS[11] criteria were used. For the dementia stage, the CDR score (Mild = 1) was applied in both conditions.

Magnetic resonance DTI (tractography)

All patients had a simple brain MRI, with T1 sequence with DTI and volumetry. The images were obtained with a 1.5T resonator (Siemens® Medical Systems). Image processing was performed by the Neuroimaging Department of the National Institute of Medical Sciences and Nutrition, Salvador Zubirán (INCMNSZ). The MRI was evaluated by a neuroradiologist (not knowing of patients being in the clinical group). A high-resolution anatomical scanner was used with a T1-weighted three-dimensional magnetization sagittal sequence, prepared with fast gradient echo (MP-RAGE), field of view (FoV) 26 mm, spatial resolution $1 \times 1 \times 1$ mm^3, repetition time of 1500 ms (TR), echo time (TE), rotation angle 9°, number of cuts 176, with 25 independent diffusion gradient directions[1,4,5,24]. The process to perform the tractography begins with the definition of an origin point (also called seed point), and the reconstruction process, which consists of taking the orientation of the voxel and advancing a certain distance until a new direction of propagation is found with the help of the orientation of neighboring voxels. From here on, it continues iteratively, adding a new segment to each step, thus forming a path with the points in each step[3,25]. The basis of the anisotropic diffusion of water in the nervous system is due to the involvement of longitudinally oriented structures within the axons, such as myelin, axonal membrane, microtubules, neurofilaments, or axonal transport, which hinders the diffusion of water. MD measures the extent of diffusion in a voxel; the increase in MD may be related to a loss of coherence in fiber alignment, a lower fiber density, or a loss of myelination[1,6,8].

The images obtained from DTI were assigned to maps of FA and MD, the tracts were established using JHU White Matter Tractography Atlas. A standard color scheme was used in the software to encode the FA maps, with blue indicating superior-inferior, red indicating transverse, and green indicating anterior-posterior[26]. Tracts were created in the Diffusion Toolkit using the Continuous Tracking Fiber Assignment method with 35° angle threshold and a unique set of fibers was generated for each patient by performing anatomically detailed analysis. It was decided to study tracts that correspond to association, commissural and projection fibers (16 brains, 48 regions of interest – ROI). Mean values of FA and MD were analyzed for each tract, these being the most important indices for determining dementia according to the previous studies[1,6]. In Figure 1, an example of the tract obtained with the proposed protocol is shown, where the cingulum is seen in red, corresponding to the left cerebral hemisphere.

Structural MRI

Cortical thickness studies

The T1 images were processed with the FreeSurfer 5.3 program, which is capable of constructing models of the cortical surface of the nervous system. This software works in an automated way and the process consists of registering the input volumes determined by the user to the atlas MNI305 (Montreal National Institute), then it performs an intensity normalization and a segmentation of the voxels according to this parameter and their spatial location to classify them in different types of tissue: gray matter, white matter, and cerebrospinal fluid[27]. For the present study, cerebral cortex was divided into 48 ROIs based on turns per hemisphere, and in this manner, the geometric information derived from

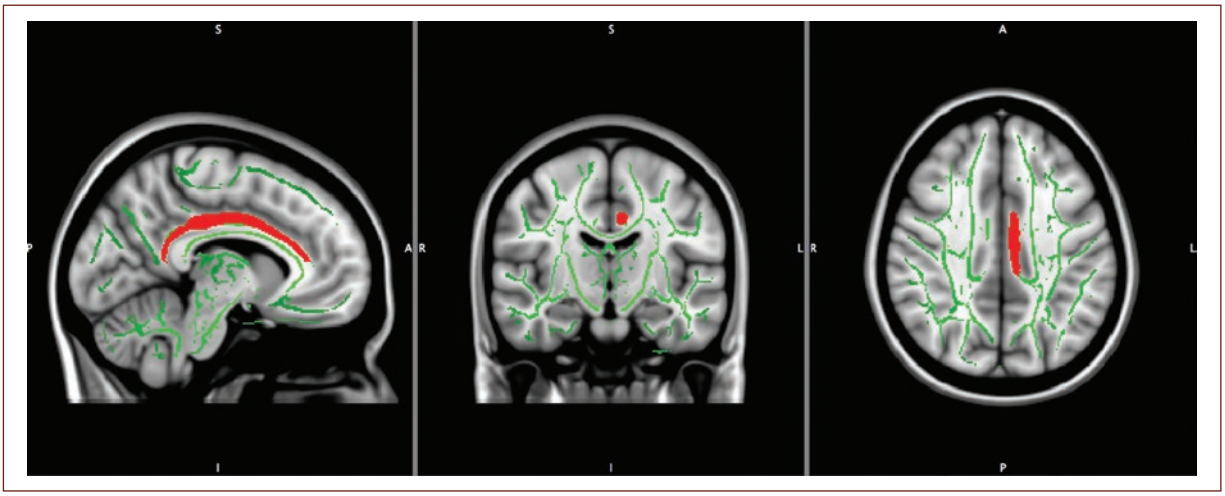

Figure 1. Left cingulum tractography (red).

the cortical model with the labels of the neuroanatomical parceling is incorporated.

The entire protocol was acquired during a single 25-min session, where the patient's brain was aligned in the stereotactic space: the anterior commissure-posterior commissure line was aligned with the axial plane, and the interhemispheric fissure was aligned along the sagittal plane and at right angles to the coronal plane.

Statistics analysis

The variables are described by using arithmetic mean and standard deviations. The Chi-square test was used for categorical variables and the Mann–Whitney U test was used for continuous variables. Clinical and demographic data were tested for normality using a Shapiro–Wilk test. The voxel statistics in the skeletonized images were performed using the randomized, an FSL tool for nonparametric permutation inference in neuroimaging data. The carcass of mean FA (threshold 0.2) was used as a mask, the permutation number was set at 5000, meaning the difference between groups was corrected for multiple comparisons using the no-threshold cluster enhancement method (TFCE) and tested a $p < 0.01$. To identify the power of the selected brain areas to discriminate between AD and MixD, a stepwise discriminant analysis was performed using the dementia subgroup (AD or MixD) as the dependent variable and the mean values of ROI of FA and MD as independent variables. Finally, by means of a Spearman correlation, the differences between the characteristics of the microstructure of the brain regions were searched in FA, MD, and the neuropsychological evaluation. Variables with a value of $p < 0.05$ were taken as significant.

SPSS version 22 for Windows® (SPSS Inc., Chicago, Illinois) was used for the analyses. The protocol was approved by the institutional Ethics Committee (REF. 3009).

Results

The average age of the AD group members was 84.3 ± 8.8 years old and of the MixD group, it was 85.3 ± 7.6 years old ($p < 0.57$) and 69% were women ($p < 0.23$). The average schooling of AD patients was 10.2 ± 7.3 and of MixD was 11.3 ± 4.8 ($p < 0.87$). In the AD group, 38% were hypertensive versus 100% in MixD ($p < 0.00$) and none had obesity in the AD group versus 38% in MixD ($p < 0.05$). Regarding the global neuropsychological assessment, patients with AD had a worse cognitive performance in the cognitive assessment (NEUROPSI) 68.5 ± 11.6 points compared to the MixD group 83.2 ± 12.4 ($p < 0.02$) (Table 1).

DTI (Tractography)

Regarding the tractography findings, significant differences were found in FA between the AD versus MixD groups in the following tracts: right cerebral peduncle 0.5733 versus 0.5557 ($p < 0.05$), left cerebral peduncle 0.5744 versus 0.5476 ($p < 0.01$), right external capsule 0.3619 versus 0.3346 ($p < 0.01$), and left cingulum gyrus 0.4049 versus 0.3756 ($p < 0.05$). Regarding MD, the differences found were in the following tracts: right thalamic posterior radiation 0.0016 versus

Table 1. Sociodemographic characteristics and cognitive performance in Alzheimer's disease and mixed dementia

	Total patients (n = 16)	Alzheimer's disease (n = 8)	Mixed dementia (n = 8)	p
Age years	84.8 ± 7.9	84.3 ± 8.8	85.3 ± 7.6	0.57
Sex (Female) %	(11) 69	(4) 25	(7) 44	0.23
Years of education, %	10.8 ± 6.0	10.2 ± 7.3	11.3 ± 4.8	0.87
Hypertension %	(11) 69	(3) 38	(8) 100	0.00
Mellitus diabetes %	(3) 19	(1) 13	(2) 25%	0.52
Heart disease %	(4) 25	(1) 13	(3) 38	0.24
Cerebral vascular event %	(1) 6	(0) 0	(1) 13	0.30
Dyslipidemia %	(9) 56	(3) 38	(6) 75	0.13
Hypothyroidism %	(4) 25	(1) 13	(3) 38	0.24
Obesity %	(3) 19	(0) 0	(3) 38	0.05
History depression %	(4) 25	(2) 25	(2) 25	1.00
GDS – 15	2.0 ± 1.5	2.6 ± 1.5	2.2 ± 1.6	0.72
Functionality KATZ LB	 6.0 ± 0.2 6.5 ± 2.5	 6 ± 0.0 5.3 ± 2.9	 5.8 ± 0.3 5.2 ± 2.3	 0.72 0.72
NEUROPSI total	75. 8 ± 13.9	68.5 ± 11.6	83.2 ± 12.4	0.02
Orientation	4.0 ± 1.7	5.1 ± 1.3	2.8 ± 1.4	0.02
Attention	13.8 ± 5.2	13.88 ± 4.9	13.75 ± 5.8	0.72
Memory	20.6 ± 5.4	23.3 ± 4.9	17.8 ± 4.7	0.03
Language	23.1 ± 4.1	21.7 ± 5.1	24.5 ± 2.6	0.23
Visuospatiality/visuospatiality	9.22 ± 1.5	9.1 ± 1.6	9.3 ± 1.6	0.87
Executive functions	11.5 ± 3.5	11.8 ± 3.6	11.2 ± 3.6	0.50
MMSE	22.5 ± 3.2	22.6 ± 3.2	22.5 ± 3.5	0.03
Semantic verbal fluency (animals)	11.0 ± 3.8	13.0 ± 2.8	9.1 ± 3.8	0.06
Phonological verbal fluency (letter F)	8.9 ± 3.8	10.6 ± 4.0	7.0 ± 3.5	0.05
Clock-drawing test	2.3 ± 1.7	2.5 ± 2.0	2.13 ± 1.3	0.95
FAB	12.4 ± 3.2	12.88 ± 3.9	12.0 ± 2.5	0.44

GDS - 15: Yesavage Geriatric Depression Scale; LB: Lawton and Brody scale; MMSE: Mini-Mental State Examination; FAB: front evaluation battery.

0.0010 ($p < 0.03$) and capsule left external capsule 0.0015 versus 0.0012 ($p < 0.03$) (Table 2).

Correlations between cognitive domains and DTI parameters

A correlation was observed between the cognitive domains and the tractography parameters (FA and MD) in both groups, finding a moderate correlation in the AD group between orientation and left cerebral peduncle with a Rho of 0.72 ($p < 0.04$); in the MixD group between orientation and left cingulate gyrus with a Rho of 0.73 ($p < 0.04$), memory and left cerebral peduncle with a Rho of –0.70 ($p < 0.05$), and phonological fluency and left cerebral peduncle with a Rho of –0.72 ($p < 0.04$) (Table 3).

Cortical thickness studies

In the volumetric analysis, significant differences were found in the following ROIs between AD and MixD: Brain segmentation volume 964.92 cm^3 versus 877.95 cm^3 ($p < 0.02$), brain segmentation volume

Table 2. Comparison of tracts, fractional anisotropy, and mean diffusivity in Alzheimer's disease and mixed dementia

	Total Patients (n = 16)	Alzheimer's disease (n = 8)	Mixed Dementia (n = 8)	p
Fractional anisotropy				
Right cerebral peduncle	0.5645 ± 0.0172	0.5733 ± 0.0176	0.5557 ± 0.0121	0.05
Left cerebral peduncle	0.5610 ± 0.0215	0.5744 ± 0.0209	0.5476 ± 0.0121	0.01
Right external capsule	0.3483 ± 0.0246	0.3619 ± 0.0246	0.3346 ± 0.0163	0.01
Left cingulum gyrus	0.3902 ± 0.0271	0.4049 ± 0.0224	0.3756 ± 0.0241	0.05
Mean diffusivity				
Right thalamic posterior radiation	0.0013 ± 0.0004	0.0016 ± 0.0005	0.0010 ± 0.0000	0.03
Left external capsule	0.0013 ± 0.0003	0.0015 ± 0.0003	0.0012 ± 0.0001	0.03

Table 3. Spearman correlation between neuropsychological testing and DTI parameters in Alzheimer's disease and mixed dementia

	Alzheimer's disease						Mixed dementia					
	Orientation		Memory		Fluency phonological		Orientation		Memory		Fluency phonological	
	Rho	p	Rho	p	Rho	p	Rho	p	Rho	P	Rho	p
Fractional anisotropy												
Right cerebral peduncle	0.31	0.44	0.03	0.93	–0.10	0.81	0.11	0.78	–0.32	0.43	–0.36	0.37
Left cerebral peduncle	0.72	0.04	0.07	0.86	–0.07	0.86	–0.27	0.51	–0.70	0.05	–0.72	0.04
Right external capsule	0.34	0.40	–0.27	0.50	–0.35	0.39	0.07	0.85	–0.18	0.67	–0.44	0.26
Left cingulum gyrus	0.60	0.11	–0.10	0.79	–0.12	0.76	0.73	0.04	0.25	0.54	0.32	0.43
Mean diffusivity												
Right thalamic posterior radiation	–0.16	0.69	–0.12	0.77	–0.10	0.81	–0.39	0.33	0.24	0.56	0.21	0.60
Left external capsule	–0.46	0.25	–0.20	0.62	–0.12	0.76	–0.54	0.16	–0.46	0.24	–0.27	0.50

without ventricles 904.25 versus 837.76 ($p < 0.01$), brain segmentation volume without ventricles from surf 903.46 versus 837.16 ($p < 0.01$), right hemisphere cerebral white matter volume 184.52 versus 168.32 ($p < 0.03$), subcortical gray matter volume 46.06 versus 42.10 ($p < 0.05$), total volume of gray matter 514.03 versus 479.94 ($p < 0.01$), total intracranial volume 1471.92 versus 1324.38 ($p < 0.05$), left thalamus 5.86 versus 5.37 ($p < 0.02$), left caudate 3.5 versus 2.71 ($p < 0.02$), fourth ventricle 2.14 versus 1.54 ($p < 0.05$), right caudate 4.01 versus 2.90 ($p < 0.02$), and right choroid plexus 0.92 versus 0.71 ($p < 0.03$) (Table 4).

Discussion

Our study compared patients with AD and MixD through a standardized acquisition protocol to investigate the parameters of FA and MD in terms of changes in the anisotropy or magnitude of water diffusion throughout the brain, demonstrating that patients with AD have alterations in specific tracts, such as right and left cerebral peduncle, right and left external capsule, left cingulate gyrus, and right thalamic posterior radiation compared to patients with MixD.

Palesi et al. demonstrated in a tractography study in patients with DVa, AD, and a control group that patients with AD had a greater affection in the parahippocampal tracts and in the knee of the corpus callosum, while patients with DVa showed a greater affection of white matter in thalamic radiation[28]. Reginold et al., in a study which objective was to evaluate the affection of white matter in patients with AD, demonstrated a greater commitment in the superficial white matter of the temporal lobe than in the members of a control group[25].

Tu et al. compared DTI parameters in members of a control group, patients with AD and subcortical ischemic vascular disease (SIVD; also called lacunar infarction), reporting a global decrease in FA in patients with SIVD; while in patients with AD, the alterations were in the left superior longitudinal bundle, knee and splenium of the corpus callosum, anterior thalamic radiation, uncinate bundle, and left cingulate gyrus, suggesting that DTI is effective in distinguishing patients with early stage of AD versus SIVD[29]. Another

Table 4. Volumetry in regions of interest in Alzheimer's disease and mixed dementia

	Total patients (n = 16)	Alzheimer's disease (n = 8)	Mixed dementia (n = 8)	p
Brain segmentation Volume, cm^3	921,44 ± 76.83	964.92 ± 69.93	877.95 ± 58.61	0.02
Brain segmentation volume without ventricles, cm^3	871.00 ± 58.93	904.25 ± 47.37	837.76 ± 51.68	0.01
Brain segmentation volume without ventricles from surf, cm^3	870.31 ± 58.87	903.46 ± 47.38	837.16 ± 51.67	0.01
Right hemisphere cerebral white matter volume, cm^3	176.42 ± 16.08	184.52 ± 14.60	168.32 ± 13.83	0.03
Subcortical gray matter volume, cm^3	44.08 ± 3.81	46.06 ± 3.36	42.10 ± 3.30	0.05
Total volume of gray matter, cm^3	496.99 ± 34.42	514.03 ± (24.91)	479.94 ± 35.42	0.01
Total intracranial volume, cm^3	1398.15 ± 150.91	1471.92 ± 118.28	1324.38 ± 149.58	0.05
Left thalamus, cm^3	5.62 ± 0.43	5.86 ± 0.39	5.37 ± 0.33	0.02
Left caudate, cm^3	3.11 ± 0.69	3.5 ± 0.64	2.71 ± 0.47	0.02
Fourth ventricle, cm^3	1.84 ± 0.53	2.14 ± 0.45	1.54 ± 0.45	0.05
Right caudate, cm^3	3.45 ± 0.10	4.01 ± 0,24	2.90 ± 0.51	0.02
Right choroid plexus, cm^3	0.82 ± 0.25	0.92 ± 0.27	0.71 ± 0.21	0.03

study by Lee et al., which compared control group members, patients with MCI and AD, showed that the latter group had a greater decrease in FA, as well as lower integrity of white matter associated with a lower hippocampal volume, indicating that the pathology in white matter follows the same degree of stages and progression of neurodegeneration[30].

Another finding in our study was the combined affection of tracts corresponding to neurodegeneration (turn of the left cingulum, cerebral peduncles)[31], as well as areas related to the vascular component (thalamic radiation)[25], a combination only observed, in our study, in patients with MixD. These alterations are the result of changes in axonal density and myelination since the homogeneity in the orientation of the axons affects the degree of FA and MD in DTI, so a decrease in FA and an increase in MD reflect a decrease in the integrity of the brain tissue[1,6,32]. An important aspect to consider is the fact that the vascular load in white matter in patients with MixD could be a potential confounder, similar to other DTI studies in neurodegenerative diseases.

In relation to the results of volumetry, a lower volume was observed in twelve ROIs in patients with MixD. Some studies have established a correlation between DTI parameters and volumetric parameters, indicating that these are measures of the same pathological process, that is, neurodegeneration[33]. The relative change in the parameters that reflect cortical integrity and diffusion is likely to vary not only depending on the methods used but also on the particular morphological process, the anatomical region being studied, and possibly the underlying molecular pathology of the disease in particular[27]. One aspect of techniques for detecting changes in white matter tracts is that they are more sensitive than measurements of cortical thickness or integrity; this is justified because the loss of cortical volume in neurodegenerative disease probably represents the loss of individual neurons (soma) and neuropil (corresponding dendrites, axons)[4,28].

Regarding the cognitive domains and the affected tracts, we found an association between FA and orientation (left cerebral peduncle) in patients with a diagnosis of AD; this anatomical structure is considered a vulnerable area from the vascular point of view[34], in addition, it is related to changes in AD[29]. An association was also observed between FA and the orientation domains (left cingulate), as well as in-memory and phonological fluency (left cerebral peduncle) in patients with MixD; these findings show that there is a disconnection of areas related to cortical memory (left cingulate) since this structure is a fundamental part of the limbic system and one of the main fascicles of white matter that connects areas of cortical association[35].

Our results suggest that DTI allows the identification of alterations in specific tracts (cerebral peduncle and cingulate) in patients with AD compared to patients with MixD. This shows that MixD shares a spectrum of neurodegeneration (due to the affection of

classic tracts affected in AD) but with alterations (decrease) in FA.

The limitations of our study are based on the cross-sectional design, which does not allow the interpretation of the causal mechanisms underlying the associations of MRI and cognitive measurements. Another important aspect is the size of the sample since this could limit the ability to detect more differences in DTI between patients with AD and MixD. Although there are various computer programs available that allow tractography to be carried out, some even free to use for research purposes, the results obtained with one or the other may vary, either due to the algorithm they use or due to their processing, since the reproducibility depends to some degree on the user's interaction with the computer program. In multicenter studies, there are also variations due to different MRI equipment and the protocol used to acquire DTI images; these can influence the quantification of the results using tractography, as mentioned by Fischer et al. and Heiervang et al., who found differences in the quantification of the cerebral fascicles applying different diffusion gradients.

Our study has several strengths: it is the first study to describe tractography characteristics and their association with cognitive performance in elderly adults with AD and MixD. Our findings could lead to future studies in which MixD can be characterized better and the role of tractography and volumetry can be understood more clearly.

Our results support existing findings reported in the literature and, most importantly, provide a complete interpretation of microstructural alterations in the white matter through the spectrum of dementia type AD and MixD using the tractography technique with parametric analysis, and it could provide a useful standard for the early diagnosis of AD and MixD in the future.

Conclusion

Tractography is an effective method to distinguish alterations in brain tracts through the measurement of the anisotropy fraction since these alterations show that MixD shares a spectrum of neurodegeneration and vascular affection.

Funding

This research received no specific grant from any funding agency in public, commercial, or not-for-profit sectors.

Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of the data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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ORIGINAL ARTICLE

Cognitive effects of chronic sleep deprivation in internal medicine residents

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Abstract

Background and objective: *Assessing the effect of chronic sleep deprivation on essential neuropsychological tests, including executive functions for performance such as selective attention, inhibitory control, processing velocity, and working memory among internal medicine residents with chronic sleep deprivation in a highly demanding academic program.* **Methods:** *We conducted a prospective study measuring neuropsychological performance among internal medicine postgraduate year 1 residents in Mexico City. The study was conducted from 2012 to 2013. We used Stroop test, as well as Wechsler Adult Intelligence Scale (WAIS III) to evaluate executive functions. We performed neuropsychological assessments at the beginning of residency, and again 12 months after the baseline assessment.* **Results:** *Chronic sleep deprivation causes an alteration in selective attention, working memory, processing speed, and inhibition. This results in longer time to perform a task, as well as impaired attention during performance of a task.* **Conclusions:** *Our findings suggest that limiting the workload of residents and giving them longer off-duty hours would reflect on better and safer patient care. Nevertheless, it is still controversial that medical errors due to fatigue cause damages to patients.*

Key words: *Sleep. Deprivation. Residents. Cognitive. Effects.*

Efectos cognitivos de la privación crónica del sueño en residentes de medicina interna

Resumen

Antecedentes y objetivo: *Evaluar el efecto de la privación crónica del sueño en pruebas neuropsicológicas esenciales, que incluyen funciones ejecutivas para el desempeño como atención selectiva, control inhibitorio, velocidad de procesamiento y memoria de trabajo entre residentes de medicina interna con privación crónica del sueño en un programa académico altamente exigente.* **Métodos:** *Realizamos un estudio prospectivo que midió el desempeño neuropsicológico en residentes del primer año de posgrado en medicina interna en la Ciudad de México. El estudio se realizó de 2012 a 2013. Utilizamos la prueba de Stroop, así como WAIS III para evaluar las funciones ejecutivas. Realizamos evaluaciones neuropsicológicas al comienzo de la residencia y nuevamente 12 meses después de la evaluación inicial.* **Resultados:** *La privación crónica del sueño provoca una alteración en la atención selectiva, la memoria de trabajo, la velocidad de procesamiento y la inhibición. Esto da como resultado un mayor tiempo para realizar una tarea, así como una atención deteriorada durante la realización de una tarea.* **Conclusiones:** *Nuestros hallazgos sugieren que limitar la carga de trabajo de los residentes y darles más*

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Date of reception: 21-11-2020
Date of acceptance: 09-02-2021
DOI: 10.24875/RMN.200001181

Available online: 17-03-2021
Rev Mex Neuroci. 2021;22(2):51-55
www.revmexneurociencia.com

horas fuera de servicio se reflejaría en una mejor y más segura atención al paciente. Sin embargo, todavía es controvertido que los errores médicos debidos a la fatiga causen daños a los pacientes.

Palabras clave: *Sueño. Privación. Residentes. Cognitivo. Efectos.*

Introduction

The exhausting effects of sleep deprivation on residents' performance have been a topic of interest and debate for a long time. In a landmark study from 1971, Friedman et al.[1] observed among medical residents, acute sleep deprivation impacts negatively on mood and performance. On average, a person sleeps seven to 8.5 h/day[2]. Acute sleep deprivation is defined as 24 h without sleep, while chronic partial sleep deprivation is defined as sleeping < 6 h per night for several consecutive nights[2].

Evidence exists about the impact in the quality of patient care by interfering with the number of errors and accidents made[3]. A meta-analysis of 60 studies of sleep deprivation effect found that sleeping 30 h or less per week reduced one standard deviation (SD) the overall performance and 1.5 SD of clinical performance[2].

In July 2003, the United States Accreditation Council for Graduate Medical Education (ACGME) instituted a maximum of 80 working hours per week, and 24 continuous hours per day[4]. Some leaders in surgical and medical specialties still think long working hours are necessary to acquire sufficient clinical exposure and prepare residents and interns for their future senior roles[5].

After an extensive debate about the medical, professional, ethical, and personal implications, the 2010 revision of the ACGME preserved an 80-h limit on the residents' workweek[6], with some reforms of shift length and time free of duty.

The effect of chronic sleep deprivation on performance among residents is not well known. The aim of our study was to assess the effect of chronic sleep deprivation on essential neuropsychological tests including executive functions for performance such as selective attention, inhibitory control, processing velocity, and working memory among internal medicine residents with chronic sleep deprivation in a highly demanding academic program.

Methods

Setting

We conducted a prospective study evaluating neuropsychological performance among Internal Medicine residents at Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City. The study was reviewed and approved by the institutional review board and ethics committee; all participants provided written informed consent. In our Institute, depending on the year of residency, Internal Medicine residents work between 60 and 130 h/week, sometimes moving between clinical duties immediately following a long call (i.e., 24 h Emergency Room shift followed by several hours at the outpatient clinic). In addition, residents have 1 or 2 h of academic lessons to prepare 5 days each week. In Mexico, the Official Norm gives total freedom to each residency-training program to decide duty hours without exceeding a maximum of nights on call to 3 times/week, at intervals not to exceed every other day[7].

Study design

First-year (postgraduate year [PGY]-1) Medical Internal Medicine residents were invited to participate. Participants were excluded if they had current depression according to Beck's depression inventory (BDI)[8], or were using psychotropic or addictive substances including recreational drugs.

Baseline neuropsychological assessment was done within 2 weeks before starting residency training and reassessed at the end of the 1st year of residency. To reduce interference of acute sleep deprivation, participants were instructed to sleep at least 8 h the night before the baseline and 12-month tests, therefore limiting the assessment to only the effects of chronic sleep deprivation.

To decrease interference of emotional state with test results, we applied BDI with scores ranging from 0 to 63, with higher scores indicative of more severe disease. Participants who had borderline clinical depression (total score of 17-20) or higher at the beginning or at the end of the study were excluded from the analysis. In addition, we applied an initial and final questionnaire of hours of sleep per day and week.

We selected a neuropsychological battery that included the Stroop test as well as subtasks from the Wechsler Adult Intelligence Scale (WAIS III) to evaluate executive functions. The Stroop test evaluates attention and concentration (words, colors, and words-colors), and selective attention by measuring interference[9]. First, subjects were asked to name a series of color words (word task);

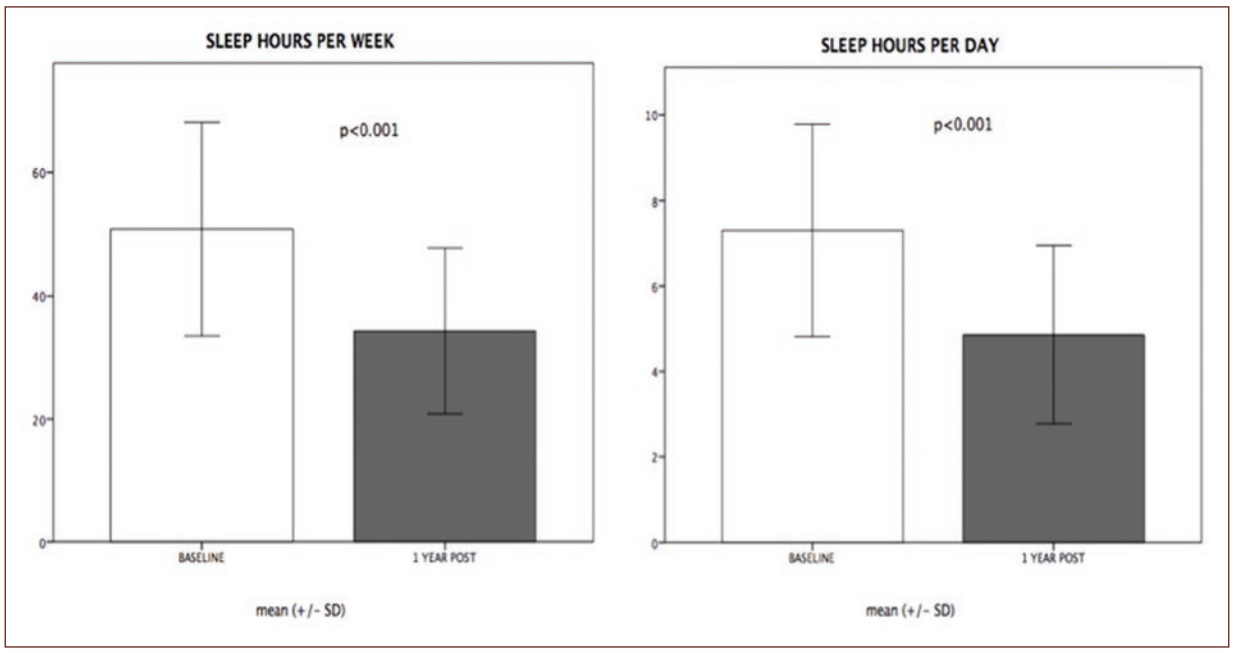

Figure 1. Sleeping time in 30 PGY1 internal medicine participants. Sleep hours per day (left panel) and per week (right panel) before starting residency training and a year thereafter. Data represented as mean ± SD. ***p < 0.001.

this component reflects basic reading rate and may be affected by speech motor problems or learning disabilities. Second, subjects were asked to name the color of a bar (colors task) of X's (e.g., XXX in blue, green, or red ink). As with the word task, performance may be affected by speech motor function; it may also be impacted by the individual's inability to name colors.

The final task was the color-word task in which subjects were shown names of colors printed in conflicting ink colors (e.g., the word "red" in blue ink) and was asked to name the color of the ink rather than the word. This component measures mental flexibility and the ability to inhibit a dominant response as well as provide a measure of the individual's ability to inhibit stimulus-bound responses and deal with interference.

WAIS III evaluates global intelligence of an individual when is fully applied. However, for the purpose of this study, we only chose tests that measured the level of activation of prefrontal cortex through selective attention, processing velocity and working memory such as digit symbol-coding, symbol search, and letter-number sequencing[10].

Statistical analysis

Parametric continuous variables are expressed as means + SD. Categorical variables are expressed as percentages. Differences in mean changes in sleep deprivation between baseline and 12-month assessments were compared in each participant by paired Student's t-test. To determine the effects of sleep deprivation on neuropsychological performance, mean changes from each neuropsychological test were compared between baseline and 12-month assessments using paired Student's t-test. All p values are two-sided and considered significant when $p < 0.05$. We used SPSS v 20.0 software for all statistical calculations.

Results

Thirty-four residents were assessed for eligibility. One subject decided not to sign the informed consent, while another one was excluded due to preexisting depression. Two participants were found to have depression at the end of the study and were also excluded from analysis. Of the 30 participants included, 16 (53.3%) were women and 14 (46.7%) were men. The mean age was 24.9 ± 1.2.

Sleep survey

As shown in figure 1, a remarkable decrease in sleeping time was observed in medical residents from the starting residency training to a year later. Residents slept

Table 1. Stroop test results in 30 PGY1 internal medicine residents at baseline (within 2 weeks before starting residency training) and after 1-year of residency

Neuropsychological test	Baseline assessment mean (± SD)	12-month follow-up assessment mean (± SD)	p
Words	49.8 (± 8.7)	47.7 (± 6.7)	0.08
Colors	48.5 (± 11.5)	42.3 (± 9.6)	0.006
Words-colors	50.6 (± 10.5)	43.8 (± 11.3)	0.002
Interference	53.2 (± 8.0)	47.8 (± 12.9)	0.02

SD: standard deviation.

Table 2. WAIS III subtasks results in 30 PGY1 internal medicine residents at baseline (within 2 weeks before starting residency training) and after 1-year of residency

Neuropsychological test	Baseline assessment mean (± SD)	12-month follow-up assessment mean (± SD)	p
Digit retention	11.8 (± 2.8)	11.3 (± 2.3)	0.10
Letter-number sequencing	12.2 (± 3.1)	10.7 (± 1.6)	0.006
Digit-symbol coding	9.1 (± 14.1)	8.6 (± 1.1)	0.03
Symbol search	13.7 (± 1.7)	11.2 (± 1.6)	< 0.001

SD: standard deviation.

around 2 h less/day ($p < 0.001$) corresponding to a decrease of around 16 sleep hours per week ($p < 0.001$).

Neuropsychological tests

Baseline scores for both Stroop and WAIS III subtask tests were normal at the beginning of medical residency training. For the Stroop test (Table 1), a significant decrease of scores was observed after 12-months of medical residency for colors, words-colors, and interference tasks ($p < 0.05$). We also observed a borderline reduction for the more elementary word task ($p = 0.08$). For the WAIS III subtask tests, the difference in the averages obtained was also significantly different between the baseline and 12-month follow-up periods for the letter-number sequencing, digit symbol-coding, and symbol-search tests ($p < 0.05$). Digit-retention task was not different between the initial and final assessments (Table 2).

Discussion

Our results indicate that chronic sleep deprivation causes an alteration in selective attention, working memory, processing speed, and inhibition. This suggests that chronically sleep-deprived medical residents not only require longer times to perform a task due to a reduced processing velocity, but also have to deal also with impaired attention during the performance of that task. Impairment of working memory may affect an individual's capacity to learn and perform simultaneous and complex procedures.

The theories about the underlying effects of sleep deprivation on the central nervous system (CNS) can be divided into two main approaches. The first one, known as the "prefrontal vulnerability hypothesis" states that lack of sleep impairs executive functions, the cognitive process mediated by the prefrontal cortex. The second, known as the "unstable state hypothesis", postulates that sleep deprivation induces a state of instability, particularly evident in tasks that require sustained attention with fluctuations of attention from time to time[11].

Executive functions include a set of self-regulation functions that enable the organization, coordination, and control of other cognitive functions, emotional responses, and behaviors[12]. The prefrontal cortex is the site of integration of such executive functions including attention, processing speed, working memory, goal-driven behavior, sequencing, inhibition, and verbal fluency.

Sleep deprivation is associated with a decline in basic cognitive functions such as sustained attention and wakefulness. These functions can be kept within certain limits despite abnormal levels of fatigue, probably because the brain is able to compensate the effects of acute sleep deprivation which may explain why a deterioration in neurocognitive function and surgical performances after acute sleep deprivation have not been proven[2,13].

Our findings add to a growing body of evidence of the underlying effects of chronic sleep deprivation on the CNS. Sleep deprivation induces a state of instability and fluctuations in tasks that require sustained attention. Given that selective attention is regulated by prefrontal cortex, our study indicates that chronic sleep deprivation interferes with executive functions such as inhibitory control, processing speed and working memory, all essential functions for complex activities, and decision-making.

Our study has limitations, including a small sample size, horizontal sampling, as well sampling from a single institution. Furthermore, performance for the digit retention subtask of the WAIS III was not apparently involved in response to chronic sleep deprivation; however, this could be because we did not make a difference among direct and inverse retention scores of this test. Instead, the score was taken as a total. The direct digit retention evaluates attention meanwhile the inverse evaluates working memory. Further studies are required to complete the data presented.

Conclusion

Our findings suggest that limiting the workload of residents and giving them longer off-duty hours will reflect on better and safer patient care. Nevertheless, it is still controversial that medical errors due to fatigue cause damages to patients. Health institutions should make every effort to reduce chronic sleep deprivation and fatigue not only among medical residents, but also medical staff at large, and assume fatigue as an unacceptable risk to both residents and patients alike.

Funding

None.

Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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REVIEW ARTICLE

Molecular basis for interpretation of fulfilled electroretinographic studies

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Abstract

Fulfilled electroretinography (ffERG) represents the phototransduction process and synaptic network in the first nerve impulse of the visual process. Little is known about its interpretations at molecular level. ffERG records potential changes within the retina in the a, b, and c waves coming from different cell types. The a-wave derives from the phototransduction of rods and cones. Depolarization of interneurons is recorded in the b-wave. The origin of the c-wave is controversial. It has been related to the retinal pigmented epithelium, but evidence of its intrinsic electrical activity is lacking. We set the hypothesis that the c-wave measures the activity of the photoreceptor ganglion cells, which results of melanopsin-based phototransduction. The evidence for this comes from experiments inhibiting synaptic transmission from rods and cones. The molecular knowledge is the basis for the interpretation of wave alterations of electroretinographic studies and intrinsically photosensitive retinal ganglion cells contribute substantially to the formation of the c-wave.

Key words: *Electroretinography. Molecular. Photoreceptors. Phototransduction. Retina. Photoreceptor ganglion cells.*

Bases moleculares para interpretar el electrorretinograma

Resumen

El electrorretinograma de campo completo (ffERG) es un método no invasivo que representa la fototransducción de los conos y bastones y la transmisión sináptica que genera el impulso nervioso en la vía visual. Se desconocen los procesos moleculares que originan las ondas del ffERG. En la fototransducción de los fotorreceptores, los canales catiónicos activados por nucléotidos cíclicos se cierran y los canales de K^+ permanecen abiertos, hiperpolarizando a estas células, originando la onda a. La onda b proviene de la despolarización de las interneuronas primarias de la retina, principalmente de las células bipolares-ON. A diferencia de estas precisas identificaciones, el origen de la onda c es elusivo, hay propuestas de asociarla con el epitelio pigmentado, aun cuando no hay evidencia de su actividad eléctrica. Este artículo tiene como objetivo analizar resultados publicados que indican que la onda c del ffERG registra la despolarización de las células ganglionares fotorreceptoras (ipRGC), que desarrollan un proceso de fototransducción mediado por la melanopsina. Se revisará evidencia experimental de registros de ffERG bajo la inhibición sináptica intrarretiniana. Los ffERG pueden verse afectados por factores fisiológicos o relacionados con el instrumento, por lo tanto, es necesario determinar la adaptación a la luz y oscuridad, tamaño de la pupila, intensidad del estímulo, tipos de electrodos y selección de fármacos y anestésicos en estudios con animales. Se concluye que la descripción de los mecanismos moleculares forma la base para interpretar las ondas a, b, c d de los ffERG, considerando que las ipRGC contribuyen a la formación de la onda c.

Palabras clave: *Electrorretinografía. Interpretación. Base molecular. Fotorreceptores. Fototraducción. Retina.*

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Date of reception: 11-05-2020
Date of acceptance: 23-06-2020
DOI: 10.24875/RMN.20000040

Available online: 17-03-2021
Rev Mex Neuroci. 2021;22(2):56-66
www.revmexneurociencia.com

Introduction

Fulfilled electroretinographic (ffERG) studies are non-invasive with a purpose of explaining the levels of functioning of retinal inner cells and they are indicated as pre-operative examinations before cataract surgeries and research into neurodegenerative diseases. Retinal structural changes can be noted before ophthalmic and behavioral signs. Protocols in animals can be short (without anesthesia), revealing mixed responses of rods and cones, while long protocols (with anesthesia) record separate waveforms, coming from the activity of second and third order neurons including that of ganglion cells. In humans, anesthesia is not used. Little is known about electroretinographic (ERG) studies interpretations at the molecular level. Knowledge of how to realize and produce ffERG will help to understand the changes that may be noticed in retinal, neurodegenerative diseases, and toxicological studies[1,2].

Development of ERG studies began with Gotch Francis in 1903, followed by that of Einthoven and Jolly in 1908. These pioneering investigations resulted in Ragnar Granit explaining an ERG study of a cat in 1933. This resulted in him being awarded the Nobel Prize in 1939[3]. ERG studies have since confirmed the hyperpolarization of rods and cones, depolarization of bipolar cells, and controversially concluded that the retinal pigmented epithelium (RPE) is involved in the formation of the **c**-wave[4]. Other tests like pupillometry are considered to complement its use in determining retinal functioning[5]. As well, retinal toxicity studies during pharmaceutical developments have been confirmed with the use of ERG studies[6].

The 21th Century has evidenced an increase in molecular biology knowledge. Research in neuro-ophthalmology was not left behind, and a third type of photoreceptor was discovered: the photoreceptor ganglion cell (PRG). This was followed by the elucidation of its phototransduction process which can be recorded and shown on an electroretinogram. ffERG studies can be used to record electrical changes related to the normal functioning of retinal cells and neurodegenerative diseases. Explaining ffERG studies at a molecular level would allow us to diagnose, apply therapies, monitor, and explain different eye diseases with more precision and certainty. There is an increase in the interest of personalized and precision medicine based on scientific- and evidence-based information and various patients can be treated with more certainty using the results obtained from ERG studies. Since the ffERG is the most utilized and most understood in clinical settings, the information provided in this review will be based on its principles. This article will show evidence that the **c**- wave, which is a depolarization phase after the **b** wave, is related to the phototransduction process of PRGs.

Methods

A literature search during the period of December 2018 and December 2019 was done and information was obtained from the databases of PubMed, Google Scholar, and Elsevier. The following keywords were used during the searches: electroretinography, interpretation, molecular basis, photoreceptors, phototransduction, and retina. Most of the articles considered were published after the year 2000, with few exceptions. A total of 300 articles were considered to have relevant information and those included in this review were having updated information about the molecular basis for the interpretation of ERG studies. Criteria used to select the articles were based on the author's contributions to the area of research and experience.

Phototransduction process in rods and cones

ERG studies are graphical representations of the phototransduction process in the retina (Figs. 1 and 2, Table 1). The phototransduction in the classical photoreceptors, cones, and rods, initiates the process of the vision. When a photon interacts with conopsin and rhodopsin the isomerization of retinaldehyde from 11-cis to trans activates the G protein, transducin. The transducing alpha subunit -GTP complex activates the phosphodiesterase (PDE), which cleaves cyclic GMP (cGMP), to 5'-GMP. The reduction in cGMP leads to cyclic nucleotide gated channels closure. Thus, rods and cones light response is the hyperpolarization and in the dark, depolarization of these photoreceptors occurs[7-11].

In the hyperpolarized photoreceptors, voltage gated Ca^{2+} channels also close and thus the Ca^{2+} mediated vesicle release of glutamate is suppressed. Because the occurrence of the distinct glutamate receptors on retinal neurons, the decrease in the amount of glutamate released by the photoreceptors causes the depolarization of the ON bipolar cells and the hyperpolarization of the OFF bipolar cells[11,12].

Table 1. Comparison of phototransduction processes within retina

Photoreceptor cell type	Rod	Cone	Photoreceptor ganglion cell
Pigment	Rhodopsin	Conopsin	Melanopsin
G protein	Gtransducin	Gtransducin	Galpha q
intracellular mechanism	Phosphodiesterase activation, cGMP hydrolysis, CNG cationic channel closure	Phosphodiesterase activation, cGMP hydrolysis, cationic channel closure	Phospholipase activation, IP3/DAG synthesis, opening TRP cationic channel
Cell response	hyperpolarization	hyperpolarization	depolarization

cGMP: cyclic guanosine monophosphate; CNG: cyclic nucleotides gated channel; IP3, inositol triphosphate; DAG: diacylglycerol; TRP: transient receptor potential channel.

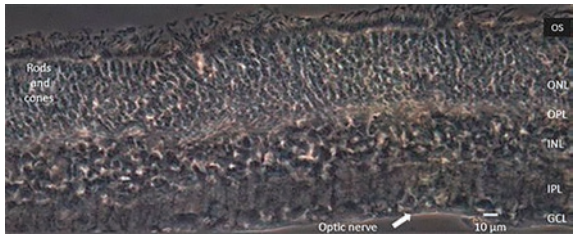

Figure 1. Vertical section of the rabbit retina showing the histological layers. photoreceptor outer segments (OS); Photoreceptor cells somata in the outer nuclear layer (ONL); the plexus for synapses between photoreceptor cells and interneurons in the outer plexiform layer (OPL); the interneurons somata (inner nuclear layer, INL); synaptic plexus of interneurons and ganglion cells (inner plexiform layer, IPL) and ganglion cell layer (GCL). Cryostat sectioning and phase contrast microscopy, image from the authors.

Phototransduction process in intrinsically photosensitive retinal ganglion cells (ipRGC)

The phototransduction process is not exclusive of rods and cones. An exceptional group of ganglion cells contains melanopsin, a homolog of conopsin, and rhodopsin. Melanopsin- activates a signal cascade that opens a cationic channel in the plasma membrane of these ganglion cells, which, thus, are intrinsically photosensitive and hence known as PRG cells. They convey the environmental illuminance information, to the suprachiasmatic nuclei by the retinohypothalamic tract (RHT) and play a key role in the photosynchronization of the circadian cycle[13].

The phototransduction process of ipRGCs (Fig. 3, Table 1) is homolog to that started by opsins in classical photoreceptors, melanopsin also uses 11-cis-retinaldhyde as chromophore. However, melanopsin activates a heterotrimeric G protein of the Gq/11 family, and results in the depolarization of the ipRGCs. The Gq/11 alpha subunit activates phospholipase C β and the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2). PIP_2 hydrolysis produces the second messenger's diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP_3)[14,15]. It is supposed that DAG opens a canonical TRP channel activating a cationic current which depolarizes the PRG as it occurs in Drosophila photoreceptors. The depolarization-induced by the light in ipRGCs exceeds the trigger threshold, generating Na^+ action potentials, which are required for these cells to signal through their long axons to higher encephalic targets.

Like other opsins, melanopsin consists of an apoprotein covalently linked 11-cis-retinal chromophore. The absorption of a photon isomerizes the chromophore in all-trans-retinal. After exposure to light, the trans-retinal in a photopigment must be re-isomerized to 11-cis-retinal to make the pigment photo excitable again. For the rods, isomerization depends on the retinal pigmented epithelium, while the cones depend on the Müller glia. In contrast, photo-activated melanopsin retains all-trans-retinal and isomerize to 11-cis-retinal after exposure to light, in a process known as photo-reversion. Apart from being a photopigment, melanopsin also has the ability to reisomerize the all-trans-retinal back to 11-cis-retinal. This capability is known as "photoisomerase activity", and it occurs also in the opsins of some invertebrate species[16].

Besides the phototransduction process described above, PRG are third order neurons which receive synaptic inputs from bipolar and amacrine cells temporal and spatial summation of these synaptic inputs can also lead to triggering of action potentials of the PRG which solely form the RHT. Therefore, it can be concluded that PRG have a synaptic and an intrinsic component (the

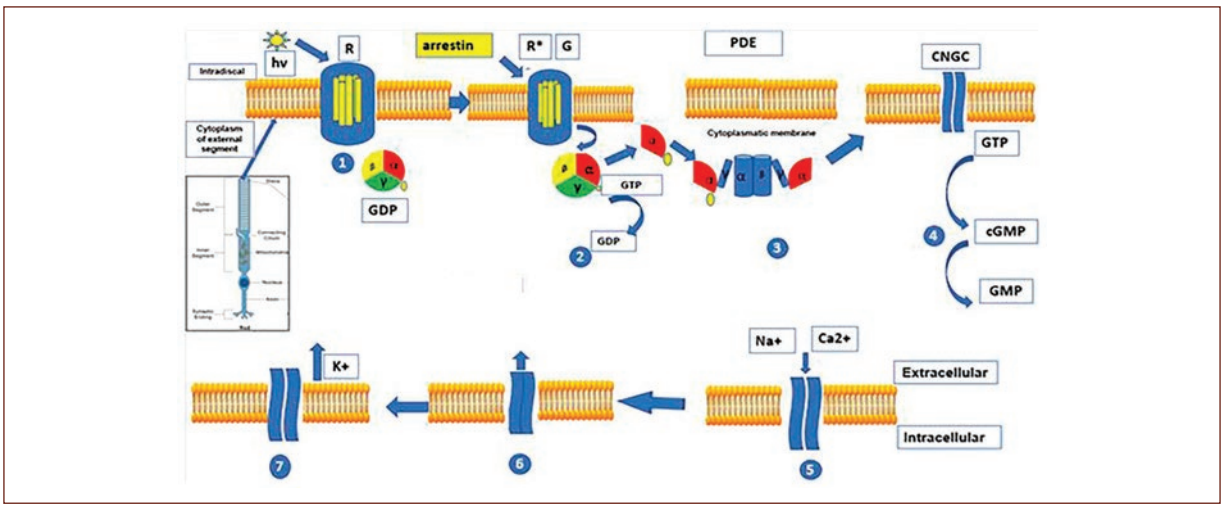

Figure 2. Phototransduction process in the outer segment of rods and cones. Light causes the isomerization of retinal and rhodopsin/conopsin. This leads to the activation of G protein transducin. The transducin alpha subunit activates the enzyme phosphodiesterase and thus the hydrolysis of cyclic guanosine monophosphate. This leads to the closing of cyclic nucleotide-gated channels. This reduces the inward current of Na^+ and Ca^{2+} and coupled with the continued outflow of K^+ through the $Ca^{2+}/K^+/Na^+$ ion exchanger, this will lead to the hyperpolarization of photoreceptors. Arrestin will bind activated rhodopsin/conopsin and will cause desensitization of this process *(adapted from* Milosavljevic*, et al. 2016)*[70].

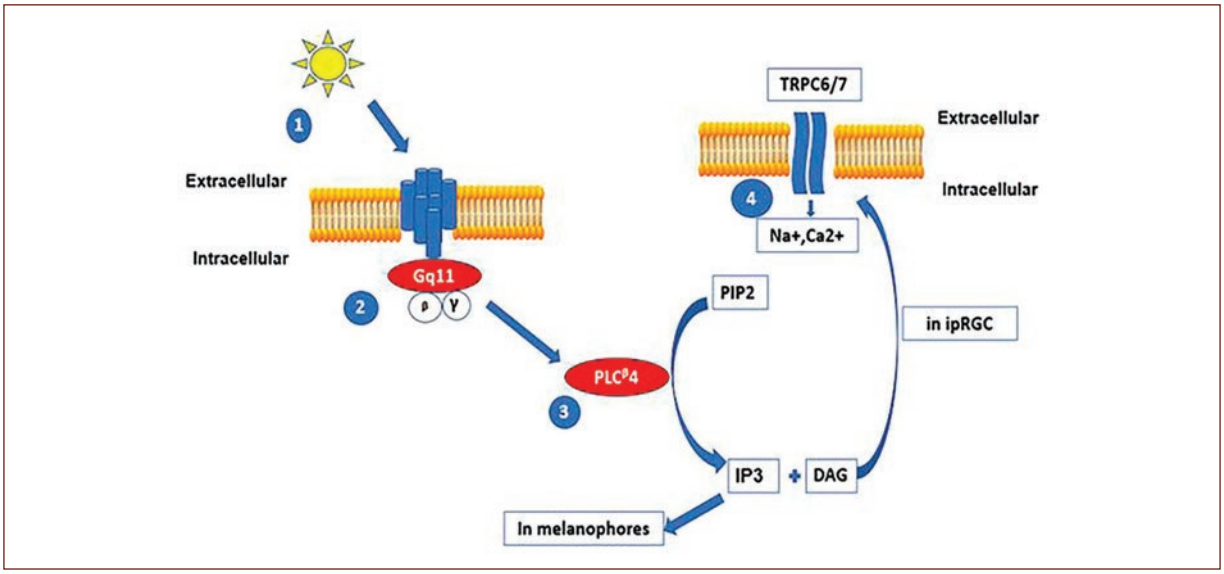

Figure 3. Phototransduction process in intrinsic photosensitive retinal ganglion cells (ipRGCs). Light induces the isomerization of retinal and melanopsin. This leads to the activation of a family of Gaq protein coupled to phospholipase C enzymes. The hydrolysis of phosphatidyl-inositol (PIP_2) to form inositol-triphosphate (IP_3) and diacyl-glycerol (DAG) will activate different intracellular transduction mechanisms in melanophores, IP3 will open Ca^{++} channels in endoplasmic reticulum. In ipRGCs, DAG will promote the opening of plasma membrane transient receptor potential channels TRPC and the inward current of Na^+ and Ca^{2+} ions. The ipRGCs response is a depolarization of membrane voltage *(adapted from Hughes, et al., 2012)*[71].

phototransduction process of melanopsin) which are reflected in their response recorded, altogether with that from the others regular ganglion cells, in the ffERG c- wave[17].

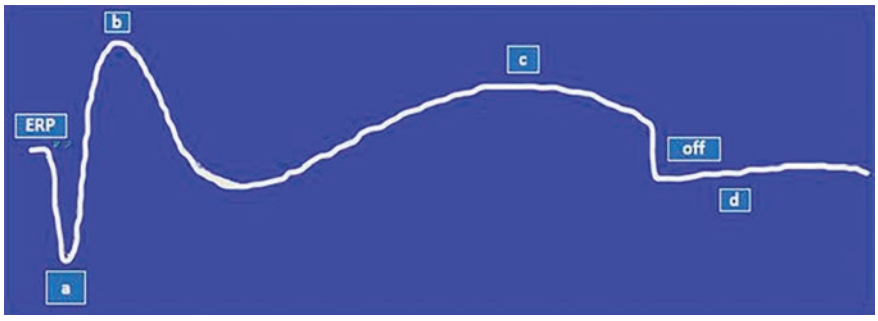

Figure 4. Schematic graphic representation of full-field electroretinogram (ffERG) study showing several types of voltage changes from different retinal cell neurons. These voltage traces are known as ERG waves. The early receptor potential (ERP) is the field potential of retinal photoreceptors in a resting phase. The a-wave, hyperpolarization is related to the phototransduction process of rods and cones while the b-wave, a depolarization, is related to changes in the potential of bipolar cells. The **c**-wave, depolarization, is being hypothesized to be related with PRG while the off portion marks the end stage of the record of ERG. The **d**-wave is the intermittent part between generations of two complete ERG studies *(adapted from Creel, 2020)*[72].

ffERG studies

ffERG studies are useful tools for assessing retinal cells *in vivo*[18] and changes in the form of the waves can be related to the distinct types of cells involved in an electroretinogram. The changes in membrane potential of the photoreceptors can be large enough to be detected by probes on the corneal surface and reference positions. In a ffERG, the recordings are average potential changes of retinal cells which result in an ERG study (Fig. 4) where there is a measurement of the amplitude of a, b, c, and d waves in microvolts (μV) and latency/implicit time in milliseconds[3].

The ffERG study is the standard for retinal electrophysiological studies while the multifocal electroretinography (mfERG) analyses specific regions of the retina at a specific moment[19]. A ffERG study will allow having a global analysis of retinal function[20]. Other retinal electrophysiological studies include ERG pattern which analyses electrical changes in the macular ganglion cells while an electrooculogram helps to analyze the RPE. Visual evoked potentials are a measurement of the electrical signals recorded at the scalp over the occipital cortex in response to light stimulus and detect changes in electrical transmission in the optic nerve and post- retinal pathways[21] while a mfERG measures specific region of the retina.

The recording of ffERG studies is composed of three stages, mainly: acquisition, fitting and amplification, and signal processing[3]. The duration of light that causes electrical changes captured in ffERG studies has a range of 50-100 ms[22]. During a ffERG study, there is variation in intensity, wavelength, and duration of stimuli. A rod driven response is recorded with a stimulus of 0.01-0.02 cd.s.m^{-2} in dark-adapted conditions, while increasing to 3 cd.s.m^{-2} would stimulate cones and result in a mixed rod-cone response. By increasing to 30 cd.s.m^{-2} would result in a pure cone response in photopic conditions. Using a flickering stimulus of 31 Hz would, as well, result in a pure cone response[23].

To make a technically viable study, calibration of the stimuli and of the ffERG machine is necessary. This would have negative effects on the measurements of latency and amplitudes of the waves. Inconsistent calibration may result in misleading data[24]. As a way of preventing accommodation of the retinal photoreceptors, the times between light stimuli should be regulated. Stimuli of up to 0.01 cd.s.m^{-2} should be separated by 2 s; up to 0.1 cd.s.m^{-2} should be by 3 s while those of up to 3 cd.s.m^{-2} should be separated by 5 s. For higher intensities, they should be separated by at least 10 s[25]. As well, measurements in an ffERG are susceptible to electrode drift, eyeball, and eyelid movements and for this reason sedatives are proposed to be used in animal studies[26].

In scotopic conditions, the b waves predominate the capture of the results[27]. Examples of ffERG recordings have demonstrated that b waves are smaller in amplitudes of young rabbits but without variation in implicit times[28]. The first part of an ERG study is a small electrical activation of the receptors and it is found near or on the baseline. It is known as an early receptor potential, ERP. The hyperpolarization of rods and cones spans the first 15 ms of the a- wave[29]. The b- wave is due to the depolarization of inner retinal neurons predominantly ON-bipolar cells. While the **c-** wave has

Table 2. Cell and molecular correlates for waves recorded in a ffERG

ffERG wave	a-wave	b-wave	c-wave
Fluctuation from baseline voltage	Hyperpolarization	Depolarization	Depolarization
Cell type involved	Rods and cones	Inner retinal neurons mostly ON bipolar cells	Photoreceptor ganglion cells
Molecular events	Closure of CNG by rod/cone-opsin phototransduction	Inhibition of metabotropic glutamate receptors	Activation of TRP channels by melanopsin phototransduction*

*Mechanism proposed based on evidence showed in this review. ffERG: full-field electroretinogram; CNG: cyclic nucleotides gated channel; TRP: transient receptor potential channel. Baseline voltage refers to the field potential recorded right before light stimulation.

been related to the RPE, but the authors show evidence below that it is rather related to ipRGCs.

In one study, intravitreal injections of 6-hydroxydopamine, a degenerating agent for dopamine neurons, in rabbits' eyes, the depletion of dopamine increased the amplitude of the **b-** wave. This was related to the suppression of lateral inhibition by dopaminergic amacrine cells which in the intact retina modulate synaptic transmission between the inner synaptic and inner nuclear layers in the retina[29-31].

The ratio of b-wave to a-wave amplitude is used to denote the normal transmission of the electrical signal of the retina. Any change will be reflected in this ratio[22].

The **c**- wave has always been confused with oscillatory potentials and a knowledge of how they originate can help to know when there is a dysfunction in the process of phototransduction. The early, intermediate, and late peaks are generated mainly by photoreceptors, action potential- independent, and action potential-dependent interactions on third-order cells on the ON pathway. Bipolar, horizontal, and neurons on the OFF pathway make a small contribution to the generation of these waves[31]. The oscillatory waves appear on the rising phase of the b waves and using the bandpass filter they can be isolated of the ERG for their analysis. They are known as well as oscillatory potentials and they are thought to arise from inner plexiform layer[22].

Earlier studies have suggested that the **c-** wave originated from the pigmented epithelium RPE, reviewed by Perlman[22]. For instance, one study which was done after the dissection of the RPE from the retinal components resulted in the disappearance of the **c-**wave and in amphibians the c- wave could be recorded after the transection of optic nerve, which leads to degeneration of retinal ganglion cells. The mechanism proposed to the origin of c- wave by RPE is based on the fact that its apical pole (facing the retinal layers) is more permeable to K^+ ions than the basal pole (facing the choroid) which leads to a transepithelial potential. The changes in extracellular K^+ following the response of rods and cones increase the transepithelial potential with the retinal face more positive, reflected as the generation of the c- wave in the ERG. Thus, even though the c- wave originated from RPE still depends on the rods and cones phototransduction mechanism. Aside that, the analysis of the c wave can tells about the integrity of the classical photoreceptors[22].

At the present, there is no sufficient evidence that the **c**-wave is exclusively contributed by the PRG. The only reports that have been put forward have been about the chemo genetic activation of PRG, a manipulation which drives changes in dark-adapted electroretinograms. There are also data from a mouse retina *ex vivo* study, in which rods and cones ablation did not prevent the appearance of c wave, which was produced in response to a light stimulus on the range of activation of melanopsin.

Therefore, it can be hypothesized that PRG contribute to the formation of the **c** waves. The authors propose that inhibition of the synaptic transmission by glutamate metabotropic and ionotropic receptors ligands and transient receptor potential canonical (TRPC) 6/7 channels can demonstrate the intrinsic properties and contribution of ipRGC to the **c-** wave.

An understating of retinal circuits can help to understand the processes that may be involved in the production of a ffERG (Table 2). In scotopic conditions, rod bipolar cells synapse with amacrine cells and the signal are then transmitted through the cone bipolar to ganglion cells[32]. The main neurotransmitter in the retina is L-glutamate and the use of agonists and antagonists can help to determine the contribution of each rod and cone pathways on the ffERG. Injection of APB and blockage of the central retinal artery caused the disappearance of the **b** waves. APB acts on the

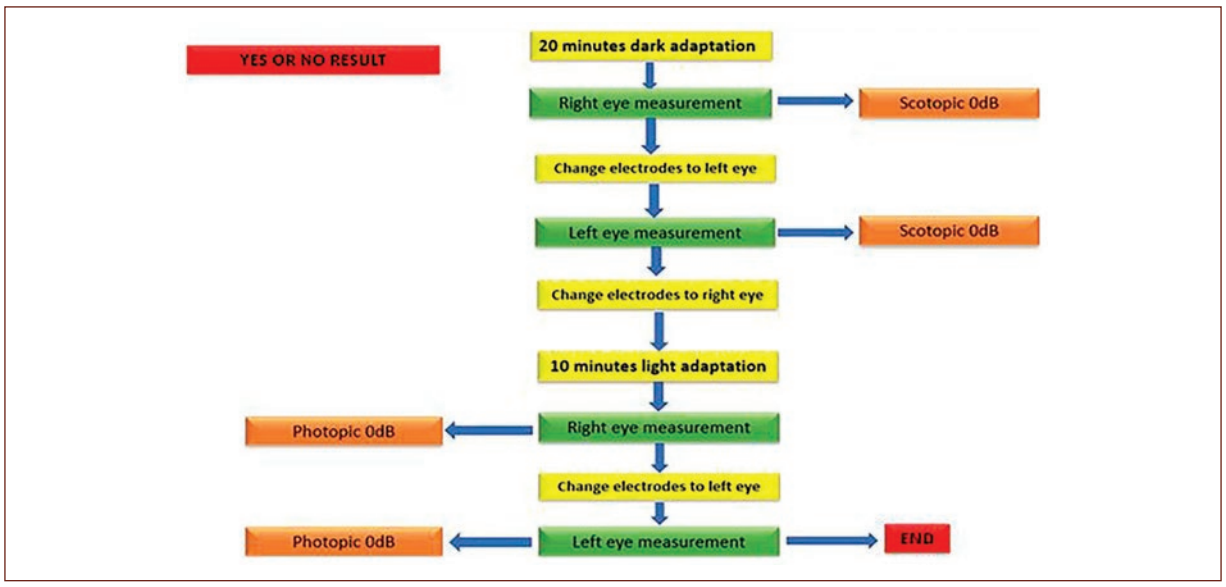

Figure 5. Short protocol for ERG studies with a standard flash of 3 cdsm^{-2}. Yellow=steps to take; Green=measurement stages; Orange=Light intensities. The procedure begins with a 20-min dark adaptation period, followed by the placement of electrodes on the right eye and measurement at 0dB. Then, the electrodes will be changed to the left side, which will then be followed by placement of corneal electrodes on the same side and measurement at 0dB. The experimental subjects are then light adapted for 10 min with measurements being done at right and left sides at 0dB. The result will be yes or no under scotopic and photopic conditions.

metabotropic receptors inhibiting the bipolar cells ON pathway while DNQX, an inhibitor of AMPA/KA glutamate type receptors, eliminates pathways that block the formation of **b** waves, enhances the appearance of the **b** waves. Tetrodotoxin would slightly reduce and would delay time to peak for **b** waves while the combination of bicuculline and strychnine would increase it[22]. Knowledge of the retinal circuits involved in the process of transmission of the light signals raises some questions on how the pigmented epithelial cells can form part of these pathways and how they can lead to the formation of the **c** waves. By retrograde labeling using alpha herpes pseudorabies virus and immunofluorescence studies, it was determined that PRG are transinaptically connected to the bipolar, amacrine, and dopaminergic cells which form part of the retinal circuits. For these reasons, the authors are putting forward the hypothesis that the PRG are the source of the formation of **c** waves.

Strategies of evaluation of ERG studies and protocols

The way in which a ffERG study is done is based on established protocols which generally can be defined as short and long. There is no use of anesthesia during the short protocol and it is mostly applied in humans. The result is a **yes** or **no** on the function of the retina. A variation of light intensity would result in variation of amplitudes of the a, b, and c waves (Figs. 5 and 6). On the other hand, the long protocol based on the European College of Veterinary Ophthalmologists guidelines includes the use of anesthesia, and is generally applied for research purposes in animals.

Factors affecting ERG studies

Factors affecting full-filled ERG studies can be divided into physiological or instrument-related and the results are a summation of the changes in potentials of the individual photoreceptors[1]. The amplitudes recorded on a ffERG study depend on the animal's eye condition, the light adaptation of the eye, the intensity of the stimulus, pupil size, and electrodes condition[33]. Eyeball movements were shown to cause changes in ffERG measurements due to changes in electrode-corneal contact and intensity of incident light. As a way of minimizing these effects, it was demonstrated that a mixture of ketamine and xylazine had the best results in rats. Minimum eye movement along with maximum level of **a** and **b** wave

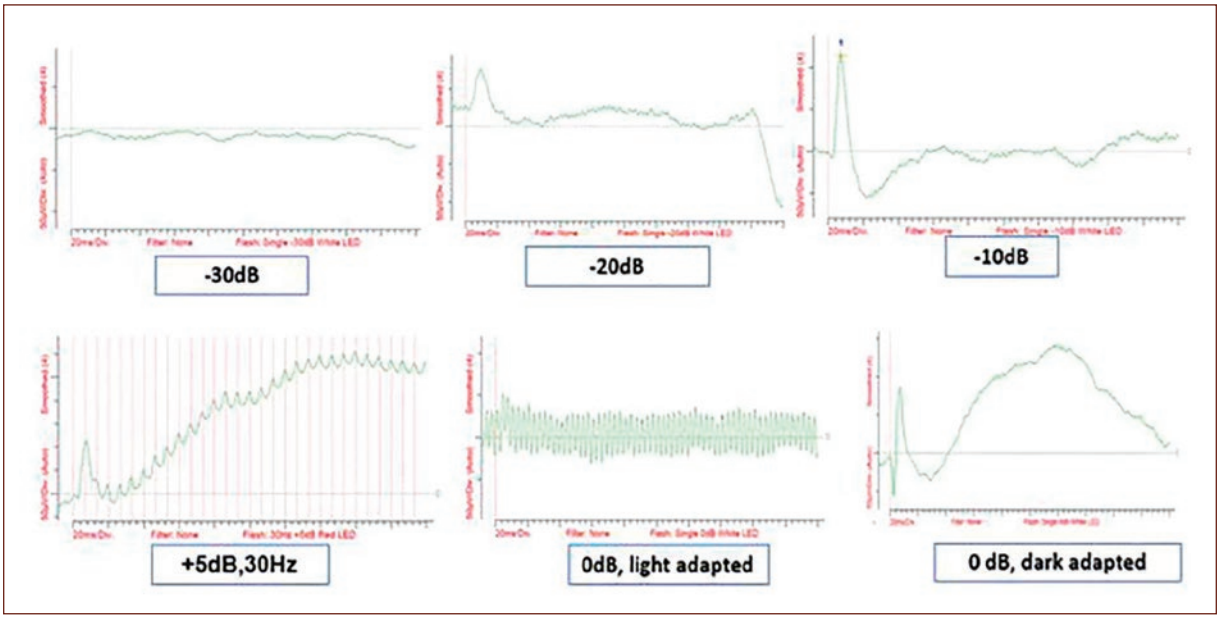

Figure 6. Example of a ffERG obtained applying the short protocol showing ERGs at different light intensities after scotopic and light adaptation. Every plot shows the changes in amplitudes (Y axis, microvolts) during recording time (X axis, milliseconds) at increasing light intensity. Subject, New Zealand rabbit, 2 months old. Results from an ongoing study of the authors.

amplitudes were recorded. There was insignificant variation in implicit times with this combination as well[34].

The ffERG is influenced by dark/light adaptation time, pupil size, stimulus intensity, electrodes, alertness level, and other factors[35]. The use of skin-electrodes produces lower amplitudes of a and b waves and longer implicit times when compared with corneal ones, making it a better option when doing ffERG studies[36]. Bipolar corneal electrodes have a high signal-noise ratio thus causing repetition of studies with longer periods and fewer stimulus elements necessary for the results to be comparable. The major cause for poor records would be related with electrode skin and corneal contact[29].

The results from recent studies have shown that there is variation between *in vivo* and *ex vivo* studies. In an *in vivo* dark-adapted study, the b- wave dominated and was four-fold in amplitude and time to peak was 60 ms when compared with a wave that had time to peak of 30ms[22]. While in an *ex vivo* studies are slower and have suppression of oscillatory potentials when compared with *in vivo* studies[37].

In a study using white New Zealand rabbits with a weight of between 2-2.5 kg and 8-10 weeks old, it was determined that the test-retest reliability was based on the levels of variability of the investigation. The largest variability, 30%, was between the subjects, followed by 20% between the repetitions and 10% between the eyes[38]. On measurement of the effects of different drugs on the **c** waves, it was demonstrated that there was no statistical difference in results of the left and right eyes of the same animal; therefore, the contralateral eye should always be used as a control[39]. A three-fold increase in pupil size equaled a nine-fold increase in light intensity reaching the retina. Therefore, pupil dilation can affect ERG studies. Another factor studied was the level of brightness of the stimulus. The brighter the stimulus the faster the ffERG and reduced time to peak levels[22].

Effects of anesthesia on ffERG studies

Since some protocols of ffERG studies are done under anesthesia, there is a great need to know their effects. Diazepam and barbiturates at high doses cause a decrease of the **a**-wave while the combination of xylazine and ketamine has been shown to have fewer effects on ffERG studies[1]. There were no significant differences in the amplitudes and implicit times of a and b waves in a study in cats when the combination of ketamine-xylazine and dexmedetomidine-ketamine was compared[40]. Propofol would cause an increase in b- wave amplitude while dexmedetomidine would have a decrease in **b** waves compared to the

tiletamine-zolazepam combination. Isoflurane would cause further decreases in all waves when combined with the others mentioned before[41].

When used intravenously at a dose of 1-2 mg/kg, ketamine cataleptic effect lasted < 1 min while at a dose of 5-10 mg/kg it took 20-40 min. These factors should be considered when conducting the study of ffERG. Halothane and thiopental would cause an increase in implicit time of waves a and b waves[42]. Lidocaine and bupivacaine are normally used as local anesthetics for retrobulbar injections and with the use of ffERG studies, it was demonstrated that they are non-toxic[43]. Initial studies on the effects of anesthesia on ffERG waves demonstrated that 1.3% vol of halothane increased the amplitudes of the b waves while during the same experiments artificial ventilation did not cause any changes[44].

Ocular and retinal diseases and their effects on ffERG studies

ffERG studies reveal the state of function of retinal cells[3] and in mammals it establishes the loss of vision related to retinal degeneration diseases which can be divided into primary (hereditary) and secondary (glaucoma-related). For example, infectious inflammatory diseases like canine distemper can cause retinal detachment[45]. Retinal detachment and attachment surgeries have also used ffERG studies as a diagnostic tool wherein one study they obtained a decrease in amplitude of **b** waves after detachment and an increase in implicit time[46].

Glaucoma is a heterogeneous group of disorders related to retinal cell apoptosis, specific optic neuropathy, and cupping of the optic disc. Although an increase in intraocular pressure has been linked to an increase in the risk of development of glaucoma, some authors argue that there is a presentation of glaucoma in humans with normal intraocular pressure. In such a situation other non-invasive tools for determining normal visual processes like ERG studies would be of beneficial use[47,48]. It is a result of interaction between genes, age, and environmental factors[49].

The following genes have been determined in various canine species to be related to the development of open-angle glaucoma: ADAMTS10, ADAMTS17 COL1A2, RAB22A, NEB, and SRBD1[50] as well as LTBP2 and TGF beta[51]. Its alluded that neuroinflammation is the key process during glaucoma. The retinal ganglion cells are the first to be affected followed by astrocytes and microglia. Microglia responds with a phagocytic process to restore homeostasis and these results in monocyte infiltration[52]. Retinal ganglion cell death is a result of apoptosis whereas cells that continue to live have the protection of neurotrophins[53].

Axonal degeneration models have demonstrated that autophagic flux impairment results from elevated intraocular pressure, TNF, traumatic injury, ischemia, oxidative stress, and aging[54]. Age-related trabecular meshwork changes have been linked to the activation of myofibroblasts which leads to the reduction of the iridocorneal angle. In primary open-angle glaucoma, there is a loss of endothelial cells due to the action of oxidants like hydrogen peroxide causing apoptosis and mitochondrial damage in the trabecular meshwork[55]. As well, long-term use of glucocorticoids has been related to iatrogenic open-angle glaucoma[56]. Possible contributors to the development of glaucoma are neurotrophins signaling, oxidative stress, excitotoxicity, mitochondrial dysfunction, protein misfolding, hypoxia, and retinal ischemia[57].

On increasing intraocular pressure in a rabbit, which is common in glaucoma, and equalizing it with the arterial blood pressure the c-wave disappeared first and then the a-wave after 1 ½ min and the b- wave will disappear[58]. Since normal standard visual field tests cannot detect initial changes related to glaucoma, it was demonstrated that there was a decrease in amplitude and increase in latency of the N2 wave of the middle area of the retina using a mfERG[59]. The diameter of the retina was between 100 and 200 μm. In general, in acute retinal degeneration diseases the amplitudes of a and b waves decrease, while implicit time is prolonged when studies are done in scotopic conditions. In chronic situations, the same changes are noticed after both scotopic and photopic adaptation[3].

In diabetic retinopathy loss of vision has been related to the elevation of vascular endothelial growth factor (VEGF), and retinal central vein occlusions as well as neovascularization of the anterior chamber has been linked to elevated VEGF in the aqueous humor. The inhibition of the proliferation of VEGF would help to analyze its effects that can be reflected in an ffERG study. Intravitreal local injection of Bevacizumab, with no systemic effects, did not show any effects on the amplitudes of a and b waves making it a better option in the treatment of age-related macular degeneration and diabetic retinopathy[60]. In diabetic retinopathy, there is inadequate glycemic control which is related to a weakening of small blood vessels which can lead to the formation of microaneurysms, bleeding, edema, and ischemia of retinal cells. In the end, this will result in neovascularization and retinal detachment[61].

One of the retinal diseases that have been demonstrated to influence the ffERG studies is retinitis pigmentosa, which is a genetic defect and the degeneration of the classic photoreceptors, rods and cones, and retinal ganglion cells[62].

Long- term administration of antiepileptic drugs like vigabatrin, a gamma-aminobutyric acid (GABA) analog and inhibitor of GABA aminotransferase have been linked to vision loss due to its effects on bipolar cells which contribute to the formation of b waves and do express GABA receptors[63,64]. Blockage of the GABA feedback system attenuates significantly the **b** waves and confirmed that it is an important pathway for the formation of b waves.

Using a multifocal type of ERG (mfERG), cataracts of distinct levels of opacity cause a reduction in amplitude of a and b waves[65]. In a flicker ffERG, cataracts reduce the intensity and scatter light on the retina. Thus, it stimulates a larger area and in contrast, increases the amplitude of ERG on the peripheral area when compared to the macular area[66]. Although these changes might have been noticed, they did not find any statistical differences before and after cataract surgeries and concluded that ffERG is a dependable pre-surgical ocular examination[67].

Age has been noted as a crucial factor during ERG studies. With the use of albino rabbits, it was noted that a wave appears during the first 2 weeks while the band oscillatory wave appears after that. The b-wave reaches its maximum amplitude at 40 days and this result justifies ERG studies to be done after 6 weeks[68]. During the development of the retina, the ganglion cells are the first to differentiate then followed by neurons of the outer retina, sequentially amacrine, classical photoreceptors, and bipolar cells. Thus, photoreceptors' responses in the recently born individual are less than those that have a matured visual system[69]. Having all the information presented thus creates a need to know the molecular basis for ERG studies.

Conclusions

It was concluded that molecular-based knowledge forms the basis for the interpretation of a, b, and c wave changes of ffERG studies and ipRGCs have a contribution to the formation of the c-wave.

Acknowledgements

The following people provided so much support and comments while this paper was being revised: Carolina Montelongo Ponce, Alejandro Botello Camacho, Marisela Aguirre Ramírez, Roxana Malpica Calderón, Gustavo Adolfo García Sánchez, and Julián Lozano Pérez.

Financing

Benn Tonderai-Katsamudanga is a fellow of Consejo Nacional de Ciencia y Tecnología (CONACyT). Work in Pérez-León lab is supported by: Programa integral de Fortalecimiento Institucional (PIFI) 2007-2008, 2909 5001-004-09. PROMEP Apoyo a Cuerpos Académicos 2013. Programa para el Fortalecimiento de la Calidad de las Instituciones Educativas, 2015 (PROFOCIE). Reconocimiento al Perfil Deseable PRODEP-SEP 2019.

Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Revista Mexicana de Neurociencia

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REVIEW ARTICLE

The role of GABA neurotransmitter in the human central nervous system, physiology, and pathophysiology

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Abstract

GABA is the main inhibitory neurotransmitter of the central nervous system (CNS) and one of the most abundant neurotransmitters in mammals is distributed in most areas of the brain and participates in 40% of the inhibitory synapses of adult vertebrates. It is produced in the CNS, through the decarboxylation of glutamic acid, catalyzed by glutamic acid decarboxylase (GAD). GABA exerts its inhibitory effect through two types of specific receptors, $GABA_A$ (ionotropic) and $GABA_B$ (metabotropic), which show different pharmacological, structural, and molecular differences. Even though GABA plays a key role in the physiology of the CNS modulating different processes, also is involved in some pathologies, furthermore, is a target for several therapeutics drugs. For instance, GABA has important involvement in sleep cycle regulation, and for decades benzodiazepines and gaboxadol have been prescribed for the treatment of insomnia. In epilepsy disease, the pharmacological and gene expression studies suggest a role in the prevention of seizures by blocking the regulation of $GABA_A$ receptors with specific antagonists. In depression and anxiety, studies indicate changes in the regulation of the genes which encoding GABA receptors. In the same way, GABA receptors have been associated with alcoholism and premenstrual syndrome. In conclusion, experimental evidence suggests, that the same subtype of GABA receptors showed a different pattern of cellular population and subcellular expression in different areas of the brain, modulating the excitability, and neuronal synchronization in different affection pathologies and conditions in humans.

Key words: *Human. GABA. GABA receptors. Central nervous system. Physiology. Pathophysiology.*

El papel del neurotransmisor GABA en el sistema nervioso central humano, fisiología, y fisiopatología

Resumen

El GABA es el principal neurotransmisor inhibitorio en el sistema nervioso central (SNC) y uno de los más abundantes en mamíferos; se distribuye en diferentes áreas del cerebro y participa en el 40% de las sinápsis de vertebrados adultos. Su síntesis se realiza en el SNC a través de la descarboxilación del ácido glutámico por acción de la descarboxilasa del ácido glutámico (GAD). El efecto ihibitorio de GABA se ejerce a través de dos tipos de receptores específicos: $GABA_A$ (ionotrópi-

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Date of reception: 27-05-2020
Date of acceptance: 14-10-2020
DOI: 10.24875/RMN.20000050

Available online: 17-03-2021
Rev Mex Neuroci. 2021;22(2):67-76
www.revmexneurociencia.com

cos) y $GABA_B$ (metabotrópicos), los cuales presentan características farmacológicas, estructurales y moleculares diferentes. A pesar de que GABA participa de manera importante regulando diferentes procesos en el SNC, también está involucrado en algunas patologías, por lo que sus receptores son un blanco terapéutico. Por ejemplo, GABA participa en la regulación del ciclo del sueño, y por décadas las benzodiazepinas y el gaboxadol se han prescrito para el tratamiento del insomnio. En epilepsia, estudios farmacológicos y de expresión génica sugieren una participación en la prevención de las crisis al bloquear los receptores $GABA_A$ con antagonistas específicos. En el caso de la depresión y ansiedad, estudios indican cambios en la regulación de genes que codifican para los receptores a GABA. En este mismo sentido, los receptores a GABA también se han asociado al alcoholismo y al síndrome premenstrual. En conclusión, la evidencia experimental sugiere que el mismo subtipo de receptor a GABA presenta patrones diferentes en las poblaciones celulares y en su expresión subcelular en diferentes áreas del cerebro, modulando la excitabilidad y sincronización neuronal en diferentes condiciones y afecciones patológicas en humanos.

Palabras clave: *Humano. GABA. Receptores GABA. CNS. Fisiología. Patofisiología.*

Introduction

Amino acids (AA) are the basic units of proteins; if there is any AA deficiency, the synthesis of proteins is altered, causing a unbalance in the cellular physiology and organism. AA have different functions and are essential for the physiology of the body; for example, hormones such as insulin, growth hormone, and glucagon, are made up of AAs. It is known that some AAs regulate the *in vitro* and *in vivo* secretion of insulin in pancreatic β-cells. AAs play an important role in different processes related to gene expression, including the modulation of proteins that regulate mRNA[1].

In the central nervous system (CNS), some AA are neurotransmitters and have a key role in neuronal communication, such as glutamate and glycine. In the case of GABA, despite that chemical structure, it is not considered like AA, because it does not conform proteins. These neurotransmitters are stored in vesicles inside the presynaptic neuron and released into the synaptic cleft by a Ca^{2+}-dependent mechanism and in response to a depolarizing stimulus. These neurotransmitters generate excitatory or inhibitory responses through binding to their specific receptors in the post-synaptic neuron or even the same presynaptic cell. A malfunction of these neurotransmitter communication systems is associated with different neurological or psychiatric disorders. For this reason, several pharmacological therapies aim to regulate release mechanisms, receptor binding, or neurotransmitter reuptake.

In this review, we will focus on the inhibitory neurotransmitter GABA (γ-aminobutyric acid), specifically in the participation of their receptors in different neurodegenerative disorders and the different pharmacological strategies aimed at their regulation for the treatment of these pathologies.

GABA

GABA is the main inhibitory neurotransmitter of the CNS and one of the most abundant neurotransmitters in mammals. It was first described in the early 1900s, while its presence and participation as a neurotransmitter in the mammalian CNS was determined until the 1950s. During the following two decades, many studies established its mechanism of action, as well as its inhibitory activity in the cerebral cortex[2]. We, currently, know that GABA is distributed in most areas of the brain and participates in 40% of the inhibitory synapses of adult vertebrates.

GABA metabolism

GABA is produced in the CNS, through the decarboxylation of glutamic acid, and catalyzed by glutamic acid decarboxylase (GAD). In general, the enzymatic activity of GAD is regulated by its expression levels and the degree of association with the cofactor pyridoxal phosphate (PLP)[3]. GAD has two isoforms, GAD_{65} and GAD_{67}, both encoded by different genes, and different expression patterns, but with similar mechanisms regulating their function[4,5]. GAD_{65} is located mainly in the synaptic terminals, where it induces vesicular GABA release in a Ca^{2+}-dependent process. GAD_{65} dissociates from PLP under physiological conditions and increases its activity depending on the demands of the synaptic release of GABA[6]. Therefore, the main regulatory mechanism for this isoform is the association with its cofactor and not it is level of expression[3].

GAD_{67} is expressed in the cytoplasm, it is associated with PLP under physiological conditions, and is regulated by its levels of expression. GAD_{67} is involved in cell metabolic activity and is responsible for most of the GABA synthesis in the brain[3]. However, it has been

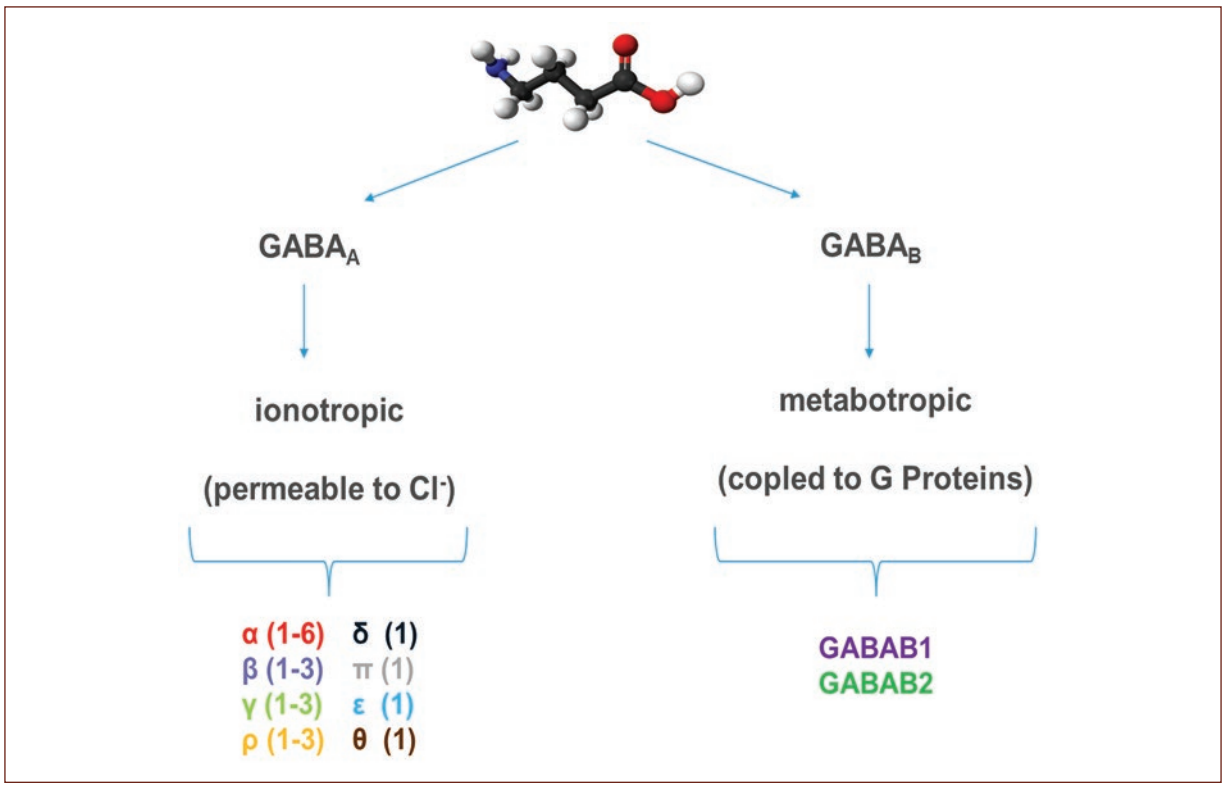

Figure 1. Representative molecular structure of GABA and GABA receptors. GABA is a full agonist of two types of receptors, named according to their mechanism of action. $GABA_A$ or ionotropic receptors, are protein channels activated by GABA and permeable to Cl-. These receptors are conformed by different subunits, six type α, three type β, three type γ, and one each of type δ, ε, π and θ. $GABA_B$, or metabotropic receptors, are receptors coupled to G proteins that modulate K^+ and Ca^{2+} channels and are conform for only two subunits. The GABA structure was from https://es.wikipedia.org/wiki/Ácido_γ-aminobutírico (Last accessed on September 20, 2020).

suggested that cytoplasmic GABA can be released by a Ca^{2+}-independent mechanism. If this is correct, then GAD_{67} may participate in inhibitory neurotransmission by activating extra-synaptic GABA receptors.

After GABA release into the synaptic cleft, it is re-uptake by the GAT-1 and GAT-3 transporters, these systems are expressed in both neurons and glial cells. GABA catabolism is carried out through the GABA transaminase (GABA-T). GABA-T is dependent on PLP, which, in turn, requires GABA and α-ketoglutarate as substrates, generating glutamic acid, and succinic semialdehyde (SSA) as by-products. SSA can be metabolized into γ-hydroxybutyric acid, which regulates $GABA_B$ receptors, or can be dehydrogenated to succinate. When GABA is dehydrogenated to succinate via SSA dehydrogenase, it is incorporated into the Krebs cycle, where it participates in cellular energy metabolism. Many clinical and preclinical researches indicate the presence of GABAergic neurons in regions such as the amygdala, hippocampus, hypothalamus, prefrontal cortex, olfactory bulb, retina, and spinal cord.

GABA receptors

GABA exerts its inhibitory effect through two types of specific receptors called $GABA_A$ and $GABA_B$, which show different pharmacological, structural, and molecular differences (Fig. 1). $GABA_A$ receptors, or ionotropic, share its structural and functional properties with the ligand-gated ion channels or "Cys-loop" family, which include glycine (Gly), acetylcholine (ACh), and serotonin ($5\text{-}HT_3$) receptors[7]. The complexity of $GABA_A$ receptors lies in the number of subunits they contain, and in the different combinations in which they are assembled. There are also variants generated by splicing or RNA editing[8]. To date, six subunits have been characterized in humans: six type α, three type β, three type γ, and one each of type δ, ε, π, and θ, giving this receptor a high degree of heterogeneity. On the other

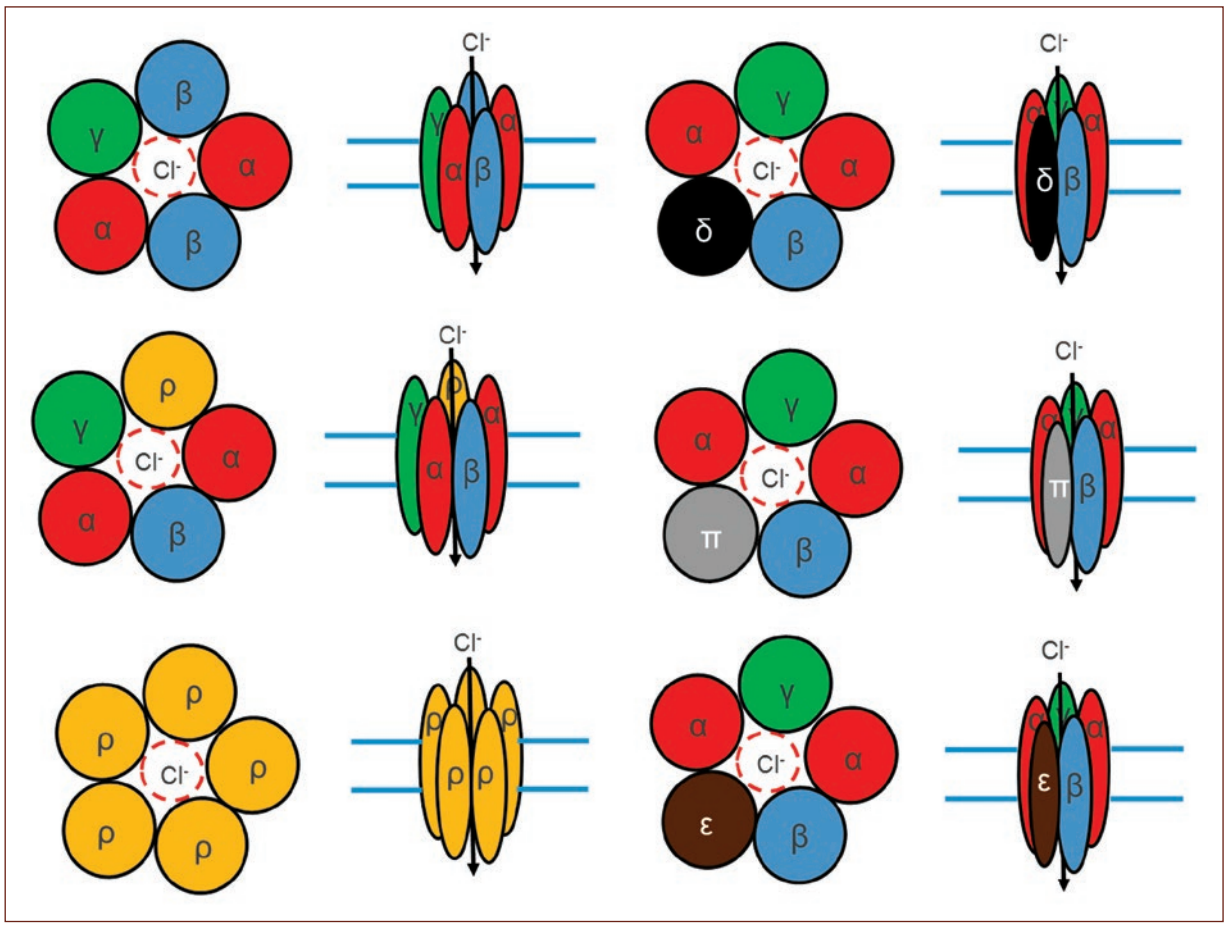

Figure 2. Structural conformation of $GABA_A$ receptors. Note the number of subunits that contain, and in the different combinations in which they are assembled. Some of them are variants from splicing. giving this receptor a high degree of heterogeneity. On the other hand, three subunits ρ have been characterized, which unlike the others form functional homopentamers. In adult mammals, the most abundant receptor conformation, composed of the subunits $\alpha_1\beta_2\gamma_2$. However, the number of $GABA_A$ receptor isoforms expressed in mammals is still unknown.

hand, three subunits ρ have been characterized, which unlike the others form functional homopentamers with different pharmacological properties. These subunits are considered a subfamily called $GABA_C$. In adult mammals, the most abundant isoform is composed of the subunits $\alpha_1\beta_2\gamma_2$. However, the number of $GABA_A$ receptor isoforms expressed in mammals is still unknown[9] (Fig. 2).

$GABA_A$ receptors are selectively blocked by bicuculline and picrotoxin, and they are allosterically modulated by neurosteroids, barbiturates, and benzodiazepines. At present, there is a continuously increasing large number of regulatory molecules for this type of receptors, due to their pathological importance[10]. In the case of $GABA_C$ receptors, they are not regulated by modulators and blockers of the $GABA_A$ receptor. However, they are sensitive to picrotoxin and 1,2,5,6-Tetrahydropyridin-4-yl)methyl phosphonic acid or TPMPA, and specific $GABA_C$ antagonist[11] (Fig. 3). $GABA_A$ receptors activation leads to the inhibition of synaptic transmission, due to hyperpolarization in response to a Cl^- influx through these receptors[12]. Interestingly, during development there is a delay in the expression of Cl^- mobilization systems, generating a high intracellular concentration of this anion[13]. Therefore, an increase of Cl^- conductance in response to the activation of $GABA_A$ receptors generates a Cl^- outflux current and consequently, cell depolarization, so GABA acts as an excitatory AA during CNS development[14].

$GABA_B$ receptors, or metabotropic, belong to the family of G-protein-coupled receptors or GPCRs, which activate slow responses through the second messengers[15], and have a limited structural diversity, unlike $GABA_A$ receptors or metabotropic glutamate receptors

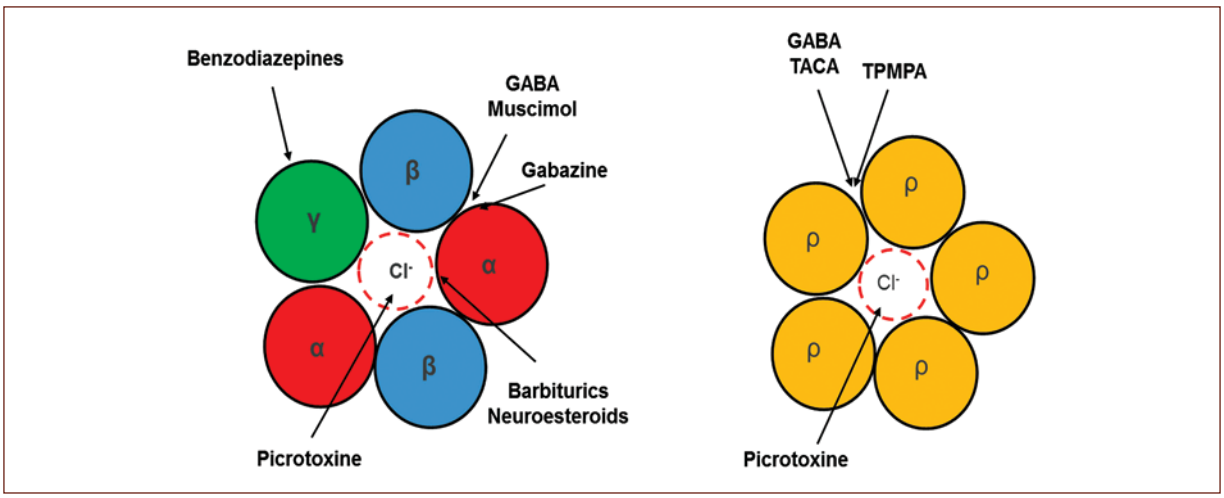

Figure 3. Pharmacology properties of $GABA_A$ receptors. At the right is the classical conformation of the $GABA_A$ receptor, observe the modulation sites were several drugs neuromodulator act to modulate the GABA physiology. These drugs exert their action in different sites of the receptor. Benzodiazepines in the γ subunit; Picrotoxin actin the pore of the channel and blocked; the Barbiturics and neurosteroids in the α subunit and, Muscimol and GABAzine compete for the GABA binding site located between α and β subunit. On the left side, are functional homopentameric receptor formed by ρ. In this case, Picrotixine, as the $\alpha_1\beta_2\gamma_2$ conformation, acts in the pore of the receptors, however, TACA and TPMA compete for the GABA binding site.

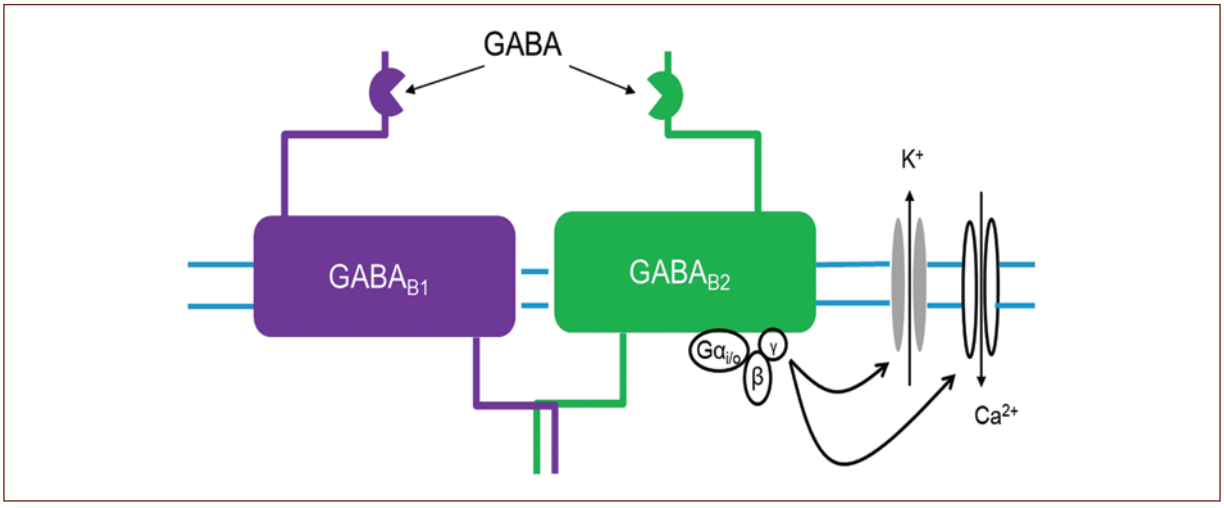

Figure 4. Structural conformation of $GABA_B$ receptor. This receptor is heterodimers composed of the $GABA_{B1}$ or $GABA_{B1}$, belong to the G-protein-coupled receptors, which activate slow responses through the second messengers, that regulate K^+ and Ca^{2+} channels. Nevertheless, despite their poor structural diversity, native $GABA_B$ receptors show a varied kinetic and pharmacological response.

(mGluRs). $GABA_B$ receptors are heterodimers composed of the $GABA_{B1a}$ or $GABA_{B1b}$ subunits combined with the $GABA_{B2}$ subunit. Nevertheless, despite their poor structural diversity, native $GABA_B$ receptors show a varied kinetic and pharmacological response (Fig. 4).

The location of $GABA_B$ receptors in the synaptic region is key to regulating neurotransmission. Depending on whether the receptor is presynaptic or postsynaptic, their activation generates an inhibition or disinhibition of synaptic activity. At the post-synaptic level, receptor activation induces a K^+ conductance increase, which is responsible for the "slow" inhibitory events of GABA in the CNS[16]. The activation of this K^+ conductance, coupled with negative regulation of Ca^{2+} influx at the presynaptic

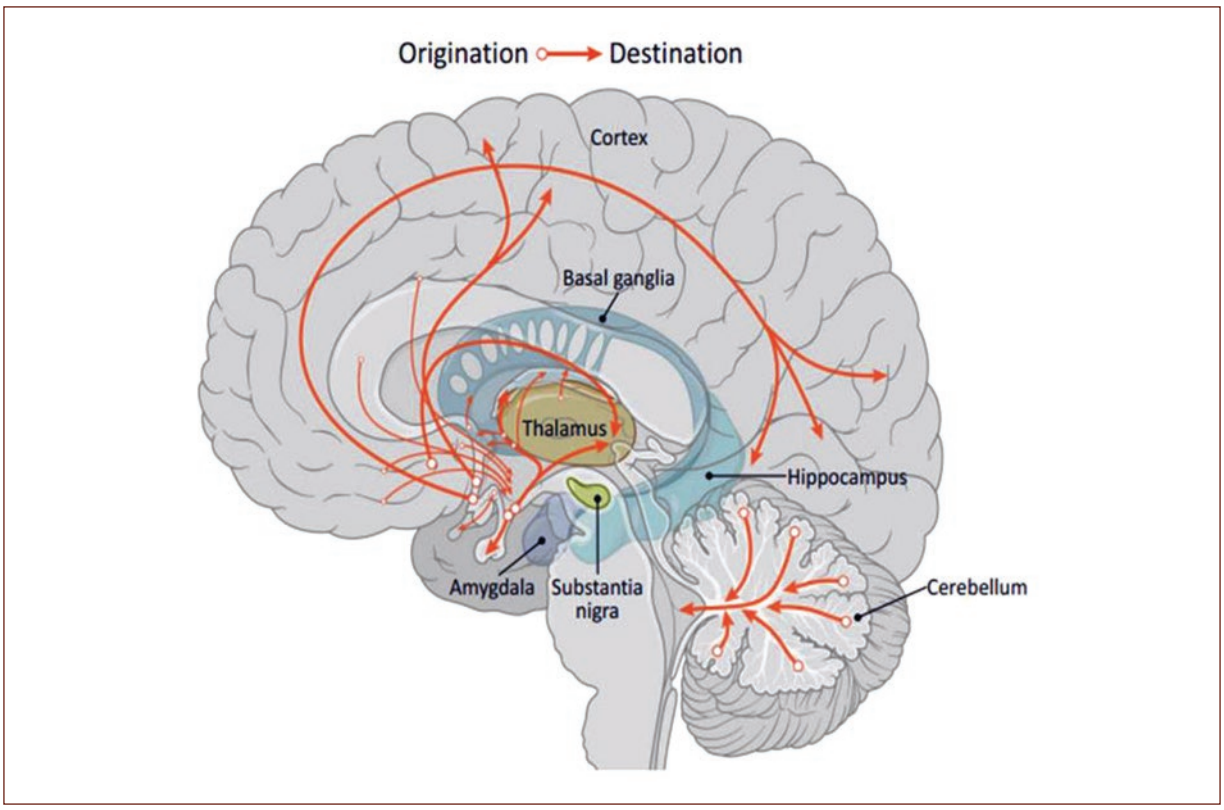

Figure 5. GABA pathways in the brain. The GABAergic system goes throughout the amygdala, hippocampus, hypothalamus, prefrontal cortex, olfactory bulb, including the spinal cord and even the retina. This wide expression of the GABAergic cells indicates the key role of this inhibitory neurotransmitter in functions in the CNS, such as behavior, motor control, mood, sleep, among others. Modified from Brain Chat. https:// thebrainchat.com/photos/gamma-aminobutyric-acid-gaba-is-a-naturally-occurring-amino-acid-that-works-as-a/1243388282530289/.

level, decreases the release of GABA by regulating the inhibitory effect mediated by this neurotransmitter[17].

GABA and the CNS

The proper functioning of the CNS depends on the balance between the excitatory and inhibitory neurotransmitter systems. The excitatory system is regulated by glutamate, while the inhibitor system is regulated by GABA through interneurons, which modulate the excitatory level generated by glutamate release. These interneurons control the flow of information and the synchronization of the cerebral cortex, despite the existence of a 1:5 proportion with glutamatergic neurons. Suggesting, that the numerical balance between neurons and interneurons determines brain functionality, in the case of the cerebral cortex, the proportion 1:5 indicates that this region is mainly excitatory.

Studies in animal models indicate the presence of GABAergic neurons in regions such as the amygdala, hippocampus, hypothalamus, prefrontal cortex, olfactory bulb, retina, and spinal cord, this observation was corroborated also in human studies. This wide expression of the GABAergic cells indicates that this inhibitory neurotransmitter is involved in many functions in the CNS (Fig. 5), for instance, the thalamocortical pathway, which regulates primordial functions such as behavior, motor control, mood, sleep, and among others.

The thalamocortical pathway consists of GABAergic neurons that project from the thalamic reticular nucleus (nRT) toward the ventral basalis (VB), generating an inhibitory "loop" for a self-modulation of the nRT. GABAergic neurons of the nRT receive the glutamatergic stimulation from the VB and the cortico-thalamic fibers that are projected from the layer six of the cerebral cortex. In this "loop context," $GABA_A$ receptors are in glutamatergic thalamocortical and cortico-thalamic neurons, as well as in the GABAergic neurons themselves[18].

Thanks to benzodiazepines it has been understood the functional differences between the $GABA_A$

receptors located in the nRT and the thalamic relay neurons. Since benzodiazepines increase the neurotransmission regulated by $GABA_A$ receptors, these compounds exacerbate absence seizures; however, they show some therapeutic efficacy in clinical and animal models. This double function of benzodiazepines is because they increase the inhibition regulated by $GABA_A$ receptors in the nRT, causing a decrease in the inhibitory stimulus of the nRT toward the thalamic relay neurons[19]. Experimental evidence indicates the participation of $GABA_B$ receptors in the pathogenesis of absence seizures[20]. $GABA_B$ receptors regulate K^+, activating low-threshold Ca^{2+} potentials and consequently, burst firing, and the oscillatory behavior of thalamic neurons. Agonists of these receptors exacerbate absence seizures in animal models in a greater proportion than $GABA_A$ receptors.

Disorders associated with GABA receptors

Sleep disorders

GABA has important involvement in sleep cycle regulation, and for decades benzodiazepines and gaboxadol (alternative to benzodiazepines) have been prescribed for the treatment of insomnia with certain tolerance; however, these compounds have side effects such as addiction, hallucinations, and disorientation. Several alterations in the thalamocortical pathway are associated with sleep problems, a common symptom in different neurological pathologies, and where it has been described an increase in GABA levels and an altered function of GABAergic receptors that contain the δ subunit (δ-$GABA_A$)[21].

Epilepsy

It is known that the mechanisms regulated by the $GABA_A$ receptor participate in the partial or generalized generation of tonic-clonic seizures. Pharmacological and gene expression studies suggest a role in the prevention of seizures by blocking the regulation of $GABA_A$ receptors with specific antagonists. Studies point to changes or differences in the expression of subunits of $GABA_A$ receptors that correlate with epileptogenesis[22]. There is a relationship between mutations in the $\gamma 2$ subunit and epilepsy in humans[23], similarly, mutations in the gene encoding the δ subunit have been associated with some forms of congenital epilepsy in humans[24].

The regulation system through the $GABA_B$ receptor participates in the prevention of tonic seizures. In humans, an upregulation of the mRNA of the $GABA_B$ R1 subunit in the CA1 region, dentate gyrus, and hilus region of the hippocampus has been determined in samples of patients with temporal lobe epilepsy[25]. A report indicates an increase in antagonist sites per neuron in the CA3 and the hilus regions of the hippocampus in patients with temporal lobe epilepsy[26]. On the other hand, it was determined that a $GABA_B$ R1 polymorphism (G1465A) confers a high susceptibility to temporal lobe epilepsy and increases the severity of the condition[27]. $GABA_B$ R1 knockout mice show several seizures per day, as well as depression-like behavior[28].

Anxiety disorders

GABA receptors are of great interest for the treatment of anxiety disorders, due to the therapeutic use of benzodiazepines for these conditions. Tests with positron emission tomography and single-photon emission tomography in patients with panic attacks showed an alteration in benzodiazepine binding sites in different areas of the brain[29]. Family studies indicate an association between inhibition of behavior and anxiety disorders. Smoller et al. found an association between an intronic polymorphism in the GAD65 gene with anxiety disorders[30].

Schizophrenia

There are numerous hypotheses about the pathophysiology of schizophrenia, one of which proposes an alteration of GABA-regulated neurotransmission[31]. Changes in the expression of genes related to the different subunits that form GABA receptors have been observed[32]. However, this is not specific for GABA, since there are also changes in gene expression of other neurotransmitters. Another evidence that supports the GABA hypothesis in schizophrenia is that the treatment of this pathology with antiepileptic drugs shows positive clinical results in schizophrenic patients[33,34]. The use of benzodiazepines in combination with antiepileptic drugs reduces considerably the symptoms of schizophrenia and anxiety. On the other hand, the administration of valproate with neuroleptics controls efficiently irritability symptoms and violent behaviors.

The genes that encode $GABA_B$ R1 are located within the loci of schizophrenia, suggesting the participation of this receptor in this disorder[35]. These observations suggest that the $GABA_B$ R1 receptor may be a pharmacological target for schizophrenia.

Depression

In recent years, different reports showed evidence about the association between GABA and depression. For example, a decrease of this neurotransmitter in the cerebrospinal fluid of patients with depression has been reported[36]. Magnetic resonance spectroscopy in depressive patients showed a reduction in GABA levels, mainly in the occipital cortex (OCC) and in some areas of the prefrontal cortex (PFC)[37]. Studies indicate changes in the regulation of the genes which encoding GABA receptors. For example, Choudary and col. showed the deregulation of the β_3, γ_2, and δ subunits in the frontal cortex[38]. In this regard, the postmortem analysis by PCR of different brain regions from suicide victims with depression showed alterations in the α_5, γ_1, and γ_2 subunits of the dorsolateral and lateral inferior PFC[39]. Fatemi and col. found an increase in the α_2, α_2, γ_3, and ε subunits in the cerebellum of non-suicidal patients with depression[40].

Premenstrual dysphoric disorder (PMDD)

Changes in cognition, mood, and sensitivity to medications throughout the premenstrual cycle have been attributed to hormonal regulation of GABAergic transmission[41]. PMDD occurs during the luteal phase of the menstrual cycle and is characterized by significant alterations in behavior, mood, and a physical limitation that compromises personal, social, and professional development. In healthy women, plasmatic levels of GABA increase from the follicular to the luteal phase, while they decrease in women suffering from PMDD[42]. However, in the CNS, GABA levels increase in this same phases[43]. An increase in cortical inhibitory activity has been reported in healthy women, whereas this effect is not observed in women with PMDD[44]. Deregulation of GABA levels possibly is secondary to the action of allopregnanolone, a metabolite of progesterone secreted during the luteal phase. This hormone acts on $GABA_A$ receptors that contain the α_4 and δ subunits[45]. During periods of fluctuation of progesterone concentrations, there are changes in the expression of the α_4 and δ subunits in the CA1 region of the hippocampus. Furthermore, a decrease in the α_1 subunit has been observed[42].

Alcoholism

It is well known that the main ethanol target in the CNS is the $GABA_A$ receptors[46], for example, studies in rodents show that the systemic administration of GABA receptors agonists or antagonists modulates the ethanol consumption[47]. $GABA_A$ receptors increase their probability of aperture and/or their affinity for the agonist after an acute ethanol intake. They are generally potentiated by low concentrations of ethanol (5-50 mM) that correspond to very low intake in humans (a couple of drinks). Chronic exposure to ethanol generates different specific neuroadaptations in GABAergic synapses in different regions of the brain. Furthermore, chronic exposure to ethanol alters the expression of different subunits of $GABA_A$ receptors at the transcriptional level in different regions of the brain involved in the development of alcohol dependence. For example, in the cerebral cortex, chronic exposure to ethanol decreases mRNA and the expression of α1, α2, and α3 subunits, and increases the expression of α4, β1, β2, β3, γ1, and γ2 subunits[48]. In humans, sequencing assays have identified different genes encoding $GABA_A$ receptors associated with alcohol consumption, such as α3 (GABRA3) in the prefrontal cortex, γ1 (GABRG1), and γ2 (GABRG2) in the hippocampus[49]. These expression changes probably alter the assembly of $GABA_A$ receptors, modifying their binding affinity to the ligand and, consequently, their function. In the case of the hippocampus, alterations in the expression of $GABA_A$ receptors depend on the time of exposure to ethanol. A 40-day chronic exposure significantly increased the levels of the α4 subunit; however, no alterations were observed in the α1, α2, α3, β (2/3), or γ2 subunits[50]. These results indicate a regulation in the expression of subunits of $GABA_A$ receptors specific to each region of the brain that is dependent on the length of exposure to ethanol.

In the case of $GABA_B$ receptors, they are determinants of the specificity of ethanol effects on certain regions of the brain. For example, Peris and col. showed that chronic ethanol treatment increases the release of GABA, decreasing long-term potentiation[51]. Exposure to baclofen ($GABA_B$ antagonist) decreases this release of GABA, whereas 2-OH-saclofen ($GABA_B$ antagonist) enhances it. This chronic effect of ethanol on the release of GABA into the synaptic cleft could explain the reduction in long-term potentiation observed after chronic exposure to ethanol.

Conclusion

Its complex structural diversity, associated with its differential expression pattern in the neural loops of the brain, makes it very difficult to understand its inhibitory

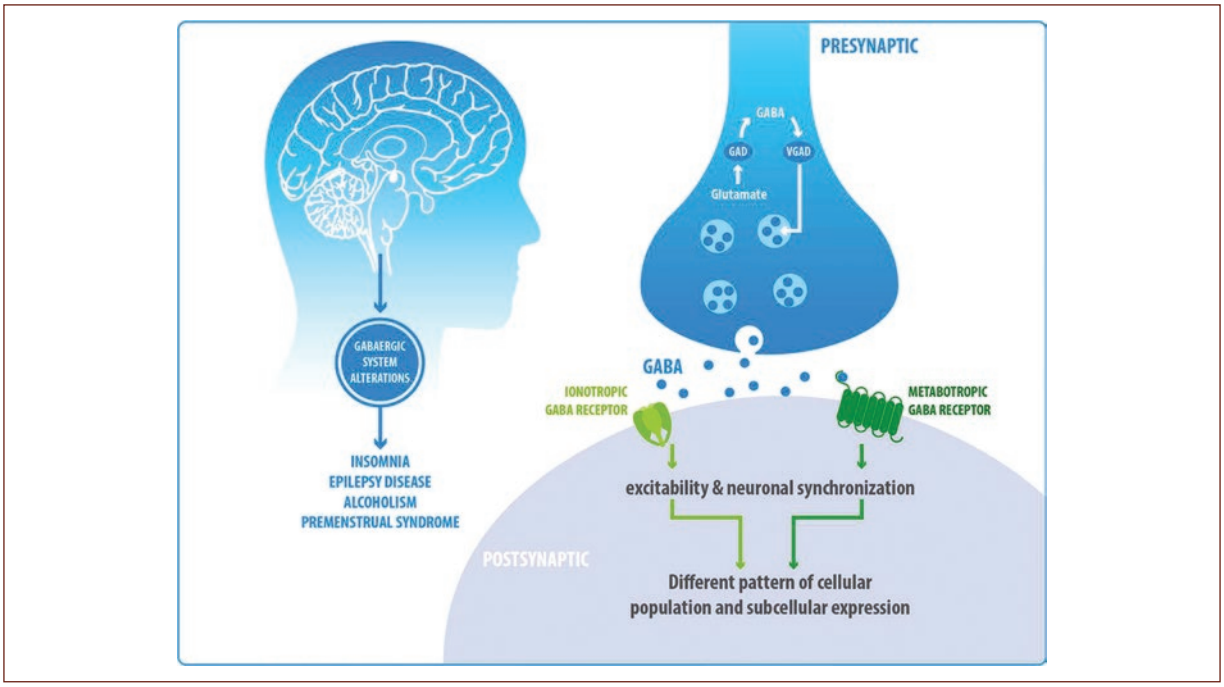

Figure 6. Its complex structural diversity, associated with its differential expression pattern in the neural loops of the brain, makes it very difficult to understand its inhibitory control in different neural networks. Although our knowledge about the specific functions of GABAergic receptors in a neural loop is limited, there is evidence that suggests that the same subtype of GABAergic receptors can be expressed in different neuronal populations with different subcellular locations and in different areas of the brain, modulating the excitability and neuronal synchronization in different ways (Fig. 6). Therefore, understanding the role that GABA plays in the brain is possible only through the specific study of a receptor subtype, a loop, or a neuronal type, considering important variables such as previous synaptic physiology, synaptic activity, sex, or the circadian cycle, are partially explored along with GABA.

control in different neural networks. Although our knowledge about the specific functions of GABAergic receptors in a neural loop is limited, there is evidence that suggests that the same subtype of GABAergic receptors can be expressed in different neuronal populations with different subcellular locations and in different areas of the brain, modulating the excitability and neuronal synchronization in different ways (Fig. 6). Therefore, understanding the role that GABA plays in the brain is possible only through the specific study of a receptor subtype, considering important variables such as synaptic activity, sex, or the circadian cycle, are partially explored along with GABA.

Funding

This article was supported by grants from PAPIIT-UNAM IA205918 and IN221820 and sponsorship by Departamento de Bioquímica, Facultad de Medicina, UNAM and Asociación para Evitar la Ceguera en México I.A.P Hospital Dr Luis Sánchez Bulnes.

Conflicts of interest

The authors declare that they have not conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare no patients data appear in this article.

Right to privacy and informed consent. The author declares that no patient data appear in this article.

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REVIEW ARTICLE

Spinal arteriovenous malformations

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Abstract

Pathologies that affect the spinal cord are diverse. In addition to trauma, common etiologies of myelopathy include autoimmune, infectious, neoplastic, vascular, and hereditary-degenerative diseases. Physicians should be aware of the possibility of a spinal arteriovenous malformation (sAVMs) when facing a challenging case. Performing an adequate clinical history and neurological examination is the key element in the proper approach to these pathologies. However, a better determination of the problem can be obtained with the help of neuroimaging studies. Given the rare nature of sAVMs, the purpose of this article is to review the spinal vascular anatomy, the pathogenesis and pathology of arteriovenous malformation, discuss key points about their classification, and summarize the imaging findings and treatment approaches available.

Key words: *Arteriovenous malformations. Spine. Review.*

Malformaciones arteriovenosas de la médula espinal

Resumen

Las patologías que afectan la médula espinal son diversas. Además del trauma, las etiologías comunes de la mielopatía incluyen enfermedades autoinmunes, infecciosas, neoplasias, vasculares y hereditarias degenerativas. Los médicos deben mantener en mente la posibilidad de una malformación arteriovenosa espinal cuando se enfrentan a un caso difícil. La historia clínica y la exploración neurológica adecuada siguen siendo pieza clave en el adecuado abordaje de estas patologías, sin embargo, puede obtenerse una mejor determinación del problema con ayuda de los estudios de neuroimagen. Dada la naturaleza rara de las malformaciones arteriovenosas espinales, el propósito de este artículo es revisar la anatomía vascular espinal, la patogenia y la patología de las malformaciones arteriovenosas, discutir puntos clave sobre su clasificación y resumir los hallazgos por imagen y los tratamientos disponibles.

Palabras clave: *Malformaciones arteriovenosas de la médula espinal. Lesiones vasculares. Médula espinal. Epidural. Revisión.*

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Date of reception: 10-06-2020
Date of acceptance: 06-08-2020
DOI: 10.24875/RMN.200000611

Available online: 17-03-2021
Rev Mex Neuroci. 2021;22(2):77-84
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Introduction

Arteriovenous malformations (AVMs) are rare lesions, which are often diagnosed late because clinicians do not think about them. The best estimates for the detection of an AVM are 1/100,000 population per year (about 3000 new cases detected each year in the U.S.), with prevalence about 10/100,000[1]. According to Moore et al., they represent 2.5% of cases of spastic paraparesis or quadriparesis compared with cervical spondylotic myelopathy (24%), tumors (16%), multiple sclerosis (18%), and motor neuron disease (4%)[2].

In this article, we briefly summarize the anatomy, clinical presentation, classification, and treatment of vascular spinal cord lesions. Given the unique nature of spinal AVM (sAVM), our purpose was to construct a bibliographic review about the spinal vascular anatomy, the pathogenesis and pathology of AVMs, discuss key points about their classification, and summarize the imaging findings and treatment approaches available. In this regard, an electronic search strategy of the literature available was conducted (January 2020) using PubMed, Access Medicine, Embase, and UpToDate to retrieve publications regarding the key points mentioned previously. The terms used in the literature search were our keywords as subject headings from the Medical Subject Headings thesaurus. Publications in both English and Spanish were included in the study. The reference sections of the included publications were also screened for potentially relevant references. In addition to the electronic search strategy, other sources were examined for potentially relevant publications. These included different books on neurology and spinal cord disorders.

Vascular anatomy of the spine

Spinal vascular anatomy incorporates not only the vascular supply to the cord but also that to the adjacent structures, including the nerve roots, dura, and paraspinal musculature[3].

Arterial supply

Anterolateral supply

Superficially, the anterior two-thirds of the spinal cord is supplied by the anterior spinal artery (ASA), which originates from a medial branch of the intracranial vertebral artery on both sides, which join together in the midcervical spine (C2-C4) and travel along the anterior medial fissure (Fig. 1). The dominant contributors to this artery are six to eight segmental radiculomedullary arteries, which typically include:

- An artery at C3 (also referred to as the artery of the cervical enlargement, from the vertebral artery),
- C6 (from the deep cervical artery),
- C8 (from the costocervical trunk),
- T4/T5
- Moreover, the anterior great radiculomedullary artery (artery of Adamkiewicz), which usually originates on the left side between the T8 and L2 level[3,4].

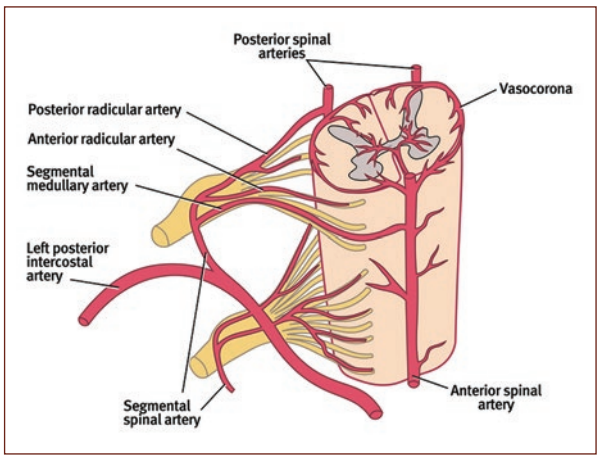

Figure 1. Blood supply of the spinal cord.

This anterior blood supply is also referred to as the "sulcocommissural system" or centrifugal system and gives rise to 200-400 sulcocommissural arteries within the ventral sulcus to supply the anterior commissure, dorsal nucleus of Clarke, corticospinal tract, spinothalamic tract, and the anterior portions of the cuneate and gracile fasciculi[5].

Posterior supply

Superficially, the posterior one-third of the spinal cord is supplied by a pair of posterior spinal arteries (PSA), which originate at the level of the foramen magnum from:

- Branches of the intradural portion of the vertebral artery or
- The posterior inferior cerebellar artery, with
- Contributions from 6 to 11 posterior radiculomedullary arteries as they descend caudally in a discontinuous manner[3].

This posterior system is also referred to as the dorsolateral pial supply or centripetal system (Fig. 1). This

network gives off radial arteries that circumferentially run along the spinal cord, reaching anastomosis with the ventral system (forming a pial plexus called the vasocorona), and giving off axially oriented perforating branches into the white matter[5].

Inferiorly on the spinal cord, the ASA and PSA systems build a reticulating network around the conus in a structure commonly referred to as the arterial basket of the conus medullaris[6,7].

ASA

The ASA descends over the central sulcus of the anterior cord all the way to the conus medullaris. En route, it acquires multiple feeders: in the cervical spine, the most prominent feeder is the artery of the cervical enlargement; in the thoracolumbar spine, the most prominent feeder are multiple anterior radiculomedullary feeders from segmental branches directly from the aorta, and in the lumbar spine, there usually are four pairs of lumbar segmental arteries from L1-L4 and lower lumbar segmental arteries arising from the common iliac arteries and the median sacral artery[4,6].

Venous drainage

The venous system of the spinal cord is not wholly analogous to the arterial anatomy. Venous drainage uses to be more regional with a central and peripheral venous system.

Peripheral system

The peripheral, or radial, veins originate in the capillaries at the gray-white junction and are directed centrifugally.

Central system

The central, or sulcal, veins drain from the medial aspects of both halves of the spinal cord, specifically from the anterior horns, anterior commissure, and the white matter in the anterior funiculus[8]. Intrinsic spinal cord veins and small superficial pial veins lead into the superficial longitudinal median spinal cord veins. Eventually, these drain into a set of veins that mirror the arterial system, but have many anastomoses creating a vast interconnected network[9]. The venous blood reaches the epidural plexus and the extraspinal veins and plexus, with a reflux-impeding mechanism within the dura mater[8]. Ultimately, the extraspinal plexus joins the cava system, mainly the innominate veins at the cervical level, the azygos vein at the thoracic level, and the ascending lumbar vein at lumbar level[6].

Pathogenesis and pathology of sAVMs

sAVMs represent an abnormal, often tiny, arteriovenous shunt located within the dura of the spinal cord. Afferent supply to the lesion is through a radicular artery, usually at a level with no associated arterial supply to the spinal cord[10]. Blood flow through the fistula runs through a radicular vein in a retrograde manner to the coronal and pial venous plexus, which becomes dilated and tortuous. Outflow impairment to the epidural drainage system or blockage of venous return in the cava system explains this venous dilatation[11]. Since there is no involvement of arterial supply to the spinal cord, no possibility of arterial steal may be invoked to explain the neurologic dysfunction in these patients[10]. Venous congestion is now recognized as a primary source of neurologic disability with sAVMs. Recent studies have validated the elevated pressure in the draining vein as a causative agent in myelopathy, and shown pathologic correlates, including hyalinized small blood vessels, perivascular/intraparenchymal lymphocytic infiltration, glial cell proliferation, and neuronal degeneration[12].

Although impairment of venous drainage from the spinal cord parenchyma is most significant at the level of the shunt, the spread of venous hypertension in the cranial and caudal directions causes damage to the cord over a long distance[11]. It is the caudal end of the spinal cord commonly first affected by congestive edema and ultimately infarction, regardless of the level of the fistula[12].

Hemorrhage can also precipitate neurologic decline: 25% of sAVMs patients will present with a ruptured lesion, as evidenced by subarachnoid hemorrhage (SAH), intraparenchymal hemorrhage, or a combination. Hemorrhage can injure neurologic structures either directly through disruption of fiber tracts or indirectly with mass effect. Venous engorgement, venous varices, and aneurysmal dilatations can further contribute to mass effect and compress the spinal cord or nerve roots[12].

Classification

The first classification system originated from Virchow in 1858, who observed lesions at autopsy that he referred as neoplasms or vascular tumors, and that was either considered angioma cavernosum if there was no parenchyma between the blood vessels, or angioma racemosum (further subdivided into telangiectasias and

angioma racemosum arteriale sive venosum [i.e., sAVM])[13,14]. In 1916, Elsberg proposed an updated scheme to include aneurysms of spinal vessels; angiomas and dilated posterior spinal veins or hemorrhoids of the spinal cord[15]. Since then, many classification systems have been reported and have changed over time in the literature. Until now, a detailed and widely accepted systematization of spinal vascular malformations has not been proposed.

During the 1960s, there was enormous progress in understanding and treating sAVMs. Three major centers (including the National Institutes of Health in Bethesda, the National Hospital for Neurology and Neurosurgery in London, and Hôpital Lariboisière in Paris), generated a combined effort of independent and collaborative investigation that resulted in what is known as the American/English/French classification system. This system separates sAVMs into three types. Type I lesions (single coiled vessel) are dural sAVMs and consist of a radicular artery draining into an engorged spinal vein on the dorsal aspect of the dural sheath of a nerve root, and comprises 80% of sAVMs. Type II lesions (spinal glomus sAVMs) consist of a medullary artery feeding into a nidus, and then into a venous drainage system; they comprise 15-20% of sAVMs and cause damage via hemorrhage, mass effect, vascular steal, and venous hypertension. Type III lesions (juvenile spinal sAVMs) are intramedullary lesions supplied by pial, dural, and paraspinal arteries with an expansive and diffuse nidus that occupies the entire cross-sectional area of the spinal cord and invade the nearby vertebral body; the particularly distinct and unique attribute within this Type III category is the intradural and extradural extension of the lesion[12]. A Type IV lesion was later described by both Djindjian et al. (1977)[16] and Heros et al. (1986)[17], consisting of a direct fistula between a spinal and a dilated perimedullary venous plexus without an intervening nidus; these lesions are intradural and extra/perimedullary. In 1980, Merland et al. further described three subtypes of Type IV lesions: in the first subtype, there is a single arterial feeder from the ASA, mild venous dilatation, and a single, small, and slow fistula. The second and third subtypes have multiple arterial feeders, the first having multiple sAVMs of medium size, whereas the latter has a single but giant sAVM and giant venous ectasia[13,18].

As we mentioned before, different authors have tried to find a clear systematization of spinal vascular lesions and several major classification systems appeared on the literature, including a description by Di Chiro et al. of sAVMs diagnosed and classified using spinal angiography in 1971[19]; a classification system by Mourier and Merland based on a case series of intradural direct perimedullary sAVM treated with endovascular intervention in 1993[20]; two classification systems based on a case series of sAVMs treated by microsurgery by Spetzler[21] or endovascular interventions in 2002 by Rodesh and Lasjaunias[22]; and a classification system based on a case series of extradural SAVMs treated by microsurgery and endovascular interventions in 2011 by Rangel-Castilla et al.[23,24]. Most remarkably, in 2002, Spetzler et al. expanded upon the prior American/English/French system, offering new classifications of spinal vascular pathological entities based on anatomical, pathophysiological or angioarchitectural features, and in 2011 Patsalides et al. offered a classification based on hemodynamic criteria[8,25].

In addition to the many classification systems reported, the Bicêtre group classified spinal cord AVMs into three main groups:

1. Genetic hereditary lesions caused by a genetic disorder affecting vascular germinal cells, such as malformations associated with hereditary hemorrhagic telangiectasia.
2. Genetic non-hereditary lesions such as the Cobb syndrome (or spinal arteriovenous metameric syndrome), Klippel-Trenaunay, and Parkes–Weber syndromes, and
3. Single lesions that may reflect the incomplete expression of one of the situations above.

These include spinal cord, nerve root, and filum terminale arteriovenous lesions. Most of the spinal vascular malformations with pial and dural arteriovenous shunts are included in this group[26,27].

Epidemiology

Little has been published regarding the epidemiology of these lesions[28]. sAVMs make up the most common vascular anomaly of the spine, with an estimated proportion of 60-80%[33]. sAVMs account for 3-4% of all intradural spinal cord mass lesions[29], and dural sAVMs are the most common vascular malformation, accounting for 50-85% of all lesions[30-32].

Lad et al. analyzed National Inpatient Sample data from 1995 to 2006, screening admissions with a primary diagnostic code for spinal vascular malformation. In these 11 years, 3291 patients were admitted with a diagnosis of spinal vascular malformation, with an average of 299 annual admissions with a new diagnosis of sAVM; the majority of admissions corresponded to male patients (57%) in the 45-64-year age range (36% of admissions)[30].

Jellema et al.[33] analyzed all reported series with more than five patients affected by direct sAVMs, detecting there were 968 men against 210 women (ratio almost 5:1). The mean age at the time of diagnosis was found to be 55-60 years; patients under the age of 30 were rarely reported (14 patients under age 30 were found in the 1178 patients or 1%). The youngest patients reported were 22 years at the time of diagnosis. In their study was concluded that no patient under the age of 20 has ever been reported.

Clinical presentation

sAVM most commonly affects the upper thoracic and cervical spine. The sAVMs can be classified in those diagnosed by their clinical presentation and those present when making a diagnostic approach. Initial symptoms are often non-specific and include symmetrical or asymmetrical sensory symptoms such as paresthesia in one or both feet, and diffuse or patchy sensory loss (17-72%), gait difficulties and motor disturbances (50-81%), pain including back or radicular pain (13-64%), and micturition difficulties (4-75%). Other but less frequent symptoms include defection problems and sexual dysfunction[33-34]. In most patients (40-63%), progression lasts for 1-3 years before the diagnosis is made, but a protracted course with a duration of > 3 years occurs in 10-34%. A gradually progressive course with stepwise deterioration is recorded in approximately 11-32% of patients[34,35]. An acute onset is reported in 5-18% of patients. If symptoms develop within minutes to hours, they can mimic an ASA syndrome[33,36]. The sudden episodes mostly occur after exercise, prolonged standing, and even singing and may disappear after rest[33].

At the time of diagnosis, there are often considerable neurological deficits and much of the clinical presentation depends on the type of the sAVM.

- Type I (dural arteriovenous fistulas [DAVFs]): the typical presentation is radiculomyelopathy followed by progressive neurological deterioration. SAH is very uncommon, and acute deterioration in neurological function is unlikely. Most are solitary lesions found in the thoracolumbar region. The fistula is located inside the dura, where a radiculomeningeal artery enters the corresponding radicular vein, close to the spinal nerve root[8,33,37].
- Type II (intramedullary glomus AVMs): the clinical course of these lesions is marked by progressive and fluctuating myelopathy, paraplegia and pain, overlaid by periods of acute neurological deterioration secondary to hemorrhage within the AVM. Sudden-onset presentation (often with profound neurological impairment and possible transverse myelopathy) is frequent. Subarachnoid and intramedullary hemorrhage often occurs in these lesions. The mortality related to type-II malformations has been reported to be 17.6%. After initial hemorrhage, the prevalence of re-bleed is 10% within the 1st month and 40% within the 1st year[8,33,37].
- Type III (Juvenile AVMs): their clinical presentation is similar to that seen with Type II sAVMs. Acute onset of symptoms can occur secondary to SAH, whereas progressive motor and sensory deterioration, as well as sphincter disturbance, usually result from vascular steal, venous hypertension, or mass effect on the spinal cord and/or nerve roots from the dilated veins[8,33,37].
- Type IV (perimedullary AVFs): presentation occurs in the third-to-sixth decade. SAH is possible, with subsequent acute neurological deterioration. A gradual but progressive neurological deterioration is common[8,33,37].

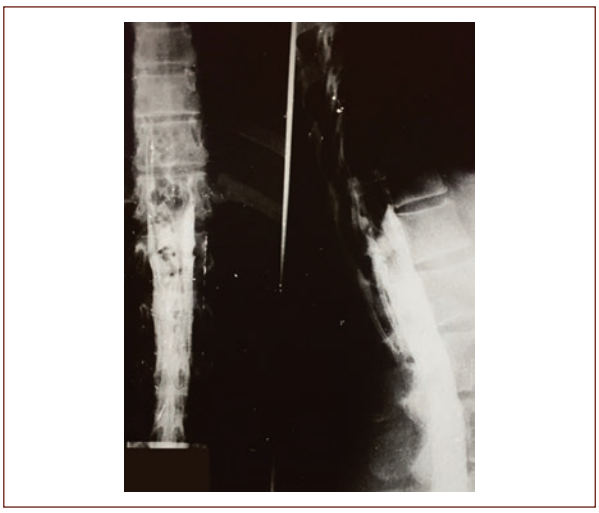

Figure 2. Myelography.

Diagnosis

Imaging is an essential component in the diagnosis, management, and follow-up of patients with AVMs in the brain and spine. A wide variety of imaging modalities are available for its use, such as computed tomography (with its permutations: non-contrast, contrast enhanced, angiography perfusion, and myelography (Fig. 1) and magnetic resonance imaging (with its permutations: non-contrast, contrast enhanced, angiography, and myelography (Fig. 2). Although advances in

Table 1. Comparison between common causes of myelopathy

Disorder	Typical age of presentation	Clinical findings	Course
Infarction	Older adults	Profound weakness with decreased pain and temperature sensation below the lesion. Intact JPS, vibration	Acute
Motor neuron disease	Adults	Upper (increased tone, hyperreflexia) and/or lower (atrophy, fasciculations) motor signs.Normal sensory findings	Progressive, rate of progression may vary
Neoplastic disease	Older adults	Weakness below the level of the lesion, sensory loss	Subacute-chronic with acute worsening
Radiation myelopathy	Any age	Weakness, spasticity	Delayed months after radiation May be slowly progressive
sAVMs	Typically adults	Focal or radicular pain, weakness, sensory symptoms Neurogenic claudication	Acute, may be step-wise or slowly progressive
Spinal epidural abscess	Any age	back pain, focal weakness, sensory symptoms, fever	Subacute with acute worsening
Spinal epidural hematoma	Any age	Focal or radicular pain, weakness	Acute
Spondylotic myelopathy	Older adults	Gait disturbance, spasticity, atrophy, and weakness of extremities	Progressive, step-wise decline
Subacute combined degeneration	Any age	Numbness, paresthesia, weakness, gait disturbance. Decreased JPS and vibration Sensory ataxia	Slowly progressive
Transverse myelitis	Young adults and children	Focal sensory and motor symptoms Sensory level at/below the lesion	Acute or subacute

this noninvasive imaging, digital subtraction angiography continues to be the gold standard for diagnosing and characterizing the detailed anatomic localization, arteriovenous transit, and venous drainage patterns of sAVMs[29,33,38].

In sAVM, spinal angiography demonstrates a tangle of vessels at the level of the spinal cord parenchyma (Fig. 3). After delineation of the lesion, the neighboring vessels should be studied to identify potential feeders and to evaluate the effect of the lesion on the spinal cord. Anteroposterior angiography should be performed to identify feeding vessels and their origin from the anterior or posterior spinal artery (if any). Mass effect can distort the midline structures on frontal projection angiography and in this situation, the lateral projection angiography may help to delineate angioarchitecture. Three-dimensional angiography may help to define the relationship between the vascular malformations, bony structures, and the spinal cord. During the selective injection, contrast reflux into the lesion helps to identify the contributing feeders immediately above and below and is critical for planning endovascular treatment[39,40].

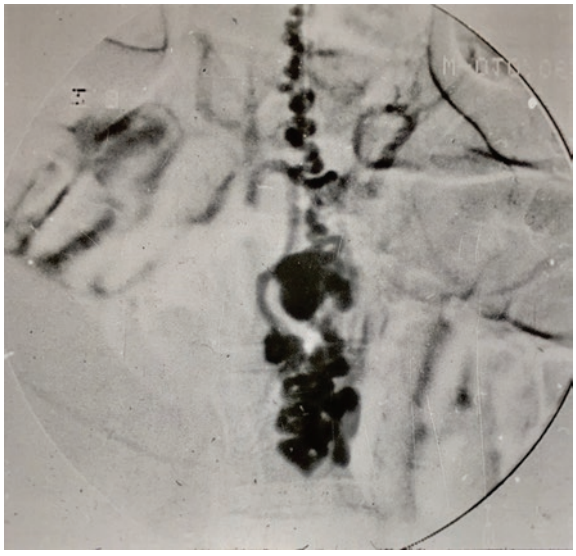

Figure 3. Angiography.

A careful diagnostic workup helps realign clinical reasoning that might otherwise lead these patients to

misdiagnosis and late specialized care, further delaying optimal management. Every patient with lower limb motor/sensory disturbances, micturition disorders, or reflex abolition not well explained by common findings (spinal stenosis, lumbar disk herniation, and spondylolisthesis) should have a focused clinical examination of the thoracic region and/or magnetic resonance imaging of the dorsal spine[41]. Table 1 summarizes the key clinical aspects of AVMs compared with their main differential diagnosis.

Management

All patients must have a thorough preoperative evaluation, including neurological, radiographic, and angiographic evaluations, to fully understand the location and anatomy of the malformations, and the strategy necessary to treat it. The decision to treat vascular malformations of the spine should be made after discussion between the various members of the team consisting of neurovascular surgeons, interventionalists, and radiation therapists, and with the patient or caregivers. Endovascular embolization should be considered as a standalone procedure or as an adjunct to microsurgical resection for spinal vascular malformations when appropriate[39-42].

Conclusion

Spinal DAVFs are rare but can cause serious gait and micturition disturbances. Delays in diagnosis and treatment result in poor clinical outcomes. When a common diagnosis fails to explain the symptoms such as thoracic myelopathy, epiconus syndrome, and conus medullaris syndrome, the possibility of sAVMs should be considered. This is particularly true after spinal trauma in young subjects or spinal stenosis or lumbar disk herniation in older individuals. A thorough exploration of abdominal reflexes or of the sensitivity of the abdominal/thoracic region, particularly when the topography of the deficit suggests a lesion of the spinal cord, should be performed.

Conflicts of interest

None.

Funding

This research has not received any specific grant from public, commercial, or non-profit agencies.

Ethical disclosures

Protection of human and animal subjects: The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data: The authors declare that no patient data appear in this article.

Right to privacy and informed consent: The authors declare that no patient data appear in this article.

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