Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: A

Neurology & Nervous System

Modifications and Optimization

Score for Accuracy and Reliability

Highlights

Networks and Rules Based System

Clinical and the Musculoskeletal Exams

Discovering Thoughts, Inventing Future

VOLUME 24 ISSUE 1 VERSION 1.0

Global Journal of Medical Research: A
Neurology and Nervous System

Global Journal of Medical Research: A
Neurology and Nervous System
Volume 24 Issue 1 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*
Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals® Headquarters
945th Concord Streets,
Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals Pvt Ltd
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investors@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Excluding Air Parcel Charges):

Yearly Subscription (Personal & Institutional)
250 USD (B/W) & 350 USD (Color)

Editorial Board

Global Journal of Medical Research

Dr. Han-Xiang Deng

MD., Ph.D
Associate Professor and Research Department
Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical Neurosciences
Northwestern University Feinberg School of Medicine
Web: neurology.northwestern.edu/faculty/deng.html

Dr. Pina C. Sanelli

Associate Professor of Radiology
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,
School of Medicine and Biomedical Sciences
Web: weillcornell.org/pinasanelli/

Dr. Roberto Sanchez

Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University
Web: mountsinai.org/

Dr. Michael R. Rudnick

M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine
Web: uphs.upenn.edu/

Dr. Feng Feng

Boston University
Microbiology
72 East Concord Street R702
Duke University
United States of America

Dr. Seung-Yup Ku

M.D., Ph.D., Seoul National University Medical College,
Seoul, Korea Department of Obstetrics and Gynecology
Seoul National University Hospital, Seoul, Korea

Dr. Hrushikesh Aphale

MDS- Orthodontics and Dentofacial Orthopedics.
Fellow- World Federation of Orthodontist, USA.

Santhosh Kumar

Reader, Department of Periodontology,
Manipal University, Manipal

Gaurav Singhal

Master of Tropical Veterinary Sciences, currently pursuing Ph.D in Medicine

Dr. Aarti Garg

Bachelor of Dental Surgery (B.D.S.) M.D.S. in Pedodontics and Preventive Dentistr Pursuing Phd in Dentistry

Sabreena Safuan

Ph.D (Pathology) MSc (Molecular Pathology and Toxicology) BSc (Biomedicine)

Getahun Asebe

Veterinary medicine, Infectious diseases, Veterinary Public health, Animal Science

Dr. Suraj Agarwal

Bachelor of dental Surgery Master of dental Surgery in Oromaxillofacial Radiology.
Diploma in Forensic Science & Oodntology

Osama Alali

PhD in Orthodontics, Department of Orthodontics, School of Dentistry, University of Damascus. Damascus, Syria. 2013 Masters Degree in Orthodontics.

Prabudh Goel

MCh (Pediatric Surgery, Gold Medalist), FISPU, FICS-IS

Raouf Hajji

MD, Specialty Assistant Professor in Internal Medicine

Surekha Damineni

Ph.D with Post Doctoral in Cancer Genetics

Arundhati Biswas

MBBS, MS (General Surgery), FCPS, MCh, DNB (Neurosurgery)

Rui Pedro Pereira de Almeida

Ph.D Student in Health Sciences program, MSc in Quality Management in Healthcare Facilities

Dr. Sunanda Sharma

B.V.Sc.& AH, M.V.Sc (Animal Reproduction, Obstetrics & gynaecology), Ph.D.(Animal Reproduction, Obstetrics & gynaecology)

Shahanawaz SD

Master of Physiotherapy in Neurology PhD- Pursuing in Neuro Physiotherapy Master of Physiotherapy in Hospital Management

Dr. Shabana Naz Shah

PhD. in Pharmaceutical Chemistry

Vaishnavi V.K Vedam

Master of dental surgery oral pathology

Tariq Aziz

PhD Biotechnology in Progress

Contents of the Issue

Global Journal of Medical Research: a
Neurology & Nervous System
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Performing the Garcia Score for Accuracy and Reliability

By Brock R. Yager, BS, Sasha A. Kondrasov, BS, Jack Jestus, BS, Sidish Venkataraman, MD, Nicholas J. Contillo, BS, Nathan P. McMullen, MS, Stephanie A. Coffman, MD, Fatima Ryalat, MD, Zhidan Xiang, PhD, Debra I. Diz, PhD & Stacey Q. Wolfe, MD

Wake Forest University School of Medicine

Abstract- Reliable, sensitive, and accurate tests are needed to assess animal models of brain injury. The Garcia score is a neurobehavioral measure that has been used in many murine studies. However, despite its widespread use, there are no detailed video descriptions of the steps to properly perform and grade the Garcia test on rats. Consequently, there has been significant variation in its performance and reliability, calling for greater standardization. The Garcia score is comprised of six measures: spontaneous activity, symmetry in the four limbs, forepaw outstretching, climbing, body sensation, and response to vibrissae touch. Each component is scored with a minimum of zero or one and a maximum of three, with the highest total score of 18. This report systematically and clearly describes how each component of the Garcia score is performed and graded with an accompanying video illustration.

Keywords: *garcia score, behavioral, brain injury.*

GJMR-A Classification: *LCC: RC394.B7*

Strictly as per the compliance and regulations of:

Performing the Garcia Score for Accuracy and Reliability

Performing the Garcia Score

Brock R. Yager, BS [α], Sasha A. Kondrasov, BS [σ], Jack Jestus, BS [ρ], Sidish Venkataraman, MD [ω], Nicholas J. Contillo, BS [¥], Nathan P. McMullen, MS [§], Stephanie A. Coffman, MD [χ], Fatima Ryalat, MD [ν], Zhidan Xiang, PhD [Θ], Debra I. Diz, PhD [ζ] & Stacey Q. Wolfe, MD [£]

Abstract- **Reliable, sensitive, and accurate tests are needed to assess animal models of brain injury. The Garcia score is a neurobehavioral measure that has been used in many murine studies. However, despite its widespread use, there are no detailed video descriptions of the steps to properly perform and grade the Garcia test on rats. Consequently, there has been significant variation in its performance and reliability, calling for greater standardization. The Garcia score is comprised of six measures: spontaneous activity, symmetry in the four limbs, forepaw outstretching, climbing, body sensation, and response to vibrissae touch. Each component is scored with a minimum of zero or one and a maximum of three, with the highest total score of 18. This report systematically and clearly describes how each component of the Garcia score is performed and graded with an accompanying video illustration. The purpose of this report is to assist researchers in implementing the Garcia test and to help standardize the use of this measure across studies.**

Keywords: garcia score, behavioral, brain injury.

I. Introduction

Behavioral testing is a critical element of assessing brain function. Reliable, sensitive, and accurate tests are needed to assess animal models of brain injury. The Garcia score is a neurobehavioral measure that has been used in many murine studies [1]. However, despite its widespread use, there are no detailed video descriptions of the steps to properly perform and grade the Garcia test on rats. Consequently, there has been significant variation in its performance and reliability. This video-illustrated article enumerates the Garcia score's methodology in clear detail to assist researchers and help standardize its use across studies. The methodology described has been tested and validated repeatedly in clinical experiments completed by experienced technicians in our lab.

Author α: Student Researcher, Department of Neurological Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, USA. e-mail: byager@wakehealth.edu
Author σ: Student Researcher, Department of Neurological Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, USA. e-mail: skondras@wakehealth.edu
Author ω: MD, Resident, Department of Neurological Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, USA. e-mail: svenkata@wakehealth.edu
Author Θ: PhD, Research Associate, Department of Neurological Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, USA. e-mail: zxiang@wakehealth.edu
Author ζ: PhD, Professor, Hypertension and Vascular Research Center, Wake Forest University School of Medicine, Winston-Salem, NC, USA. e-mail: ddiz@wakehealth.edu
Corresponding Author £: MD, FAANS, Professor, Department of Neurological Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, USA. e-mail: sqwolfe@wakehealth.edu

II. General Overview

The Garcia score is comprised of six measures: spontaneous activity, symmetry in the four limbs, forepaw outstretching, climbing, body sensation, and response to vibrissae touch. Each component is scored with a minimum of zero or one and a maximum of three. The overall score is the sum of the scores for each component—the highest possible is 18. (Table 1) A higher score indicates that the animal is closer to baseline or "normal" behavior. A lower score indicates greater neurobehavioral deficit. The following is a detailed description of each component of the Garcia test and how it is scored. Spontaneous activity is often performed first to avoid changes from testing fatigue, but the remaining components can be completed in any order. The explanations center around a rat model, but this is also valid (and commonly used) for murine models. A video is provided to demonstrate the performance and grading of this test.
Video link: https://drive.google.com/file/d/1-UEtnk7twwyEz0_oHy5lcKGASFCdqsiV/view?usp=sharing

III. Acclimation

A period of time prior to beginning the Garcia test is necessary to allow the rats to acclimate to the rat handler and their surroundings. This involves bringing the rat into the room in which the testing takes place and letting it relax in its cage, then placing it on the testing table, and holding and interacting with it. This allows the rat to become accustomed to its handler and environment, in turn ensuring that performance on the Garcia test is not impacted by external factors that may have otherwise agitated the rat if acclimation had not been achieved. The length of the acclimation period may vary and is complete only when the rat becomes

calm, indicated by grooming itself while held by the handler and cessation of frantic or agitated movement. At this time, the rat is ready for the Garcia test.

IV. Spontaneous Activity

Place the rat in its cage for the beginning of the Garcia test, to perform spontaneous activity first (0:04 in video). The goal is to observe the rat approach all four walls of the cage within a 5-minute time span. Typically, an awake rat will do this within a few seconds. If the rat approaches all four walls or corners, it will be scored a 3. If it explores one or two walls, it will receive a 2. If it does not explore any and/or circles in place, it will receive a score of 1. A score of 0 is given to a rat that does not move.

V. Symmetry in the Four Limbs

Symmetry in the four limbs is another component of the Garcia score which measures coordination and strength in each limb without an applied stimulus (0:46). The rat is suspended by its tail and its spontaneous movements are observed for at least 30 seconds. A rat will receive a 3 if it extends and moves each of its limbs equally. This can be observed if you begin by noting a side preference and subsequently examining each limb as the rat moves. The rat will receive a 2 if one paw extends but is markedly slower or more strained than the contralateral side. If there is essentially no movement on one side with minimal twitching, the rat will receive a 1. A score of 0 is given if the rat cannot move its limbs. A helpful tip for this test is to allow proper time between assessments to accurately assess symmetry.

VI. Forepaw Outstretching

Forepaw outstretching is another component of the Garcia test which measures strength and coordination in the forepaws (1:19). The rat is suspended slightly by the tail so that the forepaws are still contacting the table, but the hind paws are not. The technician pushes the rat forward so that the rat may begin walking on their forepaws three separate times. The rat will receive a score of 3 if both forelimbs are outstretched and the rat walks symmetrically. A score of 2 is if one side outstretches less than the other, or if there is some deviation in walking. The rat will receive a 1 if a forelimb significantly lacks the ability to help the rat move forward, but still twitches to some degree. A score of 0 is given if one forelimb does not move. To ensure accuracy, an important tip is to avoid applying an unequal force in any direction so that symmetry between the forepaws can be assessed.

VII. Climbing

Climbing is another component of the Garcia test which tests strength and balance (1:53). This necessitates a grid wall. The rat is placed on the center of the grid and allowed to climb to the top while the technician holds the grid upright in such a manner that it is perpendicular to the table. This is completed for three trials. The rat will receive a 3 if it reaches the top of the grid in three trials with symmetric gripping power. It will receive a 2 if it reaches the top in three trials with asymmetric gripping power; for example, the rat may move towards the right while climbing to the top. The rat will receive a 1 if it tends to circle or move downwards instead of climbing upwards. A score of 0 is given if the rat fails to move. A helpful tip for this test is to standardize the grid wall in its position and incline because data can be skewed profoundly without consistency among serial testing.

VIII. Body Proprioception

Body proprioception is another component of the Garcia test which measures coordination and strength in each limb without an applied stimulus (2:32). It is similar to testing symmetry in the movement of limbs, but tests whether the rat will respond to an applied stimulus to each side of its lower body. If the rat responds to each side equally, it will receive a 3. It will be given a 2 if it reacts more slowly to one side. The rat will receive a 1 if it does not react to one side. It is also important for this test to allow proper time between assessments to accurately assess symmetry in response to the applied stimulus.

IX. Response to Vibrissae Touch

Vibrissae touch is the remaining component of the Garcia test which tests symmetry in motor response to a stimulus applied to the whiskers (3:00). It entails slightly elevating the rat by its tail about 1-2 inches off the table in such a way that the hindlimbs are suspended but the forelimbs are still contacting the table. Once the rat is no longer moving, the technician slowly approaches one side sweeping a probe caudally to cranially along the whiskers. This procedure is repeated on the contralateral side. A response from the rat is any blinking, startling, or turning its head ipsilateral to the stimulus. The rat will receive a score of 3 if it responds equally to the stimulus on both sides. A score of 2 is given if there is a slower response on one side. It will receive a 1 if there is no response on one side. This test is technically the most challenging, and observing the reaction can be difficult due to its subtlety. Being mindful of the positioning and ensuring that the rat is calm and facing directly opposite from the technician while still achieving an easily visualized side-profile will provide the most consistent and efficient results.

X. Conclusion

The Garcia score is a widely used, validated, multi-component measure of neurobehavior in rodent

models of cerebral injury. This article systematically and clearly explains and illustrates the proper performance and grading of this test so that researchers can implement and standardize this measure across studies.

Conflict of Interest
None

Author's Contributions
Brock Yager*

GROUP 1: Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data

GROUP 2: Drafting the work, Revising the work critically for important intellectual content

GROUP 3: Final approval of the version to be published

GROUP 4: Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Sasha Kondrasov*

GROUP 1: Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data

GROUP 2: Drafting the work, Revising the work critically for important intellectual content

GROUP 3: Final approval of the version to be published

GROUP 4: Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

*Brock Yager and Sasha Kondrasov contributed equally to the work of this study.

Sidish Venkataraman, MD

GROUP 1: Acquisition of data

GROUP 2: Revising the work critically for important intellectual content

GROUP 3: Final approval of the version to be published

GROUP 4: Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Zhidan Xiang, PhD

GROUP 1: Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data

GROUP 2: Drafting the work, Revising the work critically for important intellectual content

GROUP 3: Final approval of the version to be published

GROUP 4: Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Debra I. Diz, PhD

GROUP 1: Acquisition of data

GROUP 2: Revising the work critically for important intellectual content

GROUP 3: Final approval of the version to be published

GROUP 4: Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Stacey Q. Wolfe, MD, FAANS

GROUP 1: Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data

GROUP 2: Drafting the work, Revising the work critically for important intellectual content

GROUP 3: Final approval of the version to be published

GROUP 4: Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Acknowledgements

The authors thank Wayne Graham for his laboratory and animal expertise.

References Références Referencias

1. Garcia Julio H, Wagner Simone, Liu Kai-Feng, Hu Xiao-jiang. Neurological Deficit and Extent of Neuronal Necrosis Attributable to Middle Cerebral Artery Occlusion in Rats. Stroke 1995; 26: 627-635.

Table 1: Garcia Score Grading System – Brief description of how each component of the Garcia test is scored.

	Spontaneous Activity	Symmetry in the Four Limbs	Forepaw Outstretching	Climbing	Body Proprioception	Response to Vibrissae Touch
3	Explores all four walls/corners	Extends and moves all limbs equally	Both forelimbs outstretched, walking symmetrically	Reaches top with symmetric gripping power	Responds to each side equally	Responds to each side equally
2	Explores one or two walls	One forelimb extends but slower/ strained	One forelimb outstretches less, or some deviation in walking	Reaches top with asymmetric gripping power	Reacts more slowly to one side	Reacts more slowly to one side
1	Moves but does not explore any walls	No movement on one side, minimal twitching	One forelimb lacking movement but twitches	Circles or moves downward	Does not react to one side	Does not react to one side
0	Does not move	Does not move forelimbs	Does not move one forelimb	Fails to move	N/A	N/A

Abbreviations: none

Global Journal of Medical Research: a
Neurology & Nervous System
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

A Neural Networks and Rules based System used to Find a Correlations, and Therefore Try to Maintain the State of Health, in Patient Affect by Multiple Sclerosis at the Origins of Well-Being at a Certain Time Daily Time with Clinical and the Musculoskeletal Exams

By Prof. PhD Eng. Francesco Pia

Abstract- In this work we will explore the fact that according to recent studies published in specialist medical journals, during the early morning sleep-wake hours, during the early morning sleep-wake hours. The idea we propose is the clinical-musculoskeletal monitoring of a certain number of MS patients for twenty-four hours so that they can collect data to train a neural network with an appropriate learning algorithm suitable for the purpose. The patients will first be hypothesized as such in the present work and in the subsequent ones a virtual patient will be developed, only in the last step will real patients be used whose data will be housed in the virtual container necessary to be able to present it to the learning system.

Keywords: *Glia.*

GJMR-A Classification: *NLM: WL 140*

Strictly as per the compliance and regulations of:

A Neural Networks and Rules based System used to Find a Correlations, and Therefore Try to Maintain the State of Health, in Patient Affect by Multiple Sclerosis at the Origins of Well-Being at a Certain Time Daily Time with Clinical and the Musculoskeletal Exams

Prof. PhD Eng. Francesco Pia

Abstract- In this work we will explore the fact that according to recent studies published in specialist medical journals, during the early morning sleep-wake hours, during the early morning sleep-wake hours. The idea we propose is the clinical-musculoskeletal monitoring of a certain number of MS patients for twenty-four hours so that they can collect data to train a neural network with an appropriate learning algorithm suitable for the purpose. The patients will first be hypothesized as such in the present work and in the subsequent ones a virtual patient will be developed, only in the last step will real patients be used whose data will be housed in the virtual container necessary to be able to present it to the learning system. This learning system will be created for two main purposes: the first is to find the real connection between the state of well-being and certain hours of the day, the second is to find other correlations between clinical tests and tests that highlight the state of well-being, or less, skeletal muscle. In this work, especially in the next two, collaboration with clinical entities capable of giving the right scientific and operational support will be fundamental in order to "*transform*" the various patients into useful data for the neural system which will be described in the next work. In the following pages we would limit ourselves to describing the main idea with the pros and cons of the choices of concept and method, the tools and methods to use them for the desired purposes of the work in its widest extension and finally the expected results. It should be underlined that the work in its entirety, when achieved, can be used by any pharmaceutical companies interested in the contents, in the results achieved or achievable in order to create drugs or concrete treatment methods, if only to improve the standard of living of the patients and their families, lightening the burden that hospitals and various treatment centers are called upon to bear on a daily basis. Furthermore, two more articles are planned: the second after this with the formalization of the chosen software and various simulations with virtual patients and clinical examinations and finally the third with real and concrete patients and clinical data acquired in actual hospital clinics.

Keywords: *Glia.*

Author: *Gonnosfanadiga (SU), Italy. e-mail: piafranc@hotmail.com*
www.gofundme.com keywords "Artificial intelligence vs Multiple Sclerosis"

I. Introduction

The undersigned has always studied, as a child, and with over a dozen works in which he is the sole author, [1]-[11], and is himself suffering from a secondary progressive form of MS that is blocking him as well as many patients and families around the world. He opened a fundraiser on "*www.gofundme.com*" with keyword "*Artificial Intelligence vs Multiple Sclerosis*" and in addition to thanking you, the author will make every effort to ensure that the funds raised are spent on the next steps which are certainly expensive.

In this article we will describe a system based on NNs and filters with production rules to train a system, therefore the NN with back-propagation learning algorithm, with three layers of perceptrons so that the state of well-being can be associated with the time *[12]* and therefore find some correlation that connects the two variables.

This is the first of a series of articles that will be written as the research continues. This is the first, the second will instead be based on the description of representation algorithms of the software that will be used for recognition and therefore with a broader description of the set of patients and the type of NN. Based on the second, the third will be created where real patients will be used instead of the virtual patient encapsulator. But all of this will be linked above all to raising funds for the continuation of the project, funds that cannot be taken for granted given the size and breadth of the undertaking. It must also be said about the scheme found in the next paragraph, that is, it is very summary and schematic, this is because the problems that will be found in the second step, therefore with virtual patients and in the third with real patients, many difficulties will be encountered and many precautions will be taken to overcome them; therefore making further graphs is currently useless because the difficulties are now unclear and many, all this is done by the undersigned who has therefore himself witnessed this pathology of what is hypothesized about the timetable *[12]*.

The following must also be considered: the incidence of MS on males and females, age, weight, residence... and other known indications, let's suppose that the incidence is 2 females and 3 males, this ratio is better implicitly present in the sample to be presented to the NN during the training phase so that this parameter is also learned.

Then, let's say that the training must be done on "healthy" patients? validation on "sick" patients? And the test on "sick but not too sick" patients. A schift must be made on the inputs because they are very many so there is a risk of either over-fitting the net or not fishing well given the small number of the output numerically speaking because it is still an evoked potential, therefore there are not a thousand inputs comparable to those we would have in output, therefore it will be necessary to appropriately choose a range to evaluate the inputs especially in the first NN.

II. Methods and Tools

This paragraph will describe the main scheme of the setup that will be used, also in the next works and the present one which mainly describes the idea, and the second one which involves the use of neural networks and the drafting of an algorithm especially by virtue of the fact that the patients will be virtually encapsulated while the third will be much more challenging because the use of "real" patients and interfacing with hospital realities with the related costs will be expected.

The following diagram is a particularly "simple" example because it only represents the preliminary project that will be described, represented in the figures *fig. 1, 2*. All this is obviously simple compared to the scale of the overall project. It is believed, as was said in the introduction, that it is not very useful to describe in depth these blocks which are part of the design represented in *fig. 1* as the difficulties that will be encountered will not be few and above all the methods used to describe and create the various components will not be simple, and the type of representation and its creation that one will want to follow is unpredictable.

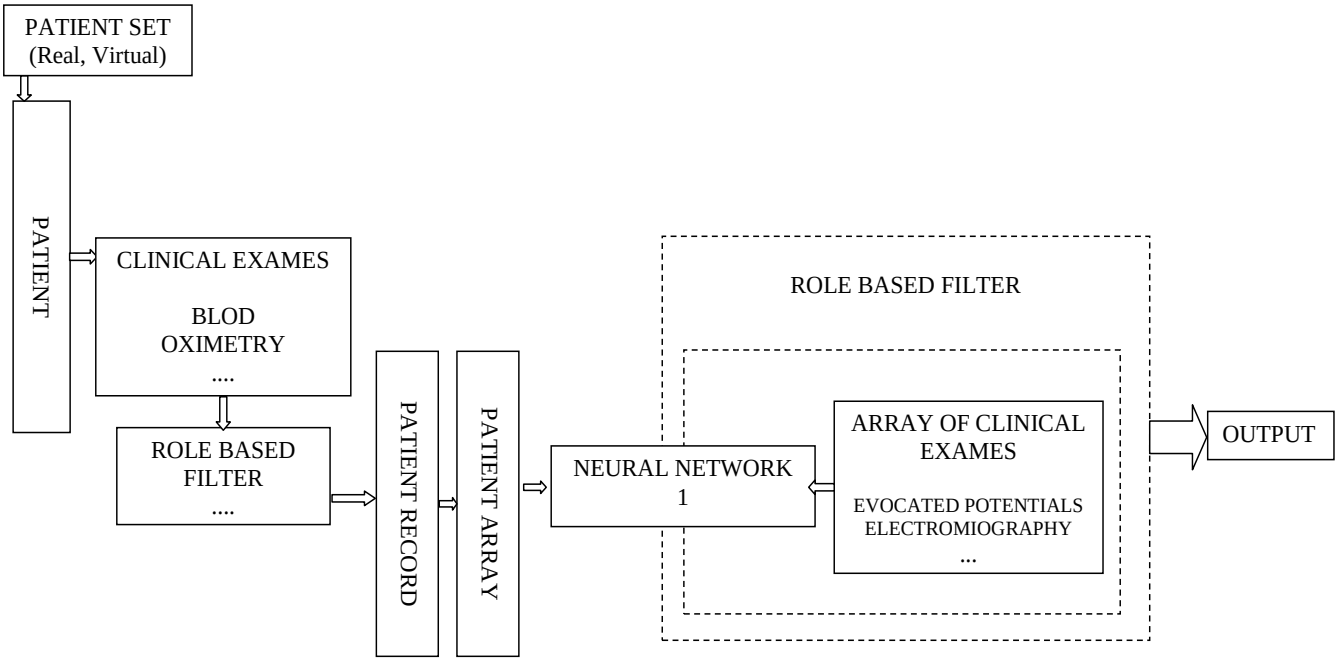


Fig. 1: This figure represents the original idea to train a neural network to distinguish an MS patient from a healthy one, as well as "memorizing" the cases seen in training.

For evoked potentials and electromyography, a filter based on output rules will be used, hoping to limit the number of inputs, and since they are numerous, it will be necessary to ensure that the NN *[12]-[44]* has a variable and selectable range for the inputs both in terms of position and as breadth; these aspects will concern the second job, not the first. Up to now we have discussed the project idea and mentioned the other two or three that will follow.

At this point the first neural network should be able to diagnose multiple sclerosis but that's just not what we would like. The following figure further highlights the potential of the diagnosis issued in figure *fig. 1*. An important factor for patient selection is the impact of MS on the male/female ratio which must be represented in the set of patients and therefore implicit in the selected sample. Training and validation should be assumed on healthy patients or testing on MS

patients. Over-fitting must be avoided thanks to a schift on the inputs and to represent the output a little widely and the inputs must be appropriately chosen with a scissor variable in amplitude and position.

The next figure *fig. 2* shows the system mentioned in the introduction, i.e. a system capable of indicating significant parameters to be indicated to the clinician.

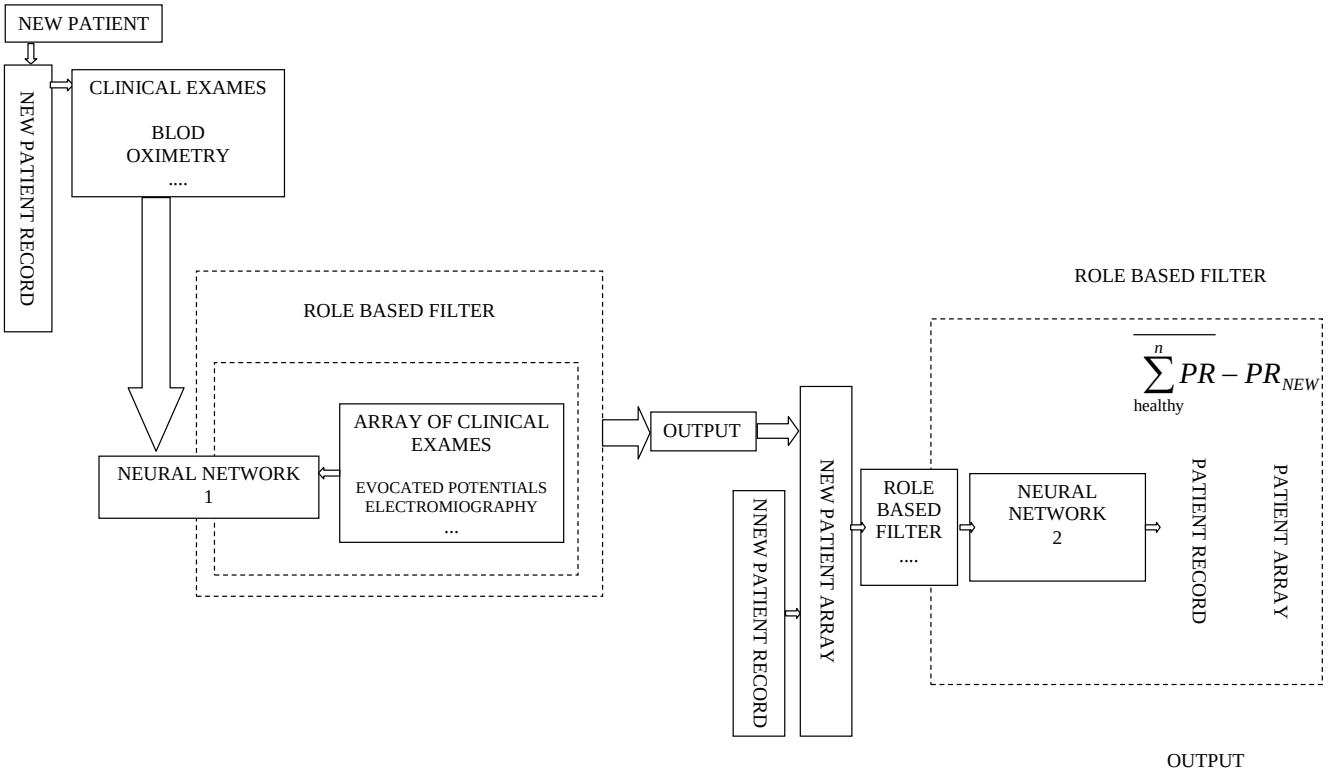


Fig. 2: This figure represents the second part of the system which could give important indications to the clinical doctor when outputs are ready.

In order to clarify better, is the training carried out on "healthy ?" patients and validation on those "sick ?" and the Test is a middle ground a little nuanced a little healthy a little sick and assuming that the system in *fig.1* is able to distinguish a healthy patient from a sick one, then where does the information reside?

The information and the result of the correct training of NN n°1 of the successful learning of the problem unknown until that moment; and up to this moment after having carried out the training: then an average is taken of the input vectors of the arrays of the patients of the healthy ones even if on the average the clue could be hidden, the truth the input of interest appears: therefore presenting a new case and at this point the network will say whether he is healthy or sick and the difference is made between the representative vector of the new case minus the average of healthy?, sick?, so we will see what are the variables in play that determine this difference between the representative vectors.

The schemes proposed in *fig. 1* and *2* should be considered a *cliché* that can also be used for other pathologies, this aspect is very important to point out.

III. Conclusion

As we intend to proceed after the pressing first step, at the end of this predominantly descriptive work on the idea of using NNs, two other steps are basically envisaged: the second will concern the IT setup of the entire set of objects relating to both the patients virtual and rule filters and also an intermediate step with the necessary simulations with a lot of work required. Once the correct functioning of the virtual patient encapsulator and the entire system has been verified, and the presence of sufficient funds has been verified, we will move on to talking about non-invasive experiments on real patients, then we will try to actually carry out the procedure that should respond, in part, to the question in the title of this work, thus giving indications to clinical doctors who are experts in the sector covered in this article.

Acknowledgment

An extraordinary thank you to all those who take care of people with physical and mental disabilities in particular a Pierpaolo eng. Furcas and family, my lovely family, Viviana S., Danilo Mallica, Maria Rita S., Alex Tomasi, MD Pierpaolo Vargiu, Alberto Urpi Caritas Diocesana di Cagliari (CA) IT.

References Références Referencias

1. Francesco Pia “I will be back”, pp. 149, Ed. Amazon® Kdp, January 2019.
2. Francesco Pia “The liar knee”, pp. 224, Ed. Amazon® Kdp, July 2019.
3. Francesco Pia “Genetic geometry”, pp. 80, Ed. Amazon® Kdp, January 2019.
4. Francesco Pia " JEWEL: a cosmological model due to the geometrical displacement of galactic object like black, white and worm holes", ICCEA 2021: 15. International Conference on Cosmology and Extragalactic Astronomy; July 19-20, 2021 in Toronto, Canada.
5. Francesco Pia "How to Try to Get Antigravity From Electrical Circuits by an Almost Magnetic Monopole or from Anti Matter" in IRJAES, Volume 7, Issue 3, Date: 25/06/2022
6. Francesco Pia "A Computer System Based On Neural Network And Rules System Based For Find An Optimum Mix of Vaccine And/Or Drugs For mRNA Like Covid-19 Virus And Other Pathologies" in IRJAES, Volume 7, Issue 2, Date, 09/06/2022
7. Francesco Pia “POLIFEMO: a Teleportation Model Based on Object Like Black, White and Worm Hole for Electric Signal and Object Also Thinking and With Conscience” ID: IRJAES-V7N3P66Y22 June-Aug 2022 Issue Vol. 7 Issue 3 https://irjaes.com/volume-7-issue-3/
8. Francesco Pia “A Symmetry of Cosmological Model Named Jewell That Allows Re Entry Into The Starting Parallel Universe With Adequate Teleportation Based on Creation of Useful Stellar Object Like Black, Withe Holes”, ICES-23-P1159, ICES2023, 6-8 February 2023, Riyadh, Saudi Arabia.
9. Francesco Pia “Neutron Concentrator, a Hypothetical Small Neutron "Star" based on the Emission of Neutrons in Minerals such as Granite and Other Commonly Available Objects” Global Journal of Researches in Engineering: E Publisher: Global Journals Civil And Structural Engineering Volume 23 Issue 1 Version 1.0 Year 2023 Type: Double Blind Peer Reviewed International Research Journal Online ISSN: 2249-4596 & Print ISSN: 0975-5861
10. Francesco Pia “A Natural Model for Dark Matter and Its Interaction with Stellar Objects such as The Galaxy Known as with Name “The Milky Way” American Journal of Biomedical Science & Research, 2023 19(5) JBSR.MS.ID.002630, DOI: 0.34297/AJBSR.2023.19.002630 Received: July 28, 2023; Published: August 07, 2023 pag. 616-621 Final PDf: PP-RW-23-0513 ISSN: 2642-1747
11. Francesco Pia “A Time-Machine, An Electro-Mechanical System That Have a Strange Behaviour Respect at Time” American Journal of Biomedical Science & Research, 2023 20(2) AJBSR.MS.ID.002681, DOI: 10.34297/AJBSR.2023.20.002681 Received: September 16, 2023; Published: September 21, 2023, pag. 122-126 Final PDf: PP-RW-23-0513 ISSN: 2642-1747
12. Luisa de Vivo, Michele Bellesi "The role of sleep and wakefulness in myelin plasticity" First published: 25 June 2019 https://doi.org/10.1002/glia.23667 GLIA WILEY
13. I. D’Antone “Intelligenza artificiale e diagnostica in sistemi complessi”, in “L’ipercomplessità tra socio-sistemica e cibernetica. A cura di A. Ardigò, G. Mazzoli”, pp.339-347, Ed. Franco Angeli, 1990.
14. A. Fanni, P. Zedda, F. Scintu “Artificial Neural Networks Approach for Anorectal Manometry Morphological Classification”; “A.I.I.M.B: Associazione Italiana di Ingegneria Medica e Biologica”, in “Proceedings of the HEALTH TELEMATICS ’95, ‘Health in the Information Society’, Luglio 1995; Ed. “M. Bracale and F. Denoth”, Area di Ricerca -CNR- Pisa.
15. P. K. Sharpe, H. E. Solberg, K. Rootwelt, M. Yearworth “Artificial Neural Networks in Diagnosis of Thyroid Function from in Vitro Laboratory”, in Clinical Chemistry, Vol. 39, n. 11, pp. 2248-2253, 1993.
16. K. Rootwelt, HE. Solberg “Free thyroxine, thyroxine/TBG ratio and other in vitro tests of thyroid function evaluated by discriminant analysis”, Scand J Clin Lab Inves 1981, pp. 41-483, 1981.
17. T. Kohonen, “Self-Organizing formation of topologically correct feature map”, Biological Cybernetics, n. 43, 1982.
18. P. Karakitsos, B. Cochand-Priollet, P. J. Cuillausseau, A. Pouliakis “Potential of the Back Propagation Neural Network in the Morphologic Examination of Thyroid Lesion”, in “Analytical and Quantitative Cytology and Histology”, vol. 18 No. 6, pp. 494-500, December 1996.
19. E. R. Carson, D. G. Cramp “The patient/clinical relationship, computing and the wider health care system”, in ‘Computer and Control in Clinical Medicine’, ed. E. R. Carson, D. G. Cramp, New York: Plenum, pp. 245-257, 1985.
20. S. El Gamal, M. Rafeh e I. Eissa “Case-based reasoning algorithms applied in a medical acquisition tool”, in ‘Medical Information’, vol. 18, no. 2, pp. 149-162, 1993.
21. S. El Gamal, M. Rafeh e E. F. Eissa “Knowledge acquisition system for clinical medicine. First national ezpert Systems and development Workshop”, tenutosi il 26-28 febbraio, grazie al Ministero dell’Agricoltura, Cairo, Egitto, 1991.
22. D. Wakerman “A Guide to Expert System”, MA: Addison Wesley, Cambridge 1985.
23. J. I. Nicolass, L. M. Perry “Tools for Knowledge Acquisition and Verfication in Medicine”, IEEE, 1986

24. F. Pia, A. Casanova, S. Mariotti “Diagnosi on line remota dell’ipertiroidismo”, 4th International Conference and Exhibition on Telemedicine, Information Technology and Telecommunications in Healthcare. 26th-28th September 2001, Genoa (Italy)
25. S. J. Russel, P. Norvig “Artificial Intelligence, A Modern Approach”, Prentice-Hall. Inc., 1995.
26. B. G. Buchanan, E. A. Feigenbaum “Dendral and Metadendral: their applications dimension”, in “Artificial Intelligence”, vol. 11, 1978.
27. G. Bara “Intelligenza artificiale”, Franco Angeli Editore, 1978.
28. R. Shank “Conceptual Information Processing”, in Fundamental Studies in Computer scienze, vol. 3, Amsterdam 1975.
29. Y. Jang “HYDY: a Hybrid system with Feedbach for Diagnosis Multiple Disorders”, M.I.T., 1993.
30. A. Casanova, P. Carta, A. Balestrieri, G. Bellu “Un sistema esperto per il supporto alla Diagnosi delle Broncopneumatopatie Professionali” Atti del Convegno AICA 1989, Vol. 2.
31. T. Khana “Fondamenti di reti neurali”, Addison-Wesley, 1991.
32. G. Rindi, E. Manni “Fisiologia Umana”, vol. 1, cap. II,III pag.9-52, cap.24 pag. 449-459, cap. 27 pag. 496-508, IV ed., Utet 1987.
33. R. Beale, T. Jackson “Neural Computing: An Introduction”, Departement of Computer Scienze, University of York, Institute of Physics Publishing Bristol and Philadelphia, 1992.
34. Robert F. Schmidt con i contributi di Josef Dudel, Wilfrid Jänig, Manfred Zimmerman “Fondamenti di Neurofisiologia” ed. Zanichelli , 1990.
35. D. Floreano “Manuale sulle reti neurali”, Il Mulino, 1996.
36. A. L. Hodgkin, A.F. Huxley “A quantitative Description of Membrane Current and its Application to Conduction and Excitation in Nerve”, in ‘Jouranal of Physiology (London)’, vol. 108, pp 500-540, 1952.
37. J. Hertz, A. Krogh e R.G. Palmer “Introduction to the Theory of Neural Computing”, Reedwood City, CA, Addison-Wesely, 1991.
38. D. E. Rumelhart, G. E. Hinton e J. R. Williams “ Learning Representations by Back-Propagation of Errors” in ‘Nature’, vol. 323, pp. 533-536, 1986.
39. H. Demuth, M. Beal “Neural Network Toolbox, for Use with Matlab®”, The Math Works Inc., january 1994.
40. D. Jones “Neural Networks for Medical Diagnosis, in Handbook of Neural Computing Applications”, a cura di A. J. Maren, C.T. Hartson e R. M. Pap, London, Academic Press, 1990.
41. C. Stergiou, D. Siganos “Neural Networks”, sito Internet (http://www-dse.doc.ic.ac.uk/~nd/surprise_96/ journal/vol4/cs11/report.html).
42. Ugo Teodori “Trattato di medicina interna” vol. 2 cap. 31 S. Mariotti L.Baschieri-E.Martino-G. Fenzi-L.Bartalena “Malattie della tiroide” pag. 1449-1512.
43. G. Delfino, E. Lanciotti, G. Liguori, M. Stefani “Biologia e medicina, dizionario enciclopedico di scienze biologiche e mediche, IT-GB, GB-IT”, Zanichelli, 1990.
44. E. Rubenstein “Malattie causate da diffettosa comunicazione tra cellule”, in “Le Scienze” n° 141, maggio 1980.

This page is intentionally left blank

Global Journal of Medical Research: a
Neurology & Nervous System
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Modifications and Optimization of an Autologous Intracerebral Hemorrhage Rat Model

By Brock R. Yager BS, Sasha A. Kondrasov BS, Jack Jestus BS, Sidish Venkataraman MD, Nicholas J. Contillo BS, Nathan P. McMullen MS, Stephanie A. Coffman MD, Fatima Ryalat MD PhD, Zhidan Xiang PhD, Debra I. Diz PhD & Stacey Q. Wolfe MD

Wake Forest University School of Medicine

Abstract- *Objective:* To date, no novel drug therapies have proven effective in improving outcomes after intracerebral hemorrhage (ICH). This has been slowed, in part, by a lack of reliable preclinical models that recapitulate the pathophysiological consequences of ICH. Limited descriptions of the methodology of preclinical models may play a role in this failed translation. The current study aimed to improve the autologous blood injection model of ICH and provide a comprehensive methodology to facilitate a reliable preclinical model that bridges the translational research gap.

Methods: A modified single-injection protocol for an autologous-blood ICH model was developed and tested. This rodent ICH model produces injury in the basal ganglia of adult rats via a stereotaxically-assisted right-sided single-injection of fresh autologous whole blood.

Keywords: *intracerebral hemorrhage, hypertension, cardiometabolic syndrome, autologous ICH model, mREN2-27 transgenic rat.*

GJMR-A Classification: *FOR Code: 1109*

Modifications and Optimization of an Autologous Intracerebral Hemorrhage Rat Model

Optimization of an Autologous Rat ICH Model

Brock R. Yager BS [α], Sasha A. Kondrasov BS [σ], Jack Jestus BS [ρ], Sidish Venkataraman MD [ω], Nicholas J. Contillo BS [¥], Nathan P. McMullen MS [§], Stephanie A. Coffman MD [χ], Fatima Ryalat MD PhD [ν], Zhidan Xiang PhD [Θ], Debra I. Diz PhD [ζ] & Stacey Q. Wolfe MD [£]

Abstract- ***Objective:*** To date, no novel drug therapies have proven effective in improving outcomes after intracerebral hemorrhage (ICH). This has been slowed, in part, by a lack of reliable preclinical models that recapitulate the pathophysiological consequences of ICH. Limited descriptions of the methodology of preclinical models may play a role in this failed translation. The current study aimed to improve the autologous blood injection model of ICH and provide a comprehensive methodology to facilitate a reliable preclinical model that bridges the translational research gap.

Methods: A modified single-injection protocol for an autologous-blood ICH model was developed and tested. This rodent ICH model produces injury in the basal ganglia of adult rats via a stereotaxically-assisted right-sided single-injection of fresh autologous whole blood. A comprehensive step-wise description of the methodology is presented and documented in a written and visual format with supporting pictorial material, to prevent common mistakes that result in failed hematoma formation. Rates of successful hematoma formation using the single-injection protocol, outlined here, were compared to those using the traditional double-injection protocol.

Results: Successful hematoma formation was observed in 48 of 52 animals (92.3% success rate) using the single-injection protocol, compared to 19 of 40 animals (47.5% success rate) using a modified double-injection protocol. ($\chi^2 = 22.94$, $p < 0.00001$). This was replicated in both male and female Sprague Dawley and (mRen2) 27 rats that exhibit cardiometabolic dysfunction, confirmed with hemoglobin assay.

Conclusions: The current report highlights a detailed description of the protocol and outlines pitfall avoidance learned throughout its development so that laboratories worldwide can use this technique while minimizing waste in research time and money.

Keywords: intracerebral hemorrhage, hypertension, cardiometabolic syndrome, autologous ICH model, mREN2-27 transgenic rat.

Author α σ ρ ω ¥ § χ: Department of Neurological Surgery, Wake Forest School of Medicine, Winston Salem, NC.
Author ν Θ: Department of Physiology, University of Jordan, Amman, Jordan.
Author ζ: Department of Surgery/Hypertension and Vascular Research, and the Cardiovascular Sciences Center, Wake Forest University School of Medicine, Winston Salem, NC.
Corresponding Author £: Department of Neurological Surgery, Wake Forest School of Medicine, Winston Salem, NC.
e-mail: sqwolfe@wakehealth.edu

Abbreviations:

Intracerebral hemorrhage (ICH)

Sprague Dawley (SD)

Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)

Personal protective equipment (PPE)

Central nervous system (CNS)

I. Introduction

Intracerebral hemorrhage (ICH) accounts for approximately 20% of all strokes, and outcomes are often catastrophic, resulting in 30-day mortality up to 40%[1] and 6-month functional independence of only 20%.[2] In addition to direct tissue damage at the time of the initial hemorrhagic event, there is profound secondary injury as the body responds to the presence of the hematoma, including cytotoxic and excitotoxic factors, oxidative stress, ferroptosis, and inflammatory pathways.[3,4] While there has been some preclinical success in identifying therapeutic targets for secondary injury, no clinical studies have demonstrated success with any trialed therapies to date; in part due to lack of preclinical models that reliably recapitulate the comorbidities seen in patients with ICH.[5,6,7]

Multiple preclinical ICH models have been developed over time, including cortical vessel avulsion,[8] microballoon,[9] bacterial collagenase[10] and several variations of an autologous blood injection,[11-14] each with distinct advantages and shortcomings. A comparative study of the cortical vessel avulsion model, the bacterial collagenase injection model, and an autologous blood injection model showed that while the models demonstrate similar temporal patterns of injury, differences in cell death, inflammatory cell infiltration, and microglial reaction showed autologous blood injection to most closely mimic spontaneous human ICH.[15] Cortical vessel avulsion demonstrated a mixed pathology of ischemia and hemorrhage, whereas, bacterial collagenase elicited a more intense and prolonged inflammatory reaction.[15]

Various modifications of a single-injection technique include blood injection via a permanently implanted needle into the basal ganglia,[11]bolus injection

through a stereotaxically-placed intracerebral needle,[16] direct circuit from an intra-arterial femoral catheter to simulate hemorrhage induced at arterial pressure,[12] and utilization of a microinfusion pump to deliver a consistent volume of blood at a specified rate.[13] Each model aimed to improve reliability and to better simulate human pathology. However, techniques based on a single continuous injection often face issues with retrograde egress of blood along the needle tract, inconsistent and insufficient hematoma volume, subarachnoid and subdural extension of blood, and overall meager reproducibility.[17,18]

To address the shortcomings of the single-injection technique, a double-injection modification was devised to allow for the formation of a "clot barrier" with time between injections, thus facilitating a more reproducible and stable hematoma during the second injection.[14] This model as initially described was performed with a stereotaxically directed catheter permanently placed into the basal ganglia with fresh autologous blood from a femoral artery cutdown and cannulation.[14] Femoral artery sampling does allow for fresh blood to be delivered, but requires an extended duration of anesthesia, which may confound ICH outcomes[20] and impairs post-surgical behavioral evaluation[21] critical to ICH studies.[22]

Our lab focuses on translational study of ICH with emphasis on biologic variables such as sex, age, and common ICH comorbidities like hypertension, insulin resistance and obesity. To accomplish these goals, we tested the modified double-injection autologous blood method against an optimized single-injection technique[24] in order to produce a reliable and cost-effective model in rats that best recapitulates human pathology in ICH. We present here our troubleshooting efforts and a granular, stepwise methodology that is not present in the current literature. This knowledge avoids a steep learning curve requiring significant time, expense, and animal expenditure.

II. Material and Methods

All procedures were conducted in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine (protocol number A19-089). Matched Hanover Sprague-Dawley (SD) normotensive rats and hypertensive (mREN2)27 rats with cardiometabolic syndrome to recapitulate human disease[23] ages 12-55 weeks, both male and female, were used for the development of this model. Rats were randomly selected into the double-injection model (n=10 per group (male SD, female SD, male (mREN2)27, female (mREN2)27): n=40 total) or the optimized single-injection model (n=13 per group (male SD, female SD, male (mREN2)27, female (mREN2)27): n=52 total). Ages were matched to account for the biologic variable of age.

Same sex litter mates were housed together in pairs in ventilated cages and maintained on a regular diurnal light cycle (12:12 light:dark) with ad libitum access to food and water. All animals were housed in the Animal Resources Program facilities at Wake Forest Baptist Health, accredited by AAALAC (The Association for Assessment and Accreditation of Laboratory Animal Care International). Animals underwent either the double injection method[14] or the optimized single-injection model described below. Following the procedure, animals underwent behavioral testing (Garcia score and Corner Turn) at 1 day and 3 days and were sacrificed at 72 hours. Brains were perfused with PBS, and serial cutting or hemoglobin assay was performed to evaluate hematoma location and volume.

a) Stepwise Optimized Single-Injection Methodology

Pre-Surgical Phase

1.1 Weigh the rat to calculate medication doses.

1.2 Transfer the rat to the anesthesia induction chamber and initiate the induction procedure with an oxygen flow rate equivalent to the volume of the chamber, approximately 1-2 L/min, and up to 3% MAC vaporized isoflurane until the rat is completely immobilized and has lost its righting reflex.

1.3 Remove rat from the induction chamber and transfer to the stereotaxic apparatus. Position the rat prone on a heating pad at the base of the stereotaxic frame and place the rat's nose into a gas adaptor stereotaxic nose cone for maintenance anesthesia.

1.4 Reduce the anesthetic to 1.5% MAC and ensure that the circuit is open to the stereotaxic nose cone adaptor. Rats weighing over 400g may require up to 2.5% MAC maintenance anesthesia.

1.5 Apply ophthalmic lubricating ointment for corneal protection.

1.6 Position the rat with all midline structures in alignment; forepaws directed anteriorly, and hindpaws directed posteriorly. The stereotaxic nose cone should be adjusted to ensure the nose, head, and spine are in line and parallel to the floor without angulation (Figure 1). The stereotaxic machine may be used to ensure that the bregma and lambda are in the same plane. Gauze may be used to support the head as needed. Ensure the nose fits snugly into the chamber. This is critical as movement throughout the procedure due to inadequate maintenance anesthesia may displace the stereotaxic apparatus and lead to poorly formed hematomas.

1.7 Place a protective earpiece to prevent otic damage with the headband wire directed posteriorly.

1.8 Ensure the blink reflex is absent and affix the head in the stereotaxic apparatus such that it is centered

in the sagittal, coronal, and axial planes. Confirm that the head is secure before proceeding.

1.9 Apply heat to the tail to dilate the tail veins. This may be achieved by placing the tail under a nitrile or latex glove filled with water and heated in a water bath at 40°C, not to exceed 43°C.

1.10 Shave the surgical site with clippers and use tape to clear the site of hair. Disinfect the surgical site with povidone-iodine solution or equivalent and use appropriate drapes for aseptic technique. At this point we aseptically inject weight appropriate SQ buprenorphine 0.01 mg/kg for post-operative pain control.

1.11 Program the automated syringe pump (we use Harvard Apparatus Model 11 Elite Syringe Pump; Holliston, MA) with an injection rate of 10 µL/min, injection volume of 100 µL, and syringe volume of 1 mL.

1.12 Attach the shortest possible segment of PE20 polyethylene catheter tubing (PE20; I.D. 0.38 mm, O.D. 1.09 mm; Intramedic Clay Adams; Sparks, MD) to a 1 mL syringe with a blunt 26-gauge needle. Flush with non-heparinized saline and ensure an uninterrupted stream with minimal resistance to light finger pressure. If there is resistance, ensure that the needle is not occluded by pieces of plastic tubing, which can be avoided by pulling the needle back in the tubing. Using another 26-gauge needle, pre-dilate the distal end of the tubing to facilitate attachment to the Hamilton needle.

1.13 Prepare a second set of flushed and pre-dilated tubing to use as a back-up, in case clotting or occlusion of the tubing occurs during the injection.

Surgical Phase

1.14 Make a 2 cm midline scalp incision starting at the posterior aspect of the eyes and extending caudally to the lambda. Incise the periosteum laterally and locate the bregma, which often appears V-shaped (Figure 2).

1.15 Mobilize the periosteum laterally, until it cannot be mobilized any further. The burr hole will be at the junction pf the posterior-most aspect of the bregma intersecting where the periosteum remains attached at is lateral aspect, approximately 4.5mm in females and 5mm male rats. (Figure 2). Use a circular motion with a 1 mm burr drill (75,000 rpm) to make the smallest burr hole that will accommodate the needle. The depth of the skull is approximately 1-1.5 mm deep at this location. We then place sterile bone wax to seal the hole and prevent blood egress.

1.16 Affix a Hamilton needle (26 gauge with hub; 50.8 mm length; #7784-08; Reno, NV) to the stereotaxic frame and insert the stylet to maintain patency of the needle during stereotaxic placement. Set the needle at a 20-degree angle along the coronal plane to the right of midline. Do not attach the needle too low on the stereotaxic frame as the needle will collide with the animal when positioning. With the stylet in place, carefully advance the needle through the center of the burr hole 6mm in males and 5.5mm in females deep to the surface of the skull. Note: The stereotaxic placement of the Hamilton needle into the striatum is completed prior to the collection of venous blood.

1.17 Using aseptic technique, disinfect the distal tail of the rat with alcohol and use sharp scissors to snip the distal-most 0.5-1 mm of the tail. Collect 0.2 mL of venous blood directly into a needleless 1 mL syringe. Note: after tail snip, it may be necessary for one lab technician to gently massage the tail from proximal to distal while another collects blood into the upright distal tip of the syringe. Avoid contacting the syringe with the epithelium of the tail. Remove any air bubbles that form in the syringe during blood collection, as air will induce clotting. A tail vein blood draw is another viable option (22-gauge needle or smaller), but we found this to prolong overall surgical time.

1.18 Connect the syringe containing fresh autologous blood to the flushed tubing with fluid-to-fluid interface, avoiding any air bubbles. Advance blood to the end of the tubing. Affix the syringe with freshly collected blood to the automated pump and P20 polyethylene catheter tubing connected to the 26 gauge Hamilton needle flushed with saline. Note: air bubbles promote hematogenous clotting and must be avoided.

1.19 Withdraw the Hamilton needle 0.5 mm to reach a final depth of 5.5mm in males and 5mm in females, creating a potential space for hematoma formation.

1.20 Remove the stylet from the stereotaxically placed Hamilton needle and connect the pre-dilated P20 tubing. A drop of saline may be needed to lubricate the needle and facilitate attaching the blood-filled tubing.

1.21 Perform a small bolus injection to get the blood to the end of the needle (one tap of the advance arrow button).

1.22 Begin the injection of 100 µL at 10 µL/min. ***Note: To ensure successful injection, note the starting volume of blood in the syringe and monitor for progression at a rate of 10 µL/min. Inspect the site of the burr hole periodically to monitor for egress of blood. If impeded flow through the tubing is suspected, manually advance the microinfusion pump to dislodge any early clot formation within the tubing. If this maneuver is unsuccessful, discard the occluded tubing and connect the back-up tubing to the apparatus. Adjust the microinfusion pump settings to account for the lost blood volume and resume the injection. Note the new starting volume

upon infusion resumption and continue to monitor for signs of injection failure. As this protocol carries a very low risk of intraventricular hematoma extrusion, we advise repeating portions of the injection ad libitum in the event of suspected injection failure.

1.23 After the injection has completed, allow 5 minutes for consolidation of the hematoma. Then, reinsert the stylet, pull back the needle 2mm and wait 2 minutes, then we remove the Hamilton needle slowly over the course of 1 minute to prevent reflux of injected blood along the needle tract.

1.24 Reapproximate the scalp edges and suture the incision. When suturing is complete, turn off the anesthetic, remove the rat from the stereotaxic apparatus, and transfer the rat from the stereotaxic apparatus to a heated recovery chamber for observation.

1.25 Flush the Hamilton syringe needle with saline between each animal and clean with 70% ethanol. It is recommended to use new tubing on every animal to prevent clotting. Do not reuse the syringe following injection, this creates a high risk of clotting.

Total procedural duration including total time under anesthesia is approximately 30-40 minutes.

III. Results

Of the 40 animals subjected to the double-injection method, successful hematoma formation was observed in only 19 animals, yielding a 47.5% success rate. Of the 21 unsuccessful procedure attempts, 13 failed due to lack of hematoma/insufficient hematoma volume, 1 due to intraventricular extension, and 7 had visible blood egress along the needle tract. Due to the unacceptably high failure rate of the double-injection protocol, the modified single-injection method was adopted and implemented in a total of 52 animals. Successful hematoma formation was observed in 48 animals, yielding a 92.3% success rate, which was significantly greater than that observed with the double-injection method ($\chi 2 = 22.94$, $p < 0.00001$). Of the 4 unsuccessful procedure attempts, 3 were deemed unsuccessful due to lack of hematoma/insufficient hematoma volume, 0 due to intraventricular extension, and 1 due to blood egress along the needle tract. Brain slices were imaged and characterized (Figure 3). Brain homogenization was performed on 14 animals with 7mRENs and 7 SDs. Hemispheric hemoglobin concentrations collected at 72 hours after ICH for SDs were 0.138 with a standard deviation of 0.018 and for, mRENs were 0.168 with a standard deviation 0.022 (Figure 4). Behavioral testing demonstrated both Garcia scores at 1-day ($p<.001$) and 3-day ($p=.023$) and Corner Turn1-day ($p=.017$) and 3-day ($p=.009$) post-ICH were significantly different from baseline.

IV. Discussion

We developed a protocol to address shortcomings in existing ICH models: an autologous, single-injection rat model that reliably creates consistent basal ganglia ICH and allows for assessment of behavioral, physiologic, and histologic outcomes in a transgenic rat model to mimic human disease. This 100uL venous injection can be performed in 30 minutes per animal by new and emerging laboratories with relatively low costs. As with previous studies, we initially encountered complications necessitating method modification to achieve reproducibility. Through a discussion of these complications and our means of overcoming them, we hope to remove barriers encountered by new researchers in order to further the field of ICH research.

For the collection of autologous blood, we found that the tail snip was the most efficient method, minimizing the duration of anesthesia, reducing the amount of handling between collection and injection, and reliably performed by all lab members. Further, it can be repeated as needed during the procedure without additional tail cutting and does not impair post-ICH functional evaluation. Early application of heat through a glove filled with warmed water improves the ease of collection.

The presence of more than one lab member to allow tasks to be completed simultaneously, including collecting the blood, flushing and connecting the tubing, and programming the pump was an additional means to minimize the opportunity for clotting. Most importantly, we found that transitioning to a continuous injection model helped circumvent the above complication, as the injection is completed in a single continuous infusion and provided more reliable results.

To overcome the concern for reflux along the needle tract described in previous studies, we found that the burr hole must be no larger than 1.5mm, initial stereotaxic placement of the Hamilton needle to a depth of 6 mm (male) and subsequent retraction by 0.5 mm to reach a final depth of 5.5 mm created a potential space for hematoma formation, minimizing reflux along the needle.

One complication encountered, as noted above, was clot formation within the blood collection vial and the injection tubing. Minimizing the amount of handling and the amount of time elapsed between blood acquisition and blood injection is imperative to prevent clot formation, regardless of the method being used. Ensuring that there is no air in the syringe or tubing is essential to prevent clotting. It is also critical to visually monitor the blood delivery throughout the injection period. By noting the starting volume of the syringe and ensuring that it is advancing appropriately through the infusion period, we prevented episodes where the appropriate volume of blood had inadvertently

not been injected. A simple bump forward with the pump if blood infused is slower than pump measurements usually dislodges any forming clot and infusion can continue uninterrupted. Having a spare flushed tubing prepared to replace any clotted tubing, as well as having extra saline for flushing the tubing, is critical. Again, as this model carries a low chance of hematoma rupture into the ventricle, and we have found that if in doubt that the full 100 µL was infused into the brain parenchyma, it is better to re-inject that volume. Failure to do this was the most common reason for failed ICH creation at the beginning of our model development.

V. Conclusions

This is the first paper that clearly details an autologous single-injection ICH model in rats with >90% rate of reproducibility. This basal ganglion ICH model results in accompanying neurobehavioral deficits on both the Garcia and corner turn testing. Importantly, we outline the pitfalls and proposed means of overcoming such complications in using this model. We hope for this report to facilitate other labs in reproducing the model effectively and efficiently and ultimately lower the expense of developing a translational animal model of ICH. This model is a form of pragmatic science that aims to recapitulate human disease, allowing for identifying possible biomarkers and potential targets for therapeutic interventions in a disease that causes significant morbidity and mortality.

Acknowledgements

This work was supported through a pilot grant from the Wake Forest Cardiovascular Sciences Center, funded in partby the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health, through Grant Award Number UL1TR001420. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Additional funds were provided by the Farley Hudson Foundation and the Hypertension & Vascular Research Center.

The authors report no conflicts of interest that could interfere with the integrity of this science.
No part of this work has been previously published.

References Références Referencias

1. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *Lancet Neurol*. 2010; 9(2): 167-176. doi: 10.1016/S1474-4422(09)70340-0
2. Fogelholm R, Murros K, Rissanen A, Avikainen S. Long term survival after primary intracerebral haemorrhage: a retrospective population based study. *J Neurol Neurosurg Psychiatry*. 2005; 76(11): 1534-1538. doi: 10.1136/jnnp.2004.055145
3. Zhu H, Wang Z, Yu J, et al. Role and mechanisms of cytokines in the secondary brain injury after intracerebral hemorrhage. *Prog Neurobiol*. 2019; 178: 101610. doi: 10.1016/j.pneurobio.2019.03.003
4. Belur PK, Chang JJ, He S, Emanuel BA, Mack WJ. Emerging experimental therapies for intracerebral hemorrhage: targeting mechanisms of secondary brain injury. *Neurosurg Focus*. 2013; 34(5): E9. doi: 10.3171/2013.2.FOCUS1317
5. Selim M, Foster LD, Moy CS, Xi G, Hill MD, Morgenstern LB, Greenberg SM, James ML, Singh V, Clark WM, Norton C, Palesch YY, Yeatts SD; i-DEF Investigators. Deferoxamine mesylate in patients with intracerebral haemorrhage (i-DEF): a multicentre, randomised, placebo-controlled, double-blind phase 2 trial. *Lancet Neurol.* 2019; 18(5): 428-438. doi: 10.1016/S1474-4422(19)30069-9.
6. Gladstone DJ, Aviv RI, Demchuk AM, Hill MD, Thorpe KE, Khoury JC, Sucharew HJ, Al-Ajlan F, Butcher K, Dowlatshahi D, Gubitz G, De Masi S, Hall J, Gregg D, Mamdani M, Shamy M, Swartz RH, Del Campo CM, Cucchiara B, Panagos P, Goldstein JN, Carrozzella J, Jauch EC, Broderick JP, Flaherty ML; SPOTLIGHT and STOP-IT Investigators and Coordinators. Effect of Recombinant Activated Coagulation Factor VII on Hemorrhage Expansion Among Patients With Spot Sign-Positive Acute Intracerebral Hemorrhage: The SPOTLIGHT and STOP-IT Randomized Clinical Trials. *JAMA Neurol*. 2019; 76(12): 1493-1501. doi: 10.1001/jamaneurol.2019.2636.
7. Sprigg N, Flaherty K, Appleton JP, Al-Shahi Salman R, Bereczki D, Beridze M, Christensen H, Ciccone A, Collins R, Czlonkowska A, Dineen RA, Duley L, Egea-Guerrero JJ, England TJ, Krishnan K, Laska AC, Law ZK, Ozturk S, Pocock SJ, Roberts I, Robinson TG, Roffe C, Seiffge D, Scutt P, Thanabalan J, Werring D, Whynes D, Bath PM; TICH-2 Investigators. Tranexamic acid for hyperacute primary IntraCerebralHaemorrhage (TICH-2): an international randomised, placebo-controlled, phase 3 superiority trial. *Lancet.* 2018; 26(391): 2107-2115. doi: 10.1016/S0140-6736(18)31033-X.
8. Funnell WR, Maysinger D, Cuello AC. Three-dimensional reconstruction and quantitative evaluation of devascularizing cortical lesions in the rat. *J Neurosci Methods*. 1990; 35(2): 147-156. doi:10.1016/0165-0270(90)90104-n
9. Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: effects of a temporary mass lesion. *J Neurosurg*. 1987; 66(4): 568-576. doi:10.3171/jns.1987.66.4.0568

10. Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990; 21(5): 801-807. doi:10.1161/01.str.21.5.801
11. Ropper AH, Zervas NT. Cerebral blood flow after experimental basal ganglia hemorrhage. *Ann Neurol*. 1982; 11(3): 266-271. doi: 10.1002/ana.410110306
12. Bullock R, Mendelow AD, Teasdale GM, Graham DI. Intracranial haemorrhage induced at arterial pressure in the rat. Part 1: Description of technique, ICP changes and neuropathological findings. *Neurol Res*. 1984; 6(4): 184-188. doi: 10.1080/01616412.1984.11739687
13. Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT. Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. *J Neurosurg*. 1994; 81(1): 93-102. doi:10.3171/jns.1994.81.1.0093
14. Deinsberger W, Vogel J, Kuschinsky W, Auer LM, Böker DK. Experimental intracerebral hemorrhage: description of a double injection model in rats. *Neurol Res*. 1996; 18(5): 475-477. doi:10.1080/01616412.1996.11740456
15. Xue M, Del Bigio MR. Comparison of brain cell death and inflammatory reaction in three models of intracerebral hemorrhage in adult rats. *J Stroke Cerebrovasc Dis Off J Natl Stroke Assoc*. 2003; 12(3): 152-159. doi: 10.1016/S1052-3057(03)00036-3
16. Masuda T, Dohrmann GJ, Kwaan HC, Erickson RK, Wollman RL. Fibrinolytic activity in experimental intracerebral hematoma. *J Neurosurg*. 1988; 68(2): 274-278. doi:10.3171/jns.1988.68.2.0274
17. Manaenko A, Chen H, Zhang JH, Tang J. Comparison of different preclinical models of intracerebral hemorrhage. *Acta Neurochir Suppl*. 2011; 111: 9-14. doi:10.1007/978-3-7091-0693-8_2
18. Ma Q, Khatibi NH, Chen H, Tang J, Zhang JH. History of preclinical models of intracerebral hemorrhage. *Acta Neurochir Suppl*. 2011; 111: 3-8. doi:10.1007/978-3-7091-0693-8_1
19. Brodbelt D. Perioperative mortality in small animal anaesthesia. *Vet J LondEngl 1997*. 2009; 182(2): 152-161. doi:10.1016/j.tvjl.2008.06.011
20. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020; 1728: 146593. doi:10.1016/j.brainres.2019.146593
21. Ohayon S, Gruenbaum SE, Artru AA, et al. Anatomical location of arterial and venous lines significantly affects motor performance in rats. *Anim Sci J Nihon ChikusanGakkaiho*. 2012; 83(9): 656-662. doi:10.1111/j.1740-0929.2012.01008.x
22. Hua Y, Schallert T, Keep RF, Wu J, Hoff JT, Xi G. Behavioral tests after intracerebral hemorrhage in the rat. *Stroke*. 2002; 33(10): 2478-2484. doi:10.1161/01.str.0000032302.91894.0f
23. Langheinrich M, Lee MA, Böhm M, Pinto YM, Ganten D, Paul M. The hypertensive Ren-2 transgenic rat TGR (mREN2)27 in hypertension research. Characteristics and functional aspects. *Am J Hypertens*. 1996; 9(5): 506-512. doi:10.1016/0895-7061(95)00400-9
24. O'Lynnger T, Hua Y, Xi G. Blood Injection Intracerebral Hemorrhage Rat Model. In: Chen J, Xu ZC, Xu X-M, Zhang JH, eds. *Animal Models of Acute Neurological Injuries*. Springer Protocols Handbooks. Humana Press; 2009: 331-336. doi:10.1007/978-1-60327-185-1_28

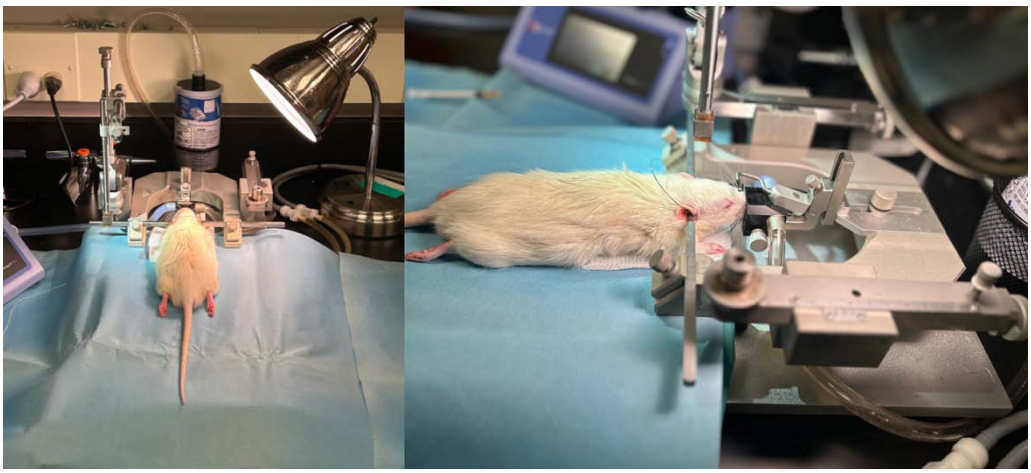


Figure 1: The rat is positioned with all midline structures in alignment; forepaws directed anteriorly, and hind paws directed posteriorly. The nose, head, and spine are in line and parallel to the floor without angulation. The nose is positioned within the apparatus adequately for anesthetic inhalation without leak.

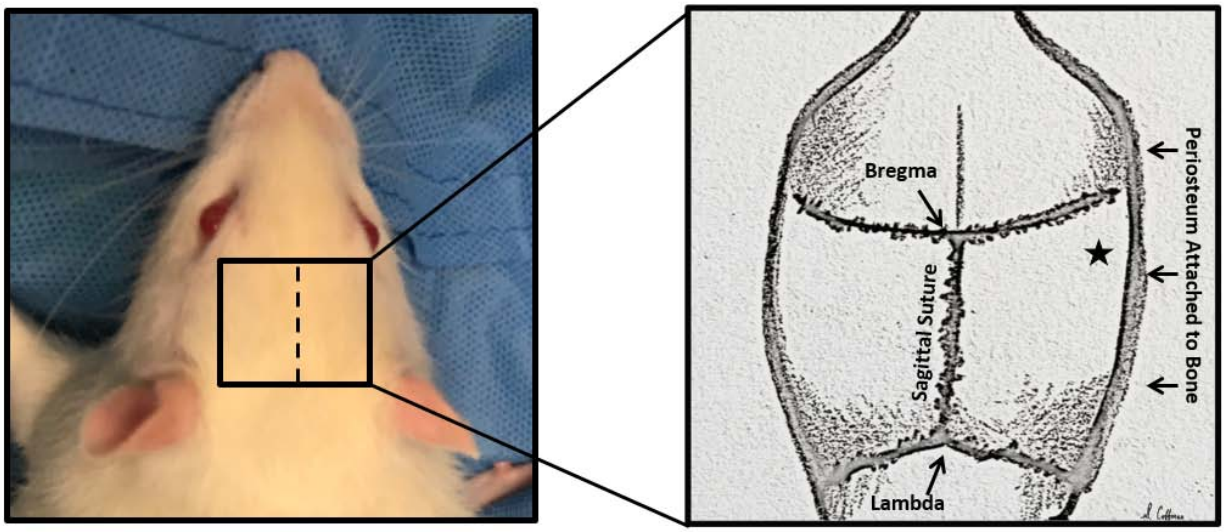


Figure 2: The incision (dashed line, left image) is approximately 2 cm long, midline on the scalp, from the back of the eyes to the lambda. After the incision is made, the periosteum is dissected and the bregma located, with the burr hole (star, right image) placed at the posterior-most point of the bregma and 5 mm to the right of the midline, where the periosteum remains attached to the skull. Original artwork by author Stephanie A. Coffman.

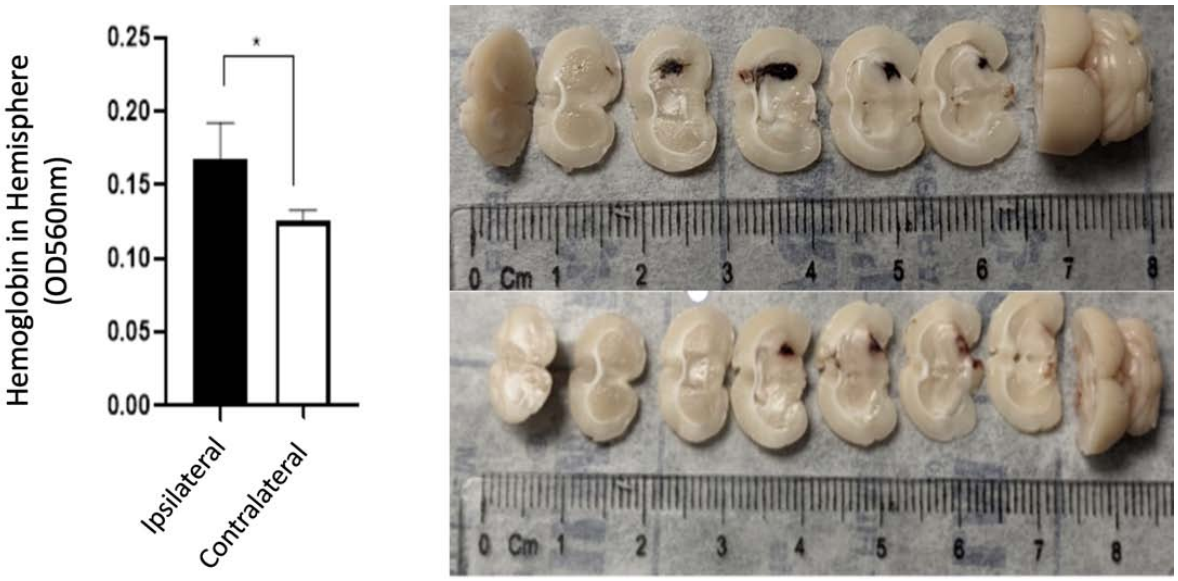


Figure 3: Graph (left) demonstrates mean total hemoglobin concentration in the ipsilateral hemisphere after induced autologous-ICH, demonstrating consistent hematoma volume and images (right) display serial brain sections demonstrating consistent basal ganglia hematoma.

Table 1: Comparative results of success rate and reasons for procedural failure in our experience of single-injection and double-injection methods for ICH creation.

	Single-Injection Method*	Double-Injection Method	X^2	p-value
# successful attempts	48	19	22.94	<0.00001
# unsuccessful attempts	4	21		
Lack of hematoma or insufficient volume	3	13		
Intraventricular extension	0	1		
Blood egress along needle tract	1	7		

**Optimized single-Injection model described by current manuscript.*

This page is intentionally left blank

Global Journal of Medical Research: a
Neurology & Nervous System
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The Scientific Discussion of Revealing of Key Issue Aspects of Features of Simulation of Inflammatory Pain through AMPA Receptor Subunits of Exosome Origin

By Nodar Sulashvili, Margarita Beglaryan, Luiza Gabunia, Nana Gorgaslidze, Ada (Adel) Tadevosyan, Nato Alavidze, Nino Abuladze, Ketevani Gabunia, Marika Sulashvili, Tamar Okropiridze, Igor Seniuk & Marina Giorgobiani

Wake Forest University School of Medicine

Abstract- The aim of the research was to study key issue aspects of features of simulation of inflammatory pain through AMPA receptor subunits of exosome origin in mice. The mechanism of functional modulation of AMPARs by their auxiliary subunits will benefit from further efforts to reach a tipping point where it will be useful for the development of improved therapies. Lipids require special attention because they may play an important role in the function of the AMPAR accessory subunit. Structural studies should only provide snapshots of complexes in action. Therefore, functional studies and molecular dynamics simulation approaches are expected to play an equally important role. Native AMPAR complexes contain more than one type of accessory subunits.

Keywords: *simulation, inflammatory, pain, AMPA receptor subunits, exosome origin.*

GJMR-A Classification: *NLM: WL 102, QU 55*

The Scientific Discussion of Revealing of Key Issue Aspects of Features of Simulation of Inflammatory Pain through AMPA Receptor Subunits of Exosome Origin

Nodar Sulashvili [α], Margarita Beglaryan [σ], Luiza Gabunia [ρ], Nana Gorgaslidze [ω], Ada (Adel) Tadevosyan [¥], Nato Alavidze [§], Nino Abuladze [χ], Ketevani Gabunia [ν], Marika Sulashvili [θ], Tamar Okropiridze [ζ], Igor Seniuk [£] & Marina Giorgobiani [€]

Abstract- The aim of the research was to study key issue aspects of features of simulation of inflammatory pain through AMPA receptor subunits of exosome origin in mice. The mechanism of functional modulation of AMPARs by their auxiliary subunits will benefit from further efforts to reach a tipping point where it will be useful for the development of improved therapies. Lipids require special attention because they may play an important role in the function of the AMPAR accessory subunit. Structural studies should only provide snapshots of complexes in action. Therefore, functional studies and molecular dynamics simulation approaches are expected to play an equally important role. Native AMPAR complexes contain more than one type of accessory subunits. Structural and functional studies of additional AMPAR subunits with complex molecular compositions, including lipids, will be required in the future. Given the strong functional modulation imposed on AMPAR by a specific accessory subunit, its regulation is expected to have significant effects on circuit activity, cognition, learning, and memory. The function of TARP γ-8 in hippocampal LTP has been extensively studied, but the role of additional non-TARP subunits in synaptic plasticity is only now being elucidated. The specific underlying molecular mechanisms that regulate circuit dynamics will be important questions to be addressed in the future. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–type (AMPA-type) glutamate receptors (AMPARs) play a crucial role in synaptic plasticity

Corresponding Author α: MD, PhD, Doctor of Theoretical Medicine In Pharmaceutical and Pharmacological Sciences, Invited Lecturer (Professor) of Scientific Research-Skills Center at Tbilisi State Medical University, Professor of Pharmacology of Faculty of Medicine at Georgian National University SEU, Associate Professor of Medical Pharmacology of Faculty of Medicine at Sulkhan-Saba Orbeliani University, Associate Professor of Division of Pharmacology of International School of Medicine at Alte University; Associate Professor of Pharmacy Program at Shota Meskhia Zugdidi State University; Associate Professor of Medical Pharmacology at School of Medicine at David Aghmashenebeli University of Georgia, Associate Professor of Biochemistry and Pharmacology Direction at the University of Georgia, School of Health Sciences. Associate Professor of Pharmacology of Faculty of Medicine at East European University, Associate Professor of Pharmacology of Faculty of Dentistry and Pharmacy at Tbilisi Humanitarian Teaching University; Tbilisi, Georgia; Researcher of Department of Pharmaceutical Management of Yerevan State Medical University after Mkhitar Heratsi, Yerevan, Armenia.
e-mail: n.sulashvili@ug.edu.ge
Orcid https://orcid.org/0000-0002-9005-8577

Author σ: MD, PhD, Doctor of Pharmaceutical Sciences, Professor of Yerevan State Medical University After Mkhitar Heratsi, Head of the Department of Pharmaceutical Management, Yerevan, Armenia.
Author ρ: MD, PhD, Doctor of Medical Sciences, Professor, Director of the Scientific Research-Skills Center at Tbilisi State Medical University, Professor of the Department of Medical Pharmacology at Tbilisi State Medical University, Clinical Pharmacologist of The First University Clinic of Tbilisi State Medical University, Tbilisi, Georgia.
https://orcid.org/0000-0003-0856-2684;
Author ω: MD, PhD, Doctor of Pharmaceutical Sciences, Professor of Tbilisi State Medical University, Head of the Department of Social and Clinical Pharmacy, Tbilisi, Georgia.
https://orcid.org/0000-0002-4563-5224
Author ¥: MD, PhD, Doctor of Medical Sciences, Academician, Professor of Yerevan State Medical University, Academician and Full Member of the International Academy of Sciences of Ecology and Life Safety (MANEB), Academician of the Republic Armenia Law Academy, Member of the Association of Psychiatrists of Armenia, World Association of Psychiatrists, International Association for Traumatic Stress, World Association for Biological Psychiatry, International Association "Stress and Behavior", Licensed Psychiatrist, Psychotherapist, Public Health Organizer; Tbilisi-Georgia, Yerevan-Armenia, Los Angeles-USA.
Author §: MD, PhD, Doctor of Pharmaceutical Sciences, Professor of AkakiTsereteli State University, Faculty of Medicine, Department of Pharmacy, Kutaisi, Georgia. Professor, Dean Faculty of Medicine at East European University, Tbilisi, Georgia.
https://orcid.org/0000-0001-6695-5924
Author χ: MD, PhD, Doctor of Pharmaceutical Sciences, Professor of AkakiTsereteli State University, Faculty of Medicine, Department of Pharmacy, Kutaisi, Georgia.
Author ν: MD, PhD, Doctor of Pharmaceutical Sciences, Professor of AkakiTsereteli State University, Faculty of Medicine, Department of Pharmacy, Kutaisi, Georgia. https://orcid.org/0000-0002-5857-6593
Author θ: MD, Doctor of Family Medicine, Invited Lecturer (Invited Professor) of Tbilisi State Medical University, Lecturer of Department of Molecular and Medical Genetics, Tbilisi, Georgia.
https://orcid.org/0000-0002-6338-4262
Author ζ: MD, PhD, Doctor Medical Sciences, Professor of the Division of Dentistry of International School of Medicine at Alte University; Professor of Teaching University Geomedi, Head of The Dental Educational Program, Head of the Department of Dentistry, Tbilisi, Georgia. Invited Professor of Dentistry Department of The School of Health Sciences at The University of Georgia, Tbilisi, Georgia.
Author £: PhD, Doctor of Pharmaceutical Sciences, Dean of faculty of Pharmacy at National University of Pharmacy of Ukraine, Associate Professor of Biological Chemistry Department at National University of Pharmacy, Kharkiv, Ukraine. https://orcid.org/0000-0003-3819-7331
Author €: MD, PhD, Doctor of Medical Sciences, Professor of Tbilisi State Medical University, Department of Hygiene and Medical Ecology, Tbilisi, Georgia. https://orcid.org/0000-0003-0686-5227

Global Journal of Medical Research (A) Volume XXIV Issue I Version I Year 2024
19

within the central nervous system. While there is anatomical evidence suggesting the presence of AMPAR expression in the peripheral nervous system, the functional significance of these receptors in vivo remains unclear. To address this knowledge gap, we used mice with specific deletions of key AMPAR subunits, GluA1, exclusively in peripheral pain-sensing neurons (nociceptors). Importantly, we maintained the expression of these subunits in the central nervous system. The nociceptor-specific deletion of GluA1 resulted in the disruption of calcium permeability and a diminished response to capsaicin stimulation in nociceptors. The deletion of GluA1, led to reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. Further investigation unveiled that GluA1-containing AMPARs played a regulatory role in the nociceptors' responses to painful stimuli in inflamed tissues, influencing the excitatory signals transmitted from the periphery into the spinal cord.

Keywords: simulation, inflammatory, pain, AMPA receptor subunits, exosome origin.

I. Introduction

Ionotropic glutamate receptors are the main mediators of excitatory synaptic transmission in the vertebrate nervous system. Glutamate receptor subunits are classified based on their pharmacological properties, biological role and sequence into those that are sensitive to the following: 1) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; GluA1–4). 2) neurotoxin kainate (GluK1–5); and 3) N-methyl-D-aspartic acid (NMDA; GluN1, GluN2A-D, GluN3A-B). Important structural details of glutamate receptors were determined first by solving the structure of the ligand-binding domain and N-terminal domain, and then the structure of the complete tetrameric GluA2 subtype of AMPA receptor. Structures in the presence of various agonists, partial agonists, and antagonists, combined with spectroscopic measurements, electrophysiological measurements, and site-directed mutagenesis, have provided insight into the relationship between structure and function. The binding domain is a bilobed structure to which the agonist binds in the cleft between the two lobes. Two linker peptide chains connect the lobes, and the lobes can close to enclose the agonist. One lobe forms the dimer interface with a second copy of the ligand-binding domain within the tetrameric structure, and the second lobe is associated with the ion channel domain. When the dimer interface is intact, the force generated by the closing of the lobes can affect the ion channel and likely open the gate, allowing ions to pass through the channel. Complexities arise from the tetrameric structure and subtle differences between glutamate receptor subtypes, but the general pattern of channel activation is likely the same for this receptor class [1-5].

The mechanism of channel activation by partial agonists remains unclear. Single-channel recording measurements of AMPA receptors have shown that three or four conductance levels can be observed from a single channel, and these conductance levels are the same for full and partial agonists. Populations with higher conductance levels are favored at higher agonist concentrations, but at any given concentration, higher conductance levels are more common among full than partial agonists. The concentration dependence is consistent with a model in which each subunit has a gate that promotes ionic conduction, and the more the gate is open, the higher the conductivity. However, since conductance levels are similar for all agonists, this suggests that gate opening is an all-or-none process. That is, the signal from the ligand-binding domain leads to a coordinated change in the structure of the channel region. The question then becomes whether this change is caused by a particular conformation of the ligand-binding domain (e.g., complete closure of the lobe), or whether multiple conformations can cause the same change, perhaps with different probabilities, or may be a combination of the two changes models [6-8].

Initial crystal structures of the GluA2 ligand-binding domain in the presence of partial agonists indicate a correlation between the degree of flap closure and ligand efficiency, suggesting that multiple conformations may control channel opening. Full closure results in a high probability of closing, while partial closure results in a significantly lower probability of opening. However, subsequent studies have shown that at least some partial agonists can induce multiple flap closures, and even the correlation in crystal structures between flap opening and efficacy is not always maintained. Most strikingly, the crystal structures of NMDA receptor partial agonists have a completely closed lobe, suggesting that partial agonism is fundamentally different for NMDA and AMPA receptors. Another view of partial agonism is that the stability of complete flap closure determines effectiveness. That is, partial agonists can potentially exist in dynamic equilibrium between two or more conformations. Some conformations may have a relatively open lobe orientation, while other conformations may be closed to the same extent as full agonists. According to this hypothesis, activation of the channel would require a completely closed form, and the stability of this form would determine efficiency. The binding of some weak partial agonists such as iodowillardiine (IW) is consistent with this idea, as a wide range of lobe closures have been observed in crystal and NMR structures and there is evidence of large-scale dynamics in the NMR spectra. In addition, mutations that reduce AMPA efficiency exhibit a range of lobe orientations measured by single-molecule FRET. On the other hand, kainate, a weak partial agonist, represents a structural barrier to flap closure. The isoprenyl group of kainate appears to block flap closure due to an apparent steric conflict with the GluA2 side chain of Leu-650. Mutation of Leu-650 to threonine increases the potency of kainate, likely

reducing the steric interaction between this position and isoprenylkainate. Additionally, little evidence of dynamics on microsecond to millisecond time scales in the presence of kainate has been observed in NMR studies. However, the structures of GluA3 and GluA4 are more closed than those of GluA2 in the presence of kainate, and the D651A mutation of GluA3 results in even greater closure of the lobe by rotation of the Leu-650 side chain (GluA2 numbering) [9-14].

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–type (AMPA-type) glutamate receptors (AMPARs) play a crucial role in synaptic plasticity within the central nervous system. While there is anatomical evidence suggesting the presence of AMPAR expression in the peripheral nervous system, the functional significance of these receptors in vivo remains unclear. To address this knowledge gap, we used mice with specific deletions of key AMPAR subunits, GluA1, exclusively in peripheral pain-sensing neurons (nociceptors). Importantly, we maintained the expression of these subunits in the central nervous system. The nociceptor-specific deletion of GluA1 resulted in the disruption of calcium permeability and a diminished response to capsaicin stimulation in nociceptors. The deletion of GluA1, led to reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. Further investigation unveiled that GluA1-containing AMPARs played a regulatory role in the nociceptors' responses to painful stimuli in inflamed tissues, influencing the excitatory signals transmitted from the periphery into the spinal cord. Consequently, the application of AMPAR antagonists to the periphery alleviated inflammatory pain by specifically targeting calcium-permeable AMPARs, without affecting physiological pain or causing central side effects. Exosomes, nanoscale particles secreted by cells (typically ranging from 30 to 150 nm in size), carry a diverse array of biological molecules, including nucleic acids, proteins, and lipids. These exosomes are recognized for their crucial roles in facilitating intercellular communication. Leveraging their inherent stability, low immunogenicity, and impressive tissue/cell penetration capabilities, exosomes show promise as advanced platforms for targeted drug and gene delivery. Despite their potential, practical applications of exosomes may encounter limitations, such as inadequate targeting ability or low efficacy in specific cases. To address these challenges, various strategies have been employed to engineer exosomes derived from cells, aiming to enhance their selectivity and effectiveness in drug and gene delivery. To address this issue, we used mice specifically lacking of the key AMPAR subunits, GluA1, in peripheral, pain-sensing neurons (nociceptors), while preserving expression of these subunits in the central nervous system. Nociceptor-specific deletion of GluA1 led to disruption of calcium permeability and reduced capsaicin-evoked activation of nociceptors. Deletion of GluA1, led to reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. We generated exosomes containing GluA1 and introduced them to mice around nociceptors, observing a reverse effect compared to GluA1 deletion. Mice treated with exosomes were more sensitive to pain [15-19].

Cysteine trapping studies (i.e., introducing two cysteines to determine whether a disulfide can form) have been used to determine the proximity of different parts of a protein or the proximity of two proteins or subunits. A criticism of this method is that proteins are dynamic structures and very rare conformations can potentially be captured. In this case, the disadvantage of the method may turn out to be an advantage. Partial agonists can activate the channel through a relatively rare transition to a fully closed lobe conformation, and then cysteine capture should be able to stabilize this form for further analysis by X-ray crystallography, NMR spectroscopy, and radioligand binding. Here we show that upon binding of glutamate, iodovyladiine, kainate, and CNQX, the A452C/S652C ligand-binding domain of GluA2 can be captured in a gated manner [20-24].

The Fast excitatory synaptic transmission in the mammalian brain is largely mediated by AMPA-type ionotropic glutamate receptors (AMPARs), which are activated by the neurotransmitter glutamate. At synapses, AMPAR function is regulated by accessory subunits, a diverse set of membrane proteins associated with the core pore-forming AMPAR subunits. Each accessory subunit provides distinct functional modulation of AMPARs, ranging from regulation of transport to modeling ion channel opening kinetics. Understanding the molecular functioning of these complexes is essential to deciphering synaptic modulation and its global role in cognitive activities such as learning and memory [25-29].

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that are activated by the neurotransmitter glutamate. Among them, the flagship of fast excitatory synaptic transmission is AMPA-type iGluR (AMPAR), which transmits signals on a millisecond time scale. The pore-forming subunits of AMPAR, known as GluA1–4, are composed of four domains. The N-terminal domain (NTD) in the extracellular space is furthest from the membrane. The function of the NTD is the least understood but is critical for subunit assembly as well as receptor clustering and synaptic localization. The C-terminal region of the NTD forms a short linker that connects the NTD to the ligand-binding domain (LBD). When bound to glutamate, the LBD undergoes conformational changes that lead to channel closure. The LBD binds to the transmembrane domain (TMD), which consists of three membrane segments (M1, M3 and M4) and a reversible helical loop (M2). In the primary structure of DNPN M1-3 is divided into two fragments: S1 and S2. The TMD forms an ion channel in

the membrane that conducts cations when it is open [30-34].

The pore-forming AMPAR subunits assemble into homo- and heterotetramers. A structural feature that generally distinguishes AMPARs and iGluRs from other ligand-gated cation-permeable tetrameric ion channels is the change in symmetry between the extracellular domains and the TMD; The NTD and LBD form dimers, and the TMD is a tetramer. Ligands that connect the LBD to the TMD and are part of the triggering mechanism compensate for this change in symmetry. Moreover, the transition between DTN and LBD involves domain swapping; Within each subunit, the NTD dimer partners differ from the LBD dimer partners. Free NTD-LBD linkers allow such domain substitution. Among the iGluRs, the architecture of GluD1 is distinct and does not exhibit domain switching, maintaining the flexibility of the NTD-LBD linker [35-38].

The biochemical property that distinguishes AMPARs and kainate receptors (KARs) from NMDARs is their solubility in detergents. NMDARs require much more aggressive cleaning agents to dissolve them than AMPARs and KARs. The properties of the membrane surrounding the receptors and the mechanism of receptor docking are likely to vary significantly between iGluR subtypes. Lipids are often found in ion channel structures. In fact, cholesterol and fatty acids modulate the function of NMDAR and KAR ion channels, respectively. Cholesterol deficiency in cultured hippocampal neurons results in redistribution of synaptic AMPARs. However, it was only recently that lipids were found to be associated with AMPARs [39-43].

Lipid density was observed in a heterotetrameric AMPAR architecture consisting of GluA1 and GluA2 in complex with TARP γ-8. These lipids must have been transferred from HEK cells in which the receptor complex was expressed. Interestingly, the lipids surrounding the GluA2-CNIH3 complexes are organized differently than the lipids of the GluA1-GluA2-TARP-γ-8 complex. These observations have led to the hypothesis that lipids may play a functional role in the assembly and action of accessory subunits and that they may play different roles in different classes of AMPAR accessory subunit complexes [44-46].

TARPs were required to keep the channel gate open in the detergent because no free TARP structures supported the open gate architecture despite being bound to an agonist plus a desensitizing blocker or potentiating toxin. AMPAR-TARP complexes exhibit higher open probabilities and longer residence times at higher conductance levels than AMPARs without TARP, suggesting that TARP stabilizes the conformation of open and activated channels. The allosteric relationship between agonist binding and blockade can be disrupted by detergent, as is known to occur with nicotinic acetylcholine receptors. Therefore, it is possible that TARP recruits lipids into the complex and creates a membrane-mimicking environment [48-52].

Some complex-stabilizing lipids may be absent in non-neuronal cells but are present in brain lipids. This is supported by the observation that different detergent conditions were optimal for AMPAR solubilization in the brain compared to recombinant expression systems such as Sf9 and HEK cells. Identification of the lipid composition of native AMPARs will be challenging but may be critical to understanding the function of AMPAR accessory subunit complexes [53-54].

The postsynaptic receptor cycle is a complex and poorly understood cell biological process. Although it is clear that disruptions in the interactions between many of the dozens of proteins that mediate exo- and endocytosis can influence synaptic function and plasticity, a clear interpretation of the outcomes requires a much more complete understanding of the role that these proteins play in post activity synaptic. The proteins such as NSF, synaptobrevin, and amphiphysin play roles in the presynaptic vesicle cycle, little is currently known about the postsynaptic localization or function of these proteins. The unexpected finding that NSF directly interacts with AMPARs suggests that other proteins involved in vesicle fusion or endocytosis also serve dual functions as receptor chaperones or play other important roles in maintaining PSD integrity [55-59].

Although there is still no consensus regarding AMPAR cycling rates and the direct role of constitutive turnover in rapid forms of synaptic plasticity, it is likely that regulated endocytosis and exocytosis will become an important mechanism for rapidly influencing synaptic strength. It is possible that AMPAR components cycle too slowly to play a role in LTP and LTD (as suggested by half-life studies). Alternatively, long-term modulation of the relative rates of exo- and endocytosis may play an important role in homeostatic forms of plasticity such as: Synaptic scaling or activity- or development-dependent modifications. in the location of receptors acting over time. Finally, it remains to be seen that the role of the regulation of AMPAR-binding proteins plays in fast and slow forms of central synaptic plasticity. It is unclear whether introducing more receptors into the membrane without the resources to trap those receptors at the synapse would be beneficial. It is possible that long-term changes in the number of receptors at the synapse require both the delivery of more receptors to the membrane and an increase in the ability to bind and immobilize these receptors [60-64].

II. Goal

The aim of the research was to study and analyze the key issue aspects of features of simulation of inflammatory pain through AMPA receptor subunits of exosome origin in mice.

III. Materials and Methods

Animals are used to the surveillance camera. The rats were injected subcutaneously (s.c.) with 15 ml of a 5% formaldehyde solution (formalin) onto the dorsal surface of the hind paw. The time spent licking the formalin-injected paw was recorded at 5-minute intervals up to 45 minutes after the formalin injection. Rats were injected with 50 ml of 5% formaldehyde and grimaces were counted at intervals ranging from 1 minute to 60 minutes, starting immediately after formaldehyde injection. Gusts at 5-min intervals were summed as average gusts per minute. The observer was not informed about treatment methods or genetic background.

In contrast to mechanical hyperalgesia, mice developed CFA-induced thermal hyperalgesia, calculated as the percentage reduction in paw withdrawal latency in the inflamed paw compared to the contralateral non-inflamed paw. SNS GluA1-/- and GluA1-/-+. exosomes ($P < 0.05$) at similar levels ($P > 0.05$ between genotypes). SNSGluA2-/− mice did not differ from their GluA1−/−+ exosome-bearing littermates in CFA-induced thermal and mechanical hypersensitivity.

Construction of a plasmid and stable MSC cell line overexpressing GluA2. Mouse bone marrow mesenchymal stem cells (BMSCs) were cultured in minimal alpha essential medium (MEM) (Gibco) containing 10% fetal bovine serum (BI) and 1% penicillin-streptomycin. GEN button) at 5% CO2 and 37 ◦C. All plasmids were provided by Genome Ditech, and the mouse GluA2 coding sequence was cloned into the PGMLV-4931 vector (Genome Ditech). GluA2 overexpression plasmid or control plasmid and Lenti-HG mixture were transfected into 293T cells using HG transgene reagent (Genome ditech). The cell culture medium was then replaced with fresh medium 20 hours after transfection. After 48 hours of incubation, the medium was collected and viruses were isolated by sequential centrifugation. The viruses were then used to infect BMSCs, and puromycin was used to screen for stable cells resistant to puromycin. The effect of gene overexpression was confirmed by qPCR and Western blotting as described below.

Characterization of BMSC-derived exosomes overexpressing GluA2. BMSC-derived exosomes (Exo) and GluA2-overexpressed BMSC-derived exosomes (GluA2) were purified using an Optima XPN-100 ultracentrifuge (Beckman Coulter). To observe the morphology, images were taken using a transmission electron microscope (TEM). Zeta potential and exosome size were determined using Zetaview-based nanoparticle tracking assay (NTA) technology (Particle Metrix). Exosome markers were identified using Western blotting. Protein was measured using a BCA protein quantitation kit (Key GEN).

All animal experiments were checked and approved by local authorities (taking into account international animal welfare regulations). All behavioral measurements were performed on awake, unrestrained adult mice of both sexes of the same age (>3 months) by persons blinded to the genotype of the mice analyzed. Before analysis, rats were habituated to the experimental setup several times. Nociceptive testing in rat models of acute and chronic pain was performed as previously described in detail (Supplementary Methods). All animal experimental protocols were approved by the local institutional review board. All behavioral measurements were performed on adult mice of both sexes, awake, unrestrained, and of the same age (>3 months). Before analysis, rats were habituated to the experimental setup several times. In all experiments, the genotypes of the mice analyzed were not taken into account.

The latency of paw withdrawal in response to noxious heat and pressure gradient was determined using the plantar test with a sensitivity of 0.1 s (n 7–14 per group). Nociceptive thresholds and dimensions of each hindpaw were recorded before and at specified intervals after intraplantar injection of CFA (20 μl). The dimensions of the hind paw were measured using a caliper and a plethysmometer. Paw edema was calculated as the change in paw volume (length-width-height) using a plastinometer as described in detail by Cirinoetal.

The nociceptive tail flick reflex was induced by noxious heat applied through an infrared light source with a sensitivity of 0.1 s as previously described. Formalin test and capsaicin test Formalin (1%, 20 L) or capsaicin (0.06%, 10 L) was injected into the plantar surface of the right hind paw and the duration of nocifentive behavior including lifting, licking, or flinching of the paw. the injected paw was measured within 5 minutes after capsaicin injection or at 5-minute intervals for 50 minutes after formalin injection as previously described.

All data are presented as mean SEM. Student's t tests or analysis of variance (ANOVA) for random measures followed by Fisher's postdoc LSD tests were used to determine statistically significant differences ($p = 0.05$).

IV. Results and Discussion

Activation of mGluR1 as a mechanism for removing CP-AMPARs from synapses is common in other systems. For example, VTA dopamine neurons express CP-AMPAR LTD, which is induced in vitro by mGluR1 agonists or in vivo by a positive allosteric modulator of mGluR1. Later, the same group provided evidence that the GluA2 subunit, which replaces internalized CP-AMPARs, is rapidly synthesized in response to mGluR1 activation through the mTOR

pathway. More detailed information about the regulatory mechanism of GluA2 synthesis and subsequent synaptic inclusion is still missing.

Another important feature of the VTA synapse is the cocaine-induced enhancement (and consequent CP-AMPAR expression) appears to persist for at least a week rather than returning to baseline levels later. It has not been directly demonstrated that this potentiation is still mediated by CP-AMPARs, but since the total amount of AMPARs is assumed to be unchanged, the remaining potentiation is likely still mediated by the greater conductance of CP- relative to CI-AMPARAS. Otherwise, cell viability is reduced 24 hours after injury in cultured neurons, making it difficult to assess surface or synaptic CP-AMPAR expression at later time points. In addition, GluA2 mRNA levels begin to decline 6 hours after ischemic stroke in vivo, promoting CP-AMPAR expression after this time. Although transport of specific subunits triggers a switch to CP-AMPAR after further disease/ischemia, this transition is largely supported by changes in GluA2 gene expression. A similar mechanism may underlie the persistence of cocaine-induced VTA plasticity, although the results do not support this. Changes in GluA1 and GluA2 mRNA expression have been reported under these conditions [65-69].

Chronic pain is a common and poorly understood medical problem. Plasticity of synaptic transmission in the nervous system during peripheral organ inflammation or nerve injury is an important component of the cellular basis of chronic pathological pain. Glutamate acts as an important excitatory neurotransmitter at several key synapses in the somatosensory nociceptive pathway, activating ionotropic and metabotropic receptors there. Recently, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA-type) glutamate receptors (AMPARs) have emerged as important mediators of synaptic plasticity in the brain. Unlike NMDA-type glutamate receptors, which always mediate Ca2+ influx when activated, AMPARs are an activity-dependent switch that controls glutamate-induced Ca2+ influx into neurons. This activity-dependent change is mediated by the regulated expression and binding of the GluA2 subunit (previously called GluR-B or GluR2), which mediates low Ca2+ permeability to AMPAR channels. In contrast, the GluA1 subunit (previously called GluR-A or GluR1) is highly expressed in regions with high densities of calcium-permeable AMPARs, including components of pain pathways. Although global genetic deletions of AMPAR subunits demonstrated that GluA1-containing AMPARs play an important role in chronic pain mechanisms, they were unable to determine anatomical localization. In fact, AMPARs are expressed in several important modulatory regions of somatosensory pathways that mediate pain, such as: peripheral nociceptive neurons, the dorsal horn of the spinal cord, the ventral horn, and several brain regions that control sensory and emotional pain. and emotional. emotional pain. However, the different relative contributions of these regions to central sensitization and chronic pain remain unclear. All peripheral sensory neurons use glutamate as a major transmitter, and large subpopulations of dorsal root ganglion (DRG) sensory neurons are known to express mRNA or be immunoreactive for ionotropic and metabotropic glutamate receptors. Electron microscopy studies have provided compelling evidence that AMPAR subunits are transported to the peripheral processes of sensory neurons, and recent ex vivo anatomical and electrophysiological data also indicate a presynaptic localization and functional involvement of AMPAR subunits in vertebral terminals. However, the functional role of AMPARs located in the central and peripheral terminals of sensory neurons in whole-body nociceptive modulation in vivo remains unclear. Moreover, because AMPARs are also expressed in peripheral sympathetic neurons, Schwann cells, and keratinocytes, the use of pharmacological agents alone does not allow for a comprehensive analysis of the contribution of AMPARs at different sites to pain modulation in vivo [70-74].

The mechanism of functional modulation of AMPARs by their auxiliary subunits will benefit from further efforts to reach a tipping point where it will be useful for the development of improved therapies. Lipids require special attention because they may play an important role in the function of the AMPAR accessory subunit. Structural studies should only provide snapshots of complexes in action. Therefore, functional studies and molecular dynamics simulation approaches are expected to play an equally important role. Native AMPAR complexes contain more than one type of accessory subunits. Structural and functional studies of additional AMPAR subunits with complex molecular compositions, including lipids, will be required in the future. Given the strong functional modulation imposed on AMPAR by a specific accessory subunit, its regulation is expected to have significant effects on circuit activity, cognition, learning, and memory. The function of TARP γ-8 in hippocampal LTP has been extensively studied, but the role of additional non-TARP subunits in synaptic plasticity is only now being elucidated. The specific underlying molecular mechanisms that regulate circuit dynamics will be important questions to be addressed in the future [75-79].

Exosomes are nanosized vesicles secreted by various cell types, including neurons, into the extracellular space. These vesicles carry a cargo of proteins, lipids, and nucleic acids, facilitating intercellular communication. Recent investigations have uncovered the presence of AMPA receptors, crucial for synaptic transmission, within exosomes, suggesting a novel mechanism of information transfer between neurons.

Here, we analyzed transgenic mice that lack the essential GluA1 subunit of AMPAR, specifically in the peripheral arm of the somatosensory pain pathway, i.e., the nervous system. System. The results showed that exosome derivatives containing GluaA1, restores nociceptive effects in GluA1 knockout mice.

Central and somatic signals received by nociceptors in paraplegia, and the consequences of bringing nociceptors into a stable hyper functional state. Nociceptors receive injury-related signals in the spinal cord (highly activated postsynaptic dorsal horn (DH) neurons, activated glial cells, and infiltrating immune cells) and in the dorsal root ganglion (DRG) (from other DRG neurons, satellite glial cells, blood, etc.). Nociceptors have strong excitatory effects on pain pathways (referred to as DG neurons) and on circuits supporting somatic and visceral functions. LTP at DG synapses can be generated by somatic and peripheral AS, as well as after-discharge, which is facilitated by the hyper functional state of the nociceptor. Nociceptor activity causes central sensitization, promotes spontaneous and evoked pain, and enhances somatic and visceral reflexes. Nociceptor activity also results in positive feedback interactions with postsynaptic neurons, other somatic DRGs, inflammatory cells (microglia, infiltrating macrophages, and T cells), astrocytes, and satellite glial cells. PN - Proprioceptive Neuron:Proprioceptive neurons are specialized sensory neurons responsible for conveying information about the position and movement of body parts to the central nervous system (CNS). They play a crucial role in proprioception, which is the sense of the relative position of neighboring parts of the body. IN - Interneuron:Interneurons are neurons that transmit signals between other neurons, acting as connectors or relays within the nervous system. In the context of the DRG, interneurons could be involved in processing and modulating sensory information before it reaches the spinal cord or higher brain centers. They contribute to the integration and coordination of signals within neural circuits [80-84].

CFA-induced mechanical hypersensitivity was tested by applying gradual point pressure to von Frey hairs, and the minimum force producing a pull-off response in at least 2 out of 5 applications of von Frey hairs was termed threshold. Answer. While exosome SNS-GluA1-/-+ mice developed significant mechanical hypersensitivity (reduced von Frey capillary threshold) 4, 12, 24 and 48 hours after CFA injection compared to their respective baseline values.

Changes in paw withdrawal latency (PWL) in response to infrared heat in the inflamed paw represented as the percentage decrease over the contralateral uninflamed paw. $P < 0.05$.

During exosome formation, the plasma membrane is invaginated and intracellular multivesicular bodies with intraluminal vesicles are formed. This endocytic pathway from the donor cell is followed by transport of transmembrane and intra vesicular proteins from the Golgi complex, leading to the formation of early endosomes. After maturation and differentiation, they become late endosomes. They are degraded by fusion with lysosomes, the plasma membrane or autophagosomes, releasing intraluminal vesicles into the extracellular environment as exosomes (40–150 nm in diameter).

Exosomes interact with recipient cells through their surface receptor molecules and ligands. Some exosomes remain on the cell membranes of donor cells after secretion, while others interact with recipient cells. Internalization of exosomes occurs through a raft- or caveolae-mediated membrane integration process or clathrin-dependent endocytosis. Micropinocytosis and phagocytosis have also been described as methods for internalization of exosomes by recipient cells. This process of physiological integration into target recipient cells is believed to have therapeutic potential as a targeted delivery system to effectively carry out biological functions. However, the exosome components responsible for cell type or organ specificity remain unclear.

Exosomes have great therapeutic potential for various diseases due to their intracellular transport ability. Nanomedicine technologies have given impetus to the study of the use of the pathogenic value of exosome particles in various diseases. Nanomedicine targeted drug delivery system focuses on the sustained release of exosomes to exert biological activity at the target site. Exosomes are used as vectors or carrier molecules to trigger a biological response.

Under certain physiological circumstances, exosomes exhibit very low immunogenicity and the ability to bypass the physiological blood-brain barrier. Thanks to the stable lipid bilayer, the cargoes contained in exosome vesicles are protected from the action of native immune cells and digestive enzymes. Artificial exosome vesicles transport the cargoes with which they are loaded to the site of action through various mechanisms of endocytosis or membrane fusion. Electric vehicles are made up of different types of cells and tissues. When injected into a specific diseased tissue, EVs trigger tissue regeneration and homeostasis under certain conditions. EVs derived from mesenchymal stromal cells exhibit cell viability, cell trophism, anti-inflammatory, immunomodulatory, and therapeutic effects. They support neo angiogenesis and cell proliferation. Exosomes exhibit the same targeting effect as the parent cell.

AMPA receptors belong to the family of ionotropic glutamate receptors and are crucial for the transmission of excitatory signals in the brain. This article provides an overview of AMPA receptor structure and function, emphasizing their contribution to synaptic

plasticity and their involvement in various neurological disorders.

Moreover, these structural insights have unveiled the dynamic nature of AMPA receptors, showcasing conformational changes that occur during various stages of receptor function. The GluA1-GluA4 subunits exhibit unique structural features that contribute to the diversity in their functional roles within the receptor complex.

Studies utilizing X-ray crystallography and cryo-electron microscopy have elucidated key interactions between the individual subunits and their binding sites for glutamate, the neurotransmitter that activates AMPA receptors. GluA2, in particular, plays a crucial role in regulating calcium permeability, impacting the overall signaling properties of the receptor.

The intricate architecture of AMPA receptors extends beyond the individual subunits, as auxiliary proteins like TARP (transmembrane AMPA receptor regulatory proteins) and cornichons have been identified as modulators of receptor activity. These auxiliary proteins influence trafficking, synaptic localization, and channel properties, further highlighting the complexity of AMPA receptor function.

Understanding the structural dynamics of AMPA receptors has significant implications for pharmacological interventions targeting neurological disorders. Drug design efforts can benefit from precise knowledge of the receptor's three-dimensional arrangement, allowing for the development of compounds that selectively modulate specific aspects of AMPA receptor function.

In summary, recent strides in structural biology have unraveled the intricacies of AMPA receptor architecture, emphasizing the importance of the arrangement of GluA1, GluA2, GluA3, and GluA4 subunits in determining the receptor's functional properties. These revelations pave the way for a deeper understanding of synaptic transmission and open avenues for the development of novel therapeutic strategies targeting neurological conditions associated with aberrant AMPA receptor activity.

Mechanisms of AMPA Receptor Function: Upon glutamate binding, AMPA receptors undergo conformational changes that lead to channel opening, allowing the influx of cations, predominantly sodium ions. The rapid activation and subsequent desensitization of AMPA receptors contribute to the fast nature of excitatory neurotransmission. Moreover, the regulation of AMPA receptor trafficking and localization is critical for synaptic plasticity, synaptic strength, and learning and memory processes.

Synaptic Plasticity and AMPA Receptors: Long-term potentiation (LTP) and long-term depression (LTD) are forms of synaptic plasticity that underlie learning and memory. AMPA receptors play a central role in these processes by modulating the strength of synaptic connections. The dynamic regulation of AMPA receptor trafficking, insertion, and removal from the synapse contribute to the fine-tuning of synaptic strength and plasticity.

AMPA Receptors in Neurological Disorders: Dysregulation of AMPA receptor function has been implicated in various neurological disorders, including epilepsy, Alzheimer's disease, and mood disorders. Understanding the molecular mechanisms underlying AMPA receptor dysfunction in these conditions provides potential targets for therapeutic intervention. Modulators of AMPA receptor activity, such as positive allosteric modulators and selective agonists, are being explored as potential treatment options.

Therapeutic Implications: Given the crucial role of AMPA receptors in synaptic transmission and plasticity, targeting these receptors holds promise for therapeutic interventions in neurological disorders. Researchers are actively investigating novel compounds and strategies to modulate AMPA receptor function selectively. The development of subtype-specific modulators and precise regulation of AMPA receptor activity may offer more targeted and effective therapeutic approaches.

The role of the AMPA receptor in painful sensations. AMPAs are transmembrane proteins made up of 4 subunits (tetramers). There are 4 different subunits in the AMPAR family, GluR1-4. Each subunit contains approximately 900 amino acids and 4 main components: a large amino-terminal extracellular domain, an adjacent ligand-binding domain, a transmembrane domain, and a carboxy-terminal cytoplasmic domain. Most native AMPARs are heterothermies, meaning they are made up of a combination of different subunits. The synthesis of AMPAR subunits and their assembly into functional receptors begins in the rough endoplasmic reticulum. A group of proteins called AMPAR transmembrane regulatory proteins (TARPs) facilitate the transport of AMPARs from the endoplasmic reticulum to the plasma membrane and anchor these receptors at the synapse. Transport of AMPARs to and from the synaptic membrane occurs in a highly regulated manner. For example, phosphorylation of residue S831 in GluR1 by Ca/calmodulin-dependent protein kinases (CaMKII) and protein kinase C has been shown to result in transport of GluR1 subunits into the synapse. By adjusting the number and type of AMPARs on the synaptic surface, a postsynaptic neuron can modify its excitability, that is, its response to presynaptic signals [85-89].

Electrophysiological properties of AMPA receptors. Most functional AMPAs are located on the postsynaptic surface. When bound to glutamate, they are permeable to Na and K ions, but usually not to Ca2 ions. Each AMPAR, when open, conducts a miniature excitatory postsynaptic current inward. Each of these small incoming currents depolarizes the cell membrane

to a small extent. When enough AMPARs bind glutamate and open, these miniature excitatory postsynaptic currents can sum and create a large depolarizing force, causing the neuron to fire an action potential. 3 Thus, AMPAR opening in response to glutamate provides the cellular basis for excitatory synaptic transmission. In addition, a subset of AMPARs, receptors without GluR2 subunits, are Ca2+ permeable. Most of these calcium-permeable AMPARs (CPARs) are composed of GluR1 homo tetramers, but they can also be formed by assembling a combination of GluR1, 3, and 4 subunits. CPARs conduct faster and larger inward currents than AMPARs. impermeable to calcium. CPARs not only exhibit faster and stronger postsynaptic currents, but through Ca2 influx they can also activate Ca2-dependent signaling cascades that lead to long-term changes in synaptic strength. Thus, CPARs act as surrogates for NMDA receptors and likely play a similar role in processes such as memory formation and central sensitization. AMPA receptors and pain. Given the critical role of AMPARs in determining the strength of synaptic transmission in various neurological systems, it is not surprising that they are involved in pain transmission. In recent years, animal studies have focused on the first synaptic contact in the pain pathway, namely the synapse between the primary afferent neuron and the dorsal horn neuron. Using sophisticated electrophysiological recordings, the spinal cord neurons expressing AMPARs receive primary afferent inputs of nociceptive origin. During this time, Polgar and his colleagues were able to provide eight quantitative estimates of AMPAR. They observed that, for example, in lamina I-II of the dorsal horn, all neurons expressed GluR2 AMPAR subunits, whereas only 65% of these neurons expressed GluR1 subunits. In lamina III, 100% of neurons express GluR2 and 80% express GluR1. They found that GluR3 and GluR4, although in smaller amounts, are also expressed in dorsal horn neurons. They also showed that these AMPARs are localized to postsynaptic density proteins, proteins that function as structures on the postsynaptic surface. Thus, their finding suggests that AMPARs are not only expressed by spinal cord neurons but likely play an active role in synaptic transmission between peripheral nociceptive neurons and spinal cord neurons [90-95].

The discovery of AMPARs at the synaptic site of the pain pathway is the first step in determining the importance of these receptors in pain. The next steps are to identify specific AMPAR changes that occur during pain and show that these changes contribute to the experience of pain. Larsson and Broman recently showed that during acute pain (induced by capsaicin), there is an increase in the number of GluR1 subunits recruited to synaptic sites. This is an important finding because the dominant AMPARs in GluR1 tend to be Ca^{2+} permeable receptors, which can trigger long-term cellular changes. According to their model, inflammation caused by capsaicin leads to the transmission of pain signals to the C-fiber neuron in the form of action potentials. The flooding of these action potentials is sufficient to recruit CPAR to the synaptic site of the dorsal horn neuron. The accumulation of CPAR in turn induces long-term memory at this synapse between the C-fiber and the spinal neuron, facilitating subsequent pain transmission. Thus, Ca^{2+}permeable AMPARs act as surrogates for NMDA receptors to mediate central pain sensitization. Additional evidence for the accumulation of Ca^{2+} -permeable AMPARs during pain conditions comes from studies focusing on chronic pain. Ca^{2+}-permeable AMPARs accumulated at spinal cord synapses in several rodent models of chronic pain. After administration of Freund's complete adjuvant, a proinflammatory agent, to the paws of rats or mice, these rodents exhibited long-lasting (2 weeks) mechanical allodynia and thermal hyperalgesia. After the onset of chronic pain, Luo and his colleagues dissected the spinal cords of these mice and found that not only did the number of GluR1 subunits in spinal cord neurons increase, but also the active part of this subunit (phosphorylated). fraction) was also increased. Thus, their results indicate that chronic pain activates AMPAR GluR1 and recruits it to the cell surface. dorsal horn neurons. Two additional studies showed that not only did the number of GluR1 subunits increase, but there was also a concomitant decrease in the number of GluR2 and GluR3 subunits at the synapse between the peripheral nociceptive neuron and the dorsal horn neuron. Regulation of the soluble factor N-ethylmaleimide fusion protein, a protein required to transport GluR2 subunits to the cell surface, was actually downregulated due to chronic pain. Moreover, Tao's group showed that GluR2-containing AMPARs can subsequently be internalized or cleared from the synaptic site over time through activation of the NMDA receptor. Thus, a complex signaling cascade begins to emerge from these studies. First, chronic pain induces intense AMPAR-mediated synaptic transmission between the peripheral nociceptive neuron and the dorsal horn neuron, activating NMDA receptors and causing Ca^{2+} influx. Ca influx in turn activates a number of downstream signaling proteins, including kinases and other transport proteins, to replace Ca-impermeable AMPARs with Ca^{2+} permeable AMPARs in the cell membrane. Finally, administration of Ca^{2+}-permeable AMPARs allows for increased Ca^{2+} influx, thereby improving synaptic transmission from peripheral neurons to spinal cord neurons. This pathway partially underlies the mechanism of central sensitization [96-99]. Modulation of AMPA receptors leads to changes in pain sensitivity. If AMPA receptors are involved in spinal cord pain pathways, and more specifically in the synaptic contact between a nociceptive afferent neuron and a spinal cord neuron, modulation of these receptors should lead to changes in pain sensitivity in animals. In

fact, researchers have been trying to administer intrathecal glutamate receptor blockers to treat pain for many years. The reason for this approach was to interrupt all synaptic transmission between peripheral nerves and spinal nerves by blocking AMPARs. For example, Sang and colleagues showed that tezampanel, a nonspecific AMPAR blocker, can be used to reduce mechanical hyperalgesia in a rodent model of inflammatory pain. This treatment modelimpairing pain transmission—requires chronic administration of the drug. However, chronic administration of an AMPAR antagonist results in unacceptable side effects by interfering with normal nociceptive and non-nociceptive sensory transmission and motor functions. In addition, these drugs can penetrate the cerebrospinal fluid and disrupt synaptic transmission in the brain. However, recent studies on the role of CPARs in the induction and maintenance of central sensitization have shed new light on the therapeutic potential of AMPAR blockade. Therefore, therapeutic AMPAR blockade may require a different strategy aimed at disrupting the molecular mechanisms of central sensitization rather than disrupting complete synaptic transmission. This strategy may only require proactive blocking of signaling events that lead to accumulation of CPARs or selective antagonism of CPARs themselves. In support of this strategy, the examined pain perception in mice carrying genetically modified GluR2 as part of an investigation into the mechanism of central sensitization in the spinal cord. They genetically modified GluR2 subunits to render these receptors unable to be internalized. Consequently, these mutated GluR2 receptors remained on the cell membrane longer and displaced GluR1 receptors. Remember that CPARs require the absence of GluR2 and the presence of GluR1. This mutation essentially results in a decrease in the amount of CPAR on the cell surface. Interestingly, but perhaps unsurprisingly, rodents with this mutation exhibited less chronic pain. Using a different genetic approach, examined the effect of selective deletion of GluR1 or GluR2 on the acute pain threshold in mice. for transmission. signs of acute pain. However, in a model of chronic inflammatory pain, genetic deletion of GluR1 subunits in mice resulted in a higher pain threshold, and deletion of GluR2 had the opposite effect. Because GluR2 is Ca2-permeable without AMPARs, these genetic data suggested that altering the number of Ca-permeable AMPARs at synaptic surfaces may alter pain transmission. The difference lies in the chronic nature of the pain. Although CPARs are interesting for acute pain signaling, they are likely to play an important role in chronic pain due to their influence on central sensitization [100-104].

AMPA receptors mediate fast excitatory synaptic transmission in the mammalian central nervous system when activated by the neurotransmitter glutamate at the postsynaptic membrane. The receptors are composed of four subunits GluA1-GluA4, which can combine with each other in various combinations to form glutamate-activated ion channels with different physiological properties. However, AMPA receptor function is also influenced by concomitant factors, such as the TARP family of AMPA receptor transmembrane regulatory proteins. For example, TARP γ8 allows AMPA receptors that have been desensitized due to the chronic presence of glutamate to return to an open state [105-107].

NMDA receptors are well expressed on the cell surface and function when double cysteine mutations are introduced into NR1 or NR2 to block lobes. The GluA2 A452C/S652C mutation is highly expressed but does not reach the cell surface. However, when expressed in bacteria, the GluA2 LBD with these mutations' folds correctly and the agonist binding site remains intact. Assuming that the protein is correctly folded but does not translocate to the cell surface, the transport defect may be due to a defect in dimer or tetramer formation or a conformational state (e.g., desensitization). The L483Y mutation appears to promote tetramerization and stabilize the interface between LBD dimers. Despite the formation of tetramers, the lack of desensitization of L483Y mutants limits their penetration to the cell surface. The formation of the A452C/S652C disulfide destabilizes the interface between LBD dimers and likely has the opposite effect on tetramerization (or even dimerization) [108-112].

The use of a disulfide bond demonstrated that it is possible to obtain an almost completely closed form of the GluA2 LBD in the presence of several partial agonists. This suggests that the flocs may exhibit transitions to multiple conformations, as previously suggested by dynamic NMR measurements and single-molecule FRET experiments. Although these experiments do not directly address the conformation required for channel activation, previous studies showing that partial agonists can adopt a range of conformations suggest that this ensemble may determine efficacy. The finding that the fully closed form is part of this set is consistent with the idea that the stability of the fully closed form determines performance.

Activation of AMPA receptors begins with agonist binding and general movement of the LBD, which in turn causes displacement of the ion channel gate and the passage of cations through the channel pore. Closure of the LBD bipartite structure is at least partially responsible for channel opening. Single-channel recording experiments showed that full and partial agonists can activate AMPA receptor channels at the same three or four different conductance levels. At saturating agonist concentrations and without desensitization, partial agonists exhibit lower currents than full agonists because lower conductance levels fill preferentially than those observed with full agonists. The

different levels of conductance were thought to be due to the activation of separate gates on each of the four subunits. That is, the highest level of conductance is achieved with the gates open for all four subunits, the next highest level of conductance is with three gates open, and so on. At saturating concentrations of agonists, all four subunits are occupied, so in the absence of desensitization, the occupancy of lower conductance states by partial agonists suggests that the activation channel is not automatically triggered upon agonist binding, but rather that the channel is open. The gate to one subunit is associated with conformational equilibrium, the energy levels of which change upon agonist binding. Partial agonism is based on a number of crystal structures that correlate lobe orientation in the GluA2 LBD with efficiency. The more sheet closures observed in a set of crystal structures, the higher the efficiency. Conduction states were shown to be identical for full and partial agonists, and the population of conductance levels followed a bionomic distribution. The success rate of a bio name can be viewed as a measure of effectiveness. The efficiency coefficient, in turn, correlates with the relative orientation of the LBD flaps. This hypothesis has been called the explanation because the relatively fixed degree of gate closure determines the likelihood of gate activation. An alternative, but not mutually exclusive, dynamic model is that each subunit has a conformational set that is modified by the binding of full and partial agonists. For full agonists, the conformational set primarily favors a closed valve state and gate activation for the subunit, whereas partial agonists include a fully closed state as well as a distribution of more open states with less frequent gate activation for the subunit. this subunit. In the simplest version of the model, the fully closed state of the LBD would be the trigger to activate the channel gate, and the probability of achieving a fully closed LBD would determine the effectiveness [113-118].

The half-life of AMPAR in cultured spinal neurons, measured by pulse receptor labeling or surface biotinylating, is approximately 30 hours. In contrast, a recent report using an antibody pulse to label surface receptors on live human embryonic kidney (HEK) cells and hippocampal neurons in culture showed that the labeled receptors were internalized very quickly, with a constant of time of approximately 40 minutes. These internalized receptors were colocalized with proteins, associated with clathrin-coated pits. This suggests that receptor endocytosis occurs much more rapidly than receptor degradation, leaving the majority of internalized AMPARs intact (and possibly functional). This, in turn, raises the possibility that internalized AMPARs may be recycled back to the synaptic membrane. Although constitutive cycling models of receptors at the NMJ emphasize a slow, stately exchange of receptors over a period of days, these recent studies suggest that central AMPARs may constantly travel between extracellular and intracellular compartments, although direct tests provide proof of this. require the reappearance of receptors on the synaptic membrane. Another reason for caution in interpreting the discrepancy between half-life and internalization rates is the possibility that the method used to measure internalization itself (the binding of antibodies to AMPARs in living cells) influences the rate of receptor internalization. For example, it would be good to know whether the receptor half-life decreases with antibody treatment. An interesting observation in cells treated with hypertonic sucrose or transfected with a dominant negative dynamin mutant (both manipulations intended to inhibit endocytosis) was that constitutive AMPAR internalization was significantly reduced, the percentage of AMPAR but the total surface area was not increased. This observation led to propose that the internalization and insertion rates of the constituent receptors are somehow linked, such that a change in one result in coordinated changes in the 'other, and that the total number of surface receptors remains constant. This interpretation could explain the lack of effect on basal transmission observed with subsequent blockade of exocytosis, but is in direct contradiction to the findings that exocytosis blockers had a profound effect on basal transmission. Insulin treatment reduced the number of surface receptors on cultured HEK or hippocampal neurons, and this reduction was sensitive to agents that disrupt endocytosis. Additionally, insulin treatment and LTD blocked in hippocampal slices. This suggests that certain agents (such as insulin and activity) are able to transiently uncouple endocytosis and exocytosis and produce a net gain or loss of cell surface receptors. These results are supported by the accompanying report that cerebellar LTD (and reduction in insulin-mediated synaptic transmission) was strongly attenuated by inhibitors of clathrin-mediated endocytosis, whereas basal transmission was not affected. The reports suggest that the synaptic plasticity mechanisms from different brain regions (hippocampus and cerebellum), using different transduction mechanisms, might ultimately converge on the same cellular mechanism to control the number of AMPARs expressed at synaptic sites [119-123].

The CP-AMPARs have been implicated in pathological processes such as ischemia for many years, their role in “normal” physiological memory processes has only recently been recognized, and CP-AMPARs are now emerging as an important additional property of various forms of synapses. The subunit composition of synaptic AMPARs can change quite rapidly due to the movement of certain subunits. Compared to the wealth of knowledge about AMPAR trafficking in general, little is known about the specific mechanisms that regulate synaptic inclusion of CP-AMPARs. As mentioned above, GluA1-dependent

mechanisms, already identified as important for LTP expression but previously thought to apply primarily to GluA1/GluA2 heteromers, may be synonymous with CP-AMPAR transport immediately following LTP induction and perhaps other forms of plasticity that also include the CP-AMPAR insert [124-127]. Internalization of GluA2, which is part of CP-AMPAR expression, may share mechanisms with the induction of LTD (which does not involve synaptic CP-AMPAR expression). It will be important to see how signaling pathways upstream of AMPAR subunits and the accessory proteins are specific for CP-AMPAR expression. Unraveling the details of the illicit trade requires a modern cell culture system that can be used for high-resolution imaging in combination with acute genetic manipulation. Although such a system is clearly available for hippocampal neurons, research on other types of neurons has lagged in this regard. However, it is already clear that there are many similarities between the neurons of the hippocampus, VTA and lateral amygdala.

V. Conclusion

So, the Modulation of AMPA receptors leads to changes in pain sensitivity. The AMPA receptors are involved in spinal cord pain pathways, and more specifically in the synaptic contact between a nociceptive afferent neuron and a spinal cord neuron, modulation of these receptors should lead to changes in pain sensitivity in animals. In fact, researchers have been trying to administer intrathecal glutamate receptor blockers to treat pain for many years. The reason for this approach was to interrupt all synaptic transmission between peripheral nerves and spinal nerves by blocking AMPARs. For example, Sang and colleagues showed that tezampanel, a nonspecific AMPAR blocker, can be used to reduce mechanical hyperalgesia in a rodent model of inflammatory pain. The nociceptor-specific deletion of GluA1 resulted in the disruption of calcium permeability and a diminished response to capsaicin stimulation in nociceptors. The deletion of GluA1, led to reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. So, the GluA1-containing AMPARs played a regulatory role in the nociceptors' responses to painful stimuli in inflamed tissues, influencing the excitatory signals transmitted from the periphery into the spinal cord.

Statement of Conflicting Interests: The authors announced, that there is no potential conflict of interest with regard to the research, and/or publication of this article.

References Références Referencias

1. Kato AS, Siuda ER, Nisenbaum ES & Bredt DS. (2008). AMPA receptor subunit-specific regulation by a distinct family of type II TARPs. Neuron 59, 986–996.
2. Khodosevich K, Jacobi E, Farrow P, Schulmann A, Rusu A, Zhang L, Sprengel R, Monyer H & von Engelhardt J. (2014). Coexpressed auxiliary subunits exhibit distinct modulatory profiles on AMPA receptor function. Neuron 83, 601–615.
3. Kielland A & Heggelund P. (2002). AMPA and NMDA currents show different short-term depression in the dorsal lateral geniculate nucleus of the rat. J Physiol 542, 99–106.
4. Kim E & Sheng M. (2004). PDZ domain proteins of synapses. Nat Rev Neurosci 5, 771–781.
5. Klaassen RV, Stroeder J, Coussen F, Hafner AS, Petersen JD, Renancio C, Schmitz LJ, Normand E, Lodder JC, Rotaru DC, Rao-Ruiz P, Spijker S, Mansvelder HD, Choquet D & Smit AB. (2016). Shisa6 traps AMPA receptors at postsynaptic sites and prevents their desensitization during synaptic activity. Nat Commun 7, 10682.
6. Korinek M, Vyklicky V, Borovska J, Lichnerova K, Kaniakova M, Krausova B, Krusek J, Balik A, Smejkalova T, Horak M &Vyklicky L. (2015). Cholesterol modulates open probability and desensitization of NMDA receptors. J Physiol 593, 2279–2293.
7. Lee MR, Gardinier KM, Gernert DL, Schober DA, Wright RA, Wang H, Qian Y, Witkin JM, Nisenbaum ES & Kato AS. (2017). Structural Determinants of the gamma-8 TARP Dependent AMPA Receptor Antagonist. ACS Chem Neurosci 8, 2631–2647.
8. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR, Desaki AL, Desta T, Diep E, Dolbeare TA, Donelan MJ, Dong HW, Dougherty JG, Duncan BJ, Ebbert AJ, Eichele G, Estin LK, Faber C, Facer BA, Fields R, Fischer SR, Fliss TP, Frensley C, Gates SN, Glattfelder KJ, Halverson KR, Hart MR, Hohmann JG, Howell MP, Jeung DP, Johnson RA, Karr PT, Kawal R, Kidney JM, Knapik RH, Kuan CL, Lake JH, Laramee AR, Larsen KD, Lau C, Lemon TA, Liang AJ, Liu Y, Luong LT, Michaels J, Morgan JJ, Morgan RJ, Mortrud MT, Mosqueda NF, Ng LL, Ng R, Orta GJ, Overly CC, Pak TH, Parry SE, Pathak SD, Pearson OC, Puchalski RB, Riley ZL, Rockett HR, Rowland SA, Royall JJ, Ruiz MJ, Sarno NR, Schaffnit K, Shapovalova NV, Sivisay T, Slaughterbeck CR, Smith SC, Smith KA, Smith BI, Sodt AJ, Stewart NN, Stumpf KR, Sunkin SM, Sutram M, Tam A, Teemer CD, Thaller C, Thompson CL, Varnam LR, Visel A, Whitlock RM, Wohnoutka PE, Wolkey CK, Wong VY, Wood M, Yaylaoglu MB, Young RC, Youngstrom BL, Yuan XF, Zhang B, Zwingman TA & Jones AR. (2007). Genome-wide

atlas of gene expression in the adult mouse brain. Nature 445, 168–176.
9. Liu YL, Fann CS, Liu CM, Chen WJ, Wu JY, Hung SI, Chen CH, Jou YS, Liu SK, Hwang TJ, Hsieh MH, Chang CC, Yang WC, Lin JJ, Chou FH, Faraone SV, Tsuang MT & Hwu HG. (2008). RASD2, MYH9, and CACNG2 genes at chromosome 22q12 associated with the subgroup of schizophrenia with non-deficit in sustained attention and executive function. Biol Psychiatry 64, 789–796.
10. Long SB, Tao X, Campbell EB & MacKinnon R. (2007). Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. Nature 450, 376–382.
11. Maher MP, Matta JA, Gu S, Seierstad M &Bredt DS. (2017). Getting a Handle on Neuropharmacology by Targeting Receptor-Associated Proteins. Neuron 96, 989–1001.
12. Matt L, Kirk LM, Chenaux G, Speca DJ, Puhger KR, Pride MC, Qneibi M, Haham T, Plambeck KE, Stern-Bach Y, Silverman JL, Crawley JN, Hell JW & Diaz E. (2018). SynDIG4/Prrt1 Is Required for Excitatory Synapse Development and Plasticity Underlying Cognitive Function. Cell Rep 22, 2246–2253.
13. McGoldrick LL, Singh AK, Saotome K, Yelshanskaya MV, Twomey EC, Grassucci RA & Sobolevsky AI. (2018). Opening of the human epithelial calcium channel TRPV6. Nature 553, 233–237.
14. Meyerson JR, Kumar J, Chittori S, Rao P, Pierson J, Bartesaghi A, Mayer ML & Subramaniam S. (2014). Structural mechanism of glutamate receptor activation and desensitization. Nature.
15. Milstein AD & Nicoll RA. (2009). TARP modulation of synaptic AMPA receptor trafficking and gating depends on multiple intracellular domains. Proc Natl Acad Sci U S A 106, 11348–11351.
16. Milstein AD, Zhou W, Karimzadegan S, Bredt DS & Nicoll RA. (2007). TARP subtypes differentially and dose-dependently control synaptic AMPA receptor gating. Neuron 55, 905–918.
17. Nakagawa T (2010). The biochemistry, ultrastructure, and subunit assembly mechanism of AMPA receptors. Mol Neurobiol 42, 161–184.
18. Cai, L.L., Courtine, G., Fong, A.J., Burdick, J.W., Roy, R.R., and Edgerton, V.R. (2006). Plasticity of functional connectivity in the adult spinal cord. Phil. Trans. R. Soc. Lond. B 361, 1635–1646.
19. Craig, A.D. (2003). Pain mechanisms: labeled lines versus convergence in central processing. Annu. Rev. Neurosci. 26, 1–30. Engelman, H.S., and MacDermott, A.B. (2004). Presynaptic ionotropic receptors and control of transmitter release. Nat. Rev. Neurosci. 5, 135–145.
20. Fetz, E.E., Perlmutter, S.I., Prut, Y., Seki, K., and Votaw, S. (2002). Roles of primate spinal interneurons in preparation and execution of voluntary hand movement. Brain Res. Rev. 40, 53–65.
21. Helms, A.W., and Johnson, J.E. (2003). Specification of dorsal spinal cord interneurons. Curr. Opin. Neurobiol. 13, 42–49.
22. Jacobs, B.L., and Fornal, C.A. (1993). 5-HT and motor control: a hypothesis. Trends Neurosci. 16, 346–352.
23. Jankowska, E. (1992). Interneuronal relay in spinal pathways from proprioceptors. Prog. Neurobiol. 38, 335–378.
24. Ji, R.R., Kohno, T., Moore, K.A., and Woolf, C.J. (2003). Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 26, 696–705.
25. Kiehn, O. (2006). Locomotor circuits in the mammalian spinal cord. Annu. Rev. Neurosci. 29, 279–306.
26. Lee, S.K., and Pfaff, S.L. (2001). Transcriptional networks regulating neuronal identity in the developing spinal cord. Nat. Neurosci. Suppl. 4, 1183–1191.
27. McLean, D.L., Fan, J., Higashijima, S., Hale, M.E., and Fetcho, J.R. (2007). A topographic map of recruitment in spinal cord. Nature 446, 71–75.
28. Millan, M.J. (2002). Descending control of pain. Prog. Neurobiol. 66, 355–474.
29. Poppele, R., and Bosco, G. (2003). Sophisticated spinal contributions to motor control. Trends Neurosci. 26, 269–276.
30. Rossignol, S., Dubuc, R., and Gossard, J.P. (2006). Dynamic sensorimotor interactions in locomotion. Physiol Rev. 86, 89–154.
31. Rudomin, P., and Schmidt, R.F. (1999). Presynaptic inhibition in the vertebrate spinal cord revisited. Exp. Brain Res. 129, 1–37.
32. Seki, K., Perlmutter, S.I., and Fetz, E.E. (2003). Sensory input to primate spinal cord is presynaptically inhibited during voluntary movement. Nat. Neurosci. 6, 1309–1316.
33. Willis, W.D. Jr., and Coggeshall, R.E. (1991). Sensory Mechanisms of the Spinal Cord, Second Edition (New York: Plenum Press).
34. Greger IH, Watson JF, Cull-Candy SG. Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. Neuron. 2017 May 17; 94(4): 713-730.
35. Chang PK, Verbich D & McKinney RA. (2012). AMPA receptors as drug targets in neurological disease--advantages, caveats, and future outlook. Eur J Neurosci 35, 1908–1916.
36. Chen C, Blitz DM &Regehr WG. (2002). Contributions of receptor desensitization and saturation to plasticity at the retinogeniculate synapse. Neuron 33, 779–788.

37. Chen C & Regehr WG. (2000). Developmental remodeling of the retinogeniculate synapse. Neuron 28, 955–966.
38. Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS & Nicoll RA. (2000). Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature 408, 936–943.
39. Chen L, Durr KL & Gouaux E. (2014). X-ray structures of AMPA receptor-cone snail toxin complexes illuminate activation mechanism. Science 345, 1021–1026.
40. Chen S & Gouaux E. (2019). Structure and mechanism of AMPA receptor - auxiliary protein complexes. CurrOpin Struct Biol 54, 104–111.
41. Chen S, Zhao Y, Wang Y, Shekhar M, Tajkhorshid E &Gouaux E. (2017). Activation and Desensitization Mechanism of AMPA Receptor-TARP Complex by Cryo-EM. Cell 170, 1234–1246 e1214.
42. Chen X, Aslam M, Gollisch T, Allen K & von Engelhardt J. (2018). CKAMP44 modulates integration of visual inputs in the lateral geniculate nucleus. Nat Commun 9, 261.
43. Chiyonobu T, Hayashi S, Kobayashi K, Morimoto M, Miyanomae Y, Nishimura A, Nishimoto A, Ito C, Imoto I, Sugimoto T, Jia Z, Inazawa J & Toda T. (2007). Partial tandem duplication of GRIA3 in a male with mental retardation. Am J Med Genet A 143A, 1448–1455.
44. Cho CH, St-Gelais F, Zhang W, Tomita S & Howe JR. (2007). Two families of TARP isoforms that have distinct effects on the kinetic properties of AMPA receptors and synaptic currents. Neuron 55, 890–904.
45. Collingridge GL, Olsen RW, Peters J & Spedding M. (2009). A nomenclature for ligand-gated ion channels. Neuropharmacology 56, 2–5.
46. Coombs ID, Soto D, Zonouzi M, Renzi M, Shelley C, Farrant M & Cull-Candy SG. (2012). Cornichons modify channel properties of recombinant and glial AMPA receptors. J Neurosci 32, 9796–9804.
47. Davies B, Brown LA, Cais O, Watson J, Clayton AJ, Chang VT, Biggs D, Preece C, Hernandez-Pliego P, Krohn J, Bhomra A, Twigg SRF, Rimmer A, Kanapin A, Consortium WGS, Sen A, Zaiwalla Z, McVean G, Foster R, Donnelly P, Taylor JC, Blair E, Nutt D, Aricescu AR, Greger IH, Peirson SN, Flint J & Martin HC. (2017). A point mutation in the ion conduction pore of AMPA receptor GRIA3 causes dramatically perturbed sleep patterns as well as intellectual disability. Hum Mol Genet 26, 3869–3882.
48. Dawe GB, Kadir MF, Venskutonyte R, Perozzo AM, Yan Y, Alexander RPD, Navarrete C, Santander EA, Arsenault M, Fuentes C, Aurousseau MRP, Frydenvang K, Barrera NP, Kastrup JS, Edwardson JM & Bowie D. (2019). Nanoscale Mobility of the Apo State and TARP Stoichiometry Dictate the Gating Behavior of Alternatively Spliced AMPA Receptors. Neuron 102, 976–992 e975.
49. Dawe GB, Musgaard M, Aurousseau MR, Nayeem N, Green T, Biggin PC & Bowie D. (2016). Distinct Structural Pathways Coordinate the Activation of AMPA Receptor-Auxiliary Subunit Complexes. Neuron 89, 1264–1276.
50. Diaz-Alonso J, Sun YJ, Granger AJ, Levy JM, Blankenship SM & Nicoll RA. (2017). Subunit-specific role for the amino-terminal domain of AMPA receptors in synaptic targeting. Proc Natl Acad Sci U S A 114, 7136–7141.
51. Durr KL, Chen L, Stein RA, De Zorzi R, Folea IM, Walz T, McHaourab HS &Gouaux E. (2014). Structure and Dynamics of AMPA Receptor GluA2 in Resting, Pre-Open, and Desensitized States. Cell 158, 778–792.
52. Erlenhardt N, Yu H, Abiraman K, Yamasaki T, Wadiche JI, Tomita S &Bredt DS. (2016). Porcupine Controls Hippocampal AMPAR Levels, Composition, and Synaptic Transmission. Cell Rep 14, 782–794.
53. Everett KV, Chioza B, Aicardi J, Aschauer H, Brouwer O, Callenbach P, Covanis A, Dulac O, Eeg-Olofsson O, Feucht M, Friis M, Goutieres F, Guerrini R, Heils A, Kjeldsen M, Lehesjoki AE, Makoff A, Nabbout R, Olsson I, Sander T, Siren A, McKeigue P, Robinson R, Taske N, Rees M & Gardiner M. (2007). Linkage and association analysis of CACNG3 in childhood absence epilepsy. Eur J Hum Genet 15, 463–472.
54. Floor K, Baroy T, Misceo D, Kanavin OJ, Fannemel M &Frengen E. (2012). A 1 Mb de novo deletion within 11q13.1q13.2 in a boy with mild intellectual disability and minor dysmorphic features. Eur J Med Genet 55, 695–699.
55. Fukaya M, Yamazaki M, Sakimura K & Watanabe M. (2005). Spatial diversity in gene expression for VDCCgamma subunit family in developing and adult mouse brains. Neurosci Res 53, 376–383.
56. Garcia-Nafria J, Herguedas B, Watson JF &Greger IH. (2016). The dynamic AMPA receptor extracellular region: a platform for synaptic protein interactions. J Physiol 594, 5449–5458.
57. Gardinier KM, Gernert DL, Porter WJ, Reel JK, Ornstein PL, Spinazze P, Stevens FC, Hahn P, Hollinshead SP, Mayhugh D, Schkeryantz J, Khilevich A, De Frutos O, Gleason SD, Kato AS, Luffer-Atlas D, Desai PV, Swanson S, Burris KD, Ding C, Heinz BA, Need AB, Barth VN, Stephenson GA, Diseroad BA, Woods TA, Yu H, Bredt D & Witkin JM. (2016). Discovery of the First alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptor Antagonist Dependent upon Transmembrane AMPA Receptor Regulatory Protein (TARP) gamma-8. J Med Chem 59, 4753–4768.

58. Gill MB, Kato AS, Roberts MF, Yu H, Wang H, Tomita S &Bredt DS. (2011). Cornichon-2 modulates AMPA receptor-transmembrane AMPA receptor regulatory protein assembly to dictate gating and pharmacology. J Neurosci 31, 6928–6938.
59. Greger IH, Watson JF & Cull-Candy SG. (2017). Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. Neuron 94, 713–730.
60. Gu X, Mao X, Lussier MP, Hutchison MA, Zhou L, Hamra FK, Roche KW & Lu W. (2016). GSG1L suppresses AMPA receptor-mediated synaptic transmission and uniquely modulates AMPA receptor kinetics in hippocampal neurons. Nat Commun 7, 10873.
61. Hackmann K, Matko S, Gerlach EM, von der Hagen M, Klink B, Schrock E, Rump A & Di Donato N. (2013). Partial deletion of GLRB and GRIA2 in a patient with intellectual disability. Eur J Hum Genet 21, 112–114.
62. Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, Park AR, Spiegelman D, Dobrzeniecka S, Piton A, Tomitori H, Daoud H, Massicotte C, Henrion E, Diallo O, Group SD, Shekarabi M, Marineau C, Shevell M, Maranda B, Mitchell G, Nadeau A, D'Anjou G, Vanasse M, Srour M, Lafreniere RG, Drapeau P, Lacaille JC, Kim E, Lee JR, Igarashi K, Huganir RL, Rouleau GA & Michaud JL. (2011). Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. Am J Hum Genet 88, 306–316.
63. Han W, Li J, Pelkey KA, Pandey S, Chen X, Wang YX, Wu K, Ge L, Li T, Castellano D, Liu C, Wu LG, Petralia RS, Lynch JW, McBain CJ & Lu W. (2019). Shisa7 is a GABAA receptor auxiliary subunit controlling benzodiazepine actions. Science 366, 246–250.
64. Hansen KB, Furukawa H &Traynelis SF. (2010). Control of assembly and function of glutamate receptors by the amino-terminal domain. Mol Pharmacol 78, 535–549.
65. Hawken NM, Zaika EI & Nakagawa T. (2017). Engineering defined membrane-embedded elements of AMPA receptor induces opposing gating modulation by cornichon 3 and stargazin. J Physiol.
66. Herguedas B, Garcia-Nafria J, Cais O, Fernandez-Leiro R, Krieger J, Ho H &Greger IH. (2016). Structure and organization of heteromeric AMPA-type glutamate receptors. Science 352, aad3873.
67. Herguedas B, Watson JF, Ho H, Cais O, Garcia-Nafria J &Greger IH. (2019). Architecture of the heteromeric GluA1/2 AMPA receptor in complex with the auxiliary subunit TARP gamma8. Science 364.
68. Hering H, Lin CC & Sheng M. (2003). Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J Neurosci 23, 3262–3271.
69. Herring BE, Shi Y, Suh YH, Zheng CY, Blankenship SM, Roche KW & Nicoll RA. (2013). Cornichon proteins determine the subunit composition of synaptic AMPA receptors. Neuron 77, 1083–1096.
70. Hollmann M, Maron C & Heinemann S. (1994). N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. Neuron 13, 1331–1343.
71. Hoshino H, Uchida T, Otsuki T, Kawamoto S, Okubo K, Takeichi M & Chisaka O. (2007). Cornichon-like protein facilitates secretion of HB-EGF and regulates proper development of cranial nerves. Mol Biol Cell 18, 1143–1152.
72. Huganir RL & Nicoll RA. (2013). AMPARs and synaptic plasticity: the last 25 years. Neuron 80, 704–717.
73. Amin JB, Salussolia CL, Chan K, Regan MC, Dai J, Zhou HX, Furukawa H, Bowen ME & Wollmuth LP. (2017). Divergent roles of a peripheral transmembrane segment in AMPA and NMDA receptors. J Gen Physiol 149, 661–680.
74. Armstrong N & Gouaux E. (2000). Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. Neuron 28, 165–181.
75. Armstrong N, Sun Y, Chen GQ & Gouaux E. (1998). Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature 395, 913–917.
76. Ayalon G & Stern-Bach Y. (2001). Functional assembly of AMPA and kainate receptors is mediated by several discrete protein-protein interactions. Neuron 31, 103–113.
77. Azumaya CM, Days EL, Vinson PN, Stauffer S, Sulikowski G, Weaver CD & Nakagawa T. (2017). Screening for AMPA receptor auxiliary subunit specific modulators. PLoS One 12, e0174742.
78. Ben-Yaacov A, Gillor M, Haham T, Parsai A, Qneibi M & Stern-Bach Y. (2017). Molecular Mechanism of AMPA Receptor Modulation by TARP/Stargazin. Neuron.
79. Blackstone CD, Moss SJ, Martin LJ, Levey AI, Price DL & Huganir RL. (2002). Biochemical characterization and localization of a non-N-methyl-D-aspartate glutamate receptor in rat brain. J Neurochem 58, 1118–1126.
80. Bonnet C, Leheup B, Beri M, Philippe C, Gregoire MJ &Jonveaux P. (2009). Aberrant GRIA3 transcripts with multi-exon duplications in a family with X-linked mental retardation. Am J Med Genet A 149A, 1280–1289.
81. Boudkkazi S, Brechet A, Schwenk J &Fakler B. (2014). Cornichon2 dictates the time course of

excitatory transmission at individual hippocampal synapses. Neuron 82, 848–858.
82. Brockie PJ, Jensen M, Mellem JE, Jensen E, Yamasaki T, Wang R, Maxfield D, Thacker C, Hoerndli F, Dunn PJ, Tomita S, Madsen DM &Maricq AV. (2013). Cornichons Control ER Export of AMPA Receptors to Regulate Synaptic Excitability. Neuron 80, 129–142.
83. Brown P, McGuire H & Bowie D. (2018). Stargazin and cornichon-3 relieve polyamine block of AMPA receptors by enhancing blocker permeation. J Gen Physiol 150, 67–82.
84. Burada AP, Vinnakota R & Kumar J. (2020). Cryo-EM structures of the ionotropic glutamate receptor GluD1 reveal a non-swapped architecture. Nat Struct Mol Biol 27, 84–91.
85. Cais O, Herguedas B, Krol K, Cull-Candy SG, Farrant M & Greger IH. (2014). Mapping the interaction sites between AMPA receptors and TARPs reveals a role for the receptor N-terminal domain in channel gating. Cell Rep 9, 728–740.
86. Carrillo E, Shaikh SA, Berka V, Durham RJ, Litwin DB, Lee G, MacLean DM, Nowak LM & Jayaraman V. (2020). Mechanism of modulation of AMPA receptors by TARP-gamma8. J Gen Physiol 152.
87. Chang PK, Verbich D & McKinney RA. (2012). AMPA receptors as drug targets in neurological disease--advantages, caveats, and future outlook. Eur J Neurosci 35, 1908–1916.
88. Chen C, Blitz DM & Regehr WG. (2002). Contributions of receptor desensitization and saturation to plasticity at the retinogeniculate synapse. Neuron 33, 779–788.
89. Chen C & Regehr WG. (2000). Developmental remodeling of the retinogeniculate synapse. Neuron 28, 955–966.
90. Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS & Nicoll RA. (2000). Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature 408, 936–943.
91. Chen L, Durr KL & Gouaux E. (2014). X-ray structures of AMPA receptor-cone snail toxin complexes illuminate activation mechanism. Science 345, 1021–1026.
92. Chen S & Gouaux E. (2019). Structure and mechanism of AMPA receptor - auxiliary protein complexes. CurrOpin Struct Biol 54, 104–111.
93. Chen S, Zhao Y, Wang Y, Shekhar M, Tajkhorshid E & Gouaux E. (2017). Activation and Desensitization Mechanism of AMPA Receptor-TARP Complex by Cryo-EM. Cell 170, 1234–1246 e1214.
94. Chen X, Aslam M, Gollisch T, Allen K & von Engelhardt J. (2018). CKAMP44 modulates integration of visual inputs in the lateral geniculate nucleus. Nat Commun 9, 261.
95. Chiyonobu T, Hayashi S, Kobayashi K, Morimoto M, Miyanomae Y, Nishimura A, Nishimoto A, Ito C, Imoto I, Sugimoto T, Jia Z, Inazawa J & Toda T. (2007). Partial tandem duplication of GRIA3 in a male with mental retardation. Am J Med Genet A 143A, 1448–1455.
96. Cho CH, St-Gelais F, Zhang W, Tomita S & Howe JR. (2007). Two families of TARP isoforms that have distinct effects on the kinetic properties of AMPA receptors and synaptic currents. Neuron 55, 890–904.
97. Collingridge GL, Olsen RW, Peters J &Spedding M. (2009). A nomenclature for ligand-gated ion channels. Neuropharmacology 56, 2–5.
98. Coombs ID, Soto D, Zonouzi M, Renzi M, Shelley C, Farrant M & Cull-Candy SG. (2012). Cornichons modify channel properties of recombinant and glial AMPA receptors. J Neurosci 32, 9796–9804.
99. Davies B, Brown LA, Cais O, Watson J, Clayton AJ, Chang VT, Biggs D, Preece C, Hernandez-Pliego P, Krohn J, Bhomra A, Twigg SRF, Rimmer A, Kanapin A, Consortium WGS, Sen A, Zaiwalla Z, McVean G, Foster R, Donnelly P, Taylor JC, Blair E, Nutt D, Aricescu AR, Greger IH, Peirson SN, Flint J & Martin HC. (2017). A point mutation in the ion conduction pore of AMPA receptor GRIA3 causes dramatically perturbed sleep patterns as well as intellectual disability. Hum Mol Genet 26, 3869–3882.
100. Dawe GB, Kadir MF, Venskutonyte R, Perozzo AM, Yan Y, Alexander RPD, Navarrete C, Santander EA, Arsenault M, Fuentes C, Aurousseau MRP, Frydenvang K, Barrera NP, Kastrup JS, Edwardson JM & Bowie D. (2019). Nanoscale Mobility of the Apo State and TARP Stoichiometry Dictate the Gating Behavior of Alternatively Spliced AMPA Receptors. Neuron 102, 976–992 e975.
101. Dawe GB, Musgaard M, Aurousseau MR, Nayeem N, Green T, Biggin PC & Bowie D. (2016). Distinct Structural Pathways Coordinate the Activation of AMPA Receptor-Auxiliary Subunit Complexes. Neuron 89, 1264–1276.
102. Hochman S. Spinal cord. Curr Biol. 2007 Nov 20; 17(22): R950-5. doi: 10.1016/j.cub.2007.10.014. PMID: 18029245.
103. Gill MB, Kato AS, Roberts MF, Yu H, Wang H, Tomita S &Bredt DS. (2011). Cornichon-2 modulates AMPA receptor-transmembrane AMPA receptor regulatory protein assembly to dictate gating and pharmacology. J Neurosci 31, 6928–6938.
104. Greger IH, Watson JF & Cull-Candy SG. (2017). Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. Neuron 94, 713–730.
105. Gu X, Mao X, Lussier MP, Hutchison MA, Zhou L, Hamra FK, Roche KW & Lu W. (2016). GSG1L suppresses AMPA receptor-mediated synaptic

transmission and uniquely modulates AMPA receptor kinetics in hippocampal neurons. Nat Commun 7, 10873.
106. Hackmann K, Matko S, Gerlach EM, von der Hagen M, Klink B, Schrock E, Rump A & Di Donato N. (2013). Partial deletion of GLRB and GRIA2 in a patient with intellectual disability. Eur J Hum Genet 21, 112–114.
107. Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, Park AR, Spiegelman D, Dobrzeniecka S, Piton A, Tomitori H, Daoud H, Massicotte C, Henrion E, Diallo O, Group SD, Shekarabi M, Marineau C, Shevell M, Maranda B, Mitchell G, Nadeau A, D'Anjou G, Vanasse M, Srour M, Lafreniere RG, Drapeau P, Lacaille JC, Kim E, Lee JR, Igarashi K, Huganir RL, Rouleau GA & Michaud JL. (2011). Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. Am J Hum Genet 88, 306–316.
108. Han W, Li J, Pelkey KA, Pandey S, Chen X, Wang YX, Wu K, Ge L, Li T, Castellano D, Liu C, Wu LG, Petralia RS, Lynch JW, McBain CJ & Lu W. (2019). Shisa7 is a GABAA receptor auxiliary subunit controlling benzodiazepine actions. Science 366, 246–250.
109. Hansen KB, Furukawa H &Traynelis SF. (2010). Control of assembly and function of glutamate receptors by the amino-terminal domain. Mol Pharmacol 78, 535–549.
110. Hawken NM, Zaika EI & Nakagawa T. (2017). Engineering defined membrane-embedded elements of AMPA receptor induces opposing gating modulation by cornichon 3 and stargazin. J Physiol.
111. Herguedas B, Garcia-Nafria J, Cais O, Fernandez-Leiro R, Krieger J, Ho H &Greger IH. (2016). Structure and organization of heteromeric AMPA-type glutamate receptors. Science 352, aad3873.
112. Herguedas B, Watson JF, Ho H, Cais O, Garcia-Nafria J & Greger IH. (2019). Architecture of the heteromeric GluA1/2 AMPA receptor in complex with the auxiliary subunit TARP gamma8. Science 364.
113. Hering H, Lin CC & Sheng M. (2003). Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J Neurosci 23, 3262–3271.
114. Herring BE, Shi Y, Suh YH, Zheng CY, Blankenship SM, Roche KW & Nicoll RA. (2013). Cornichon proteins determine the subunit composition of synaptic AMPA receptors. Neuron 77, 1083–1096.
115. Hollmann M, Maron C & Heinemann S. (1994). N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. Neuron 13, 1331–1343.
116. Hoshino H, Uchida T, Otsuki T, Kawamoto S, Okubo K, Takeichi M & Chisaka O. (2007). Cornichon-like protein facilitates secretion of HB-EGF and regulates proper development of cranial nerves. Mol Biol Cell 18, 1143–1152.
117. Huganir RL & Nicoll RA. (2013). AMPARs and synaptic plasticity: the last 25 years. Neuron 80, 704–717.
118. Jackson AC & Nicoll RA. (2011). The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. Neuron 70, 178–199.
119. Jin R, Singh SK, Gu S, Furukawa H, Sobolevsky AI, Zhou J, Jin Y & Gouaux E. (2009). Crystal structure and association behaviour of the GluR2 amino-terminal domain. Embo J 28, 1812–1823.
120. Kalashnikova E, Lorca RA, Kaur I, Barisone GA, Li B, Ishimaru T, Trimmer JS, Mohapatra DP & Diaz E. (2010). SynDIG1: an activity-regulated, AMPA-receptor-interacting transmembrane protein that regulates excitatory synapse development. Neuron 65, 80–93.
121. Kang MG, Nuriya M, Guo Y, Martindale KD, Lee DZ &Huganir RL. (2012). Proteomic analysis of AMPA receptor complexes. J Biol Chem.
122. Kato AS, Burris KD, Gardinier KM, Gernert DL, Porter WJ, Reel J, Ding C, Tu Y, Schober DA, Lee MR, Heinz BA, Fitch TE, Gleason SD, Catlow JT, Yu H, Fitzjohn SM, Pasqui F, Wang H, Qian Y, Sher E, Zwart R, Wafford KA, Rasmussen K, Ornstein PL, Isaac JT, Nisenbaum ES, Bredt DS & Witkin JM. (2016). Forebrain-selective AMPA-receptor antagonism guided by TARP gamma-8 as an antiepileptic mechanism. Nat Med 22, 1496–1501.
123. Kamalova A, Nakagawa T. AMPA receptor structure and auxiliary subunits. J Physiol. 2021 Jan; 599(2): 453-469. doi: 10.1113/JP278701. Epub 2020 Feb 18. PMID: 32004381; PMCID: PMC7392800.
124. Kato AS, Gill MB, Ho MT, Yu H, Tu Y, Siuda ER, Wang H, Qian YW, Nisenbaum ES, Tomita S &Bredt DS. (2010). Hippocampal AMPA Receptor Gating Controlled by Both TARP and Cornichon Proteins. Neuron 68, 1082–1096.
125. Jackson AC & Nicoll RA. (2011). The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. Neuron 70, 178–199.
126. Hanley JG. Subunit-specific trafficking mechanisms regulating the synaptic expression of Ca(2+)-permeable AMPA receptors. Semin Cell Dev Biol. 2014 Mar; 27:14-22.
127. J.D. Shepherd, R.L. Huganir The cell biology of synaptic plasticity: AMPA receptor trafficking Annu Rev Cell Dev Biol, 23 (2007), pp. 613-643.

This page is intentionally left blank

Global Journal of Medical Research: a
Neurology & Nervous System
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Redefining Psychopathology from an Anatomical and Functional Perspective

By Michael Raymond Binder, M.D.

Abstract- After more than a century of scientific study and philosophical debate, the distinction between psychological and psychiatric illness remains unclear. The challenge of distinguishing between these seemingly different forms of pathology continues to cause errors in patient referral, delays in therapeutic progress, and, in some cases, an actual worsening of symptoms due to the application of inappropriate treatment techniques. The fundamental cause of the confusion has been a lack of clarity about the anatomy of the cognitive-emotional system and the mechanism by which psychological, emotional, and behavior abnormalities are produced. Also lacking is an understanding of how intrapsychic tension affects neurophysiology and vice-versa, as this too is dependent upon a more comprehensive understanding of the cognitive-emotional system.

Keywords: pathophysiology of psychiatric disorders, neuronal hyperexcitability, biomarkers, mood stabilizers, genetic engineering, cognitive-emotional system, mind-brain duality, neurosis, psychotherapy, stigma.

GJMR-A Classification: LCC: RC454, RC455, RC456

Strictly as per the compliance and regulations of:

Redefining Psychopathology from an Anatomical and Functional Perspective

Michael Raymond Binder, M.D.

Abstract- After more than a century of scientific study and philosophical debate, the distinction between psychological and psychiatric illness remains unclear. The challenge of distinguishing between these seemingly different forms of pathology continues to cause errors in patient referral, delays in therapeutic progress, and, in some cases, an actual worsening of symptoms due to the application of inappropriate treatment techniques. The fundamental cause of the confusion has been a lack of clarity about the anatomy of the cognitive-emotional system and the mechanism by which psychological, emotional, and behavior abnormalities are produced. Also lacking is an understanding of how intrapsychic tension affects neurophysiology and vice-versa, as this too is dependent upon a more comprehensive understanding of the cognitive-emotional system. In this discussion, the anatomical and functional relationship between the mind and the brain will be more clearly elucidated, and the different forms of psychopathology will be more clearly defined. Achieving a better understanding of human psychophysiology and greater clarity about what pathological processes are being treated could streamline the care of patients and lead to a more speedy and sustained resolution of symptoms. It could also increase patient confidence and patient compliance, two factors that are sorely lacking in an era in which less than half of all patients are receiving the help they really need. Also presented will be an evidence-based way to objectively determine which patients are at increased risk of developing any of a wide range of psychiatric and chronic medical conditions and the measures that they can take to reduce that risk before symptoms even begin.

Keywords: pathophysiology of psychiatric disorders, neuronal hyperexcitability, biomarkers, mood stabilizers, genetic engineering, cognitive-emotional system, mind-brain duality, neurosis, psychotherapy, stigma.

I. Introduction

After more than a century of scientific study and philosophical debate, psychological, emotional, and behavioral disorders are still being classified descriptively and treated with various psychological, behavioral, and medical techniques without a clear understanding of what pathological process is being treated or how the therapy imparts its therapeutic effects. Unsurprisingly, treatment outcomes continue to be unacceptably poor [1, 2], and patient faith in the behavioral healthcare system continues to be concerningly low, with nearly half of all symptomatic persons failing to seek professional help [3]. Moreover, their lack of faith in the behavioral healthcare system is partly shared by third-party payers, as behavioral health services continue to be reimbursed at lower rates than most other health conditions. In the meantime, the mental health of the world's population continues to decline, and the mental health crisis affecting many subpopulations continues to spin out of control. This underscores the need for a radical change in the way that mental illness is understood and the way that various therapies are matched to patients who present for treatment.

In this article, the multifaceted disorder called "psychopathology" will be defined in a radically new way. It will be reconceptualized as a group of psychological, emotional, and behavioral abnormalities that can be divided into two structurally different but functionally overlapping categories; namely, those that are rooted in psychological abnormalities, and those that are rooted in biological abnormalities. Based on this reconceptualization, which is guided by a more comprehensive understanding of the anatomy of the cognitive-emotional system, the assessment of psychological, emotional, and behavioral disorders will be markedly simplified, and the treatment of these disorders will become far more targeted. In a field that continues to rely on symptoms rather than pathology to guide treatment, the new paradigm that will be discussed could be transformative. We will see how it could completely reshape the way that psychiatric disorders are treated, reduce the stigma of mental illness, and bring an end to the mental health crisis.

II. Anatomy of the Cognitive-Emotional System

The logical place to start in reconceptualizing mental illness is with a reevaluation of the structural and functional anatomy of the cognitive-emotional system. Although many of the early pioneers in the medical field, such as Socrates, Plato, Descartes, Popper, and Eccles, believed that the essence of the mind was different than that of the brain, the concept of a mind-brain duality was largely replaced by the reductionist view that all psychopathology could ultimately be understood through a more comprehensive understanding of brain structure and function. Yet despite moderns advances in neuroscience, we are still no closer to distinguishing the psychological from the biological underpinnings of psychiatric disorders.

Author: Adult and Adolescent Psychiatry, 5 Revere Drive, Suite 200, Northbrook, USA. e-mail: mbinder@drmichaelbinder.com

However, the recent explosion of near-death testimonials is beginning to reshape the way we conceptualize the cognitive-emotional system. According to researchers who have studied these experiences, consciousness continues even after the brain stops working and the heart stops beating [4-9]. Most of those who claim to have had a near-death experience (NDE) say that they had left their physical bodies and continued to think, perceive, and remember things that, based on a reductionist view of brain function, would have been physically impossible [4-9]. However, many of these accounts have been corroborated by factual information that the NDErs could not possibly have known had they not actually separated from their physical bodies and retained their cognitive, sensory, and memory functions [4-9]. The evidence is now so strong that, in 2022, the New York Academy of Sciences published a multidisciplinary consensus statement concluding that "NDEs are not hallucinations or illusions but rather evidence that life continues after death" [10].

In an effort to experimentally explore the possibility that the mind is a separate entity that interacts with the brain, Cerf et al. [11] found that willful thoughts and their associated emotions readily stimulated specific neurons when subjects were asked to perform specific mental tasks. Conversely, Wilder Penfield [12], about a century earlier, had found that stimulating the brain in specific places caused his patients to experience specific thoughts and emotions. More recently, it was demonstrated that the behavior of laboratory animals could be influenced by stimulating or inhibiting specific neurons [13, 14]. These later experiments helped clarify the means by which the touch of Penfield's electrical probe was stimulating related thoughts and emotions. Taken together, these observations provide compelling evidence that the mind and the brain are separate entities that influence each other.

Another line of evidence in support of a mind-brain duality is the tremendous explanatory power that it has. To begin with, it seems intuitively obvious that the mind and the brain are not the same thing. Notice that in referring to the brain, one naturally says "my brain" just as one says "my heart," "my lungs," or "my kidneys." Thus, one naturally refers to the brain as a body-part rather than as "the self." That begs the question: who am I? Also, it is intuitively obvious that we train our brains rather than being robotically led around by our brains. We teach our brains to coordinate walking, talking, reading, writing, and complex athletic movements. Though most of us come into the world with a fully functioning brain, we cannot perform any of the aforementioned skills until we teach our brains to help us perform them. So that raises the question: who is doing the teaching?

The same question was asked by Nobel Prize laureate Francis Crick in his thought-provoking article, "Function of the Thalamic Reticular Complex: The Searchlight Hypothesis" [15]. In the article, Crick references the pioneering work of Anne Treisman and her colleagues, which suggests that there is an "attentional searchlight" that scans and then selects information coming into the thalamic reticular nucleus (TRN). Recall that the thalamus is the central hub of the brain where nearly all sensory input is relayed directly [15]. The searchlight is not proposed to light up areas of a completely dark landscape but rather, like a searchlight at dusk, is thought to illuminate those parts of a dimly lit landscape that are of particular interest to it. According to the investigators, it does this by stimulating select assemblies of cells in the TRN. Although the collaterals of these cells are largely (if not entirely) inhibitory, specialized burst activity allows them to enhance the activation of select neural networks when stimulated [16]. The mechanism by which this occurs is based on the unique physiology of thalamic neurons. Elegant studies on thalamic slices from the guinea pig have confirmed that when hyperpolarized thalamic neurons are stimulated, they respond by producing a single spike (or short burst of rapid spikes) followed by a brief period during which they are unresponsive to continued stimulation [17-19]. This implies that when the attentional searchlight turns its attention to a point of interest, the excitatory phase initiates a wave of inhibition that turns down irrelevant information, while the refractory phase allows activity in select circuits to be turned up. In this way, the TRN allows the searchlight, which could be nothing other than the human mind, to scan the information coming into the thalamus, highlight select inputs, and then shift attention to other areas of potential interest. This could explain how, on a psychophysiological basis, the mind is able to contemplate or, conversely, repress various thoughts, emotions, and images. It suggests that the mind, like a pilot seated in the cockpit of an airplane, interacts with the brain in much the same way that we interact with our computers. The TRN would be the computer monitor, and the individual neurons would be the keys on the keyboard. Furthermore, the high degree of specificity of various neuron-types [20], and the equally precise topography of the TRN [15, 20, 21], could help explain why damage to specific neurons causes functional deficits that can only be recovered by training other neurons to take over the functions of the damaged neurons.

Another line of evidence for a mind-brain duality is the ability of mental and emotional stress to dysregulate brain function. What we call "stress" cannot logically be experienced by the brain because the brain is merely a collection of fats, proteins, and carbohydrates. Stress is a human emotional experience,

and this experience, like other emotional experiences, such as love, joy, fear, and anger, cannot possibly be attributed to the physical components of the brain. However, from the perspective of a mind-brain duality, the stressed mind, like a whirling wind, could be hyper-activating specific circuits in the brain. This effect would be akin to using mental effort to increase the force with which a muscle contracts.

III. Mind-Brain Communications

However, this raises the same question that René Descartes was asked in the 17th century: if the mind is neither visible nor tangible, how could it communicate with the physical brain?

The answer to this question appears to be provided by several key discoveries that were made in biology, chemistry, and physics over the last few centuries. From the field of biology came the discovery of the neuron; from the field of chemistry came the discovery of electrochemical processes; and from the field of physics came the discovery of electromagnetic energy. Taken together, these discoveries seem to provide the answer to the mind-brain problem. The mind and the brain could communicate with each other via the induction of magnetic fields. The mind, being an energy body, could induce magnetic fields as it thinks and emotes; and the brain, being an electrical organ, could induce magnetic fields as neurons depolarize and repolarize. Of course, the idea that electromagnetic energy could communicate intelligible information should come as no surprise to us. Electromagnetic signals are the means by which television programming is relayed to our television sets and intelligible information is relayed across the internet. The idea of mind-brain communication via electromagnetic energy is also supported by the fact that all forms of sensory input, including vibration (sound), mechanical (touch), and chemo (taste), are converted into electrical signals en-route to the brain. This conversion prepares the information to be relayed to the mind via electromagnetic energy.

IV. Integrating Mental Processes with Neurological Processes

The sharing of electromagnetic energy by the mind and the brain helps to explain, for the first time, the psychophysiological distinction between conscious, preconscious, and unconscious thoughts as described by the renowned Austrian psychiatrist Sigmund Freud. Although Freud used his structural theory of the psyche to better understand and treat his patients, he did not, in his work, relate it to the workings of the brain [22]. However, a mind-brain duality of the cognitive-emotional system offers a coherent anatomical and functional explanation of these three operations of the mind. Conscious mental processing would describe the state in which neurologically-induced magnetic fields were fully synchronized with mentally-induced magnetic fields; preconscious mental processing would describe the state in which neurologically-induced magnetic fields were partially synchronized with mentally-induced magnetic fields; and unconscious mental processing would describe the state in which neurologically-induced magnetic fields were minimally or completely unsynchronized with mentally-induced magnetic fields. This could help explain why, for instance, there is sometimes a delay between the time that one tries to recall someone's name and the time that one actually recalls the name. The delay would represent the time that it took for neurological signals that had previously been associated with the person's name to synchronize with the mental signals that were attempting to reactivate them. The mind-brain duality could also explain why most of our thought-life would, as Freud theorized, be unconscious. The mind, being a body of electromagnetic energy, would be processing information millions of times faster than the brain.

Now then, if we were to seriously consider the possibility of a mind-brain duality, the brain would be reduced to a neurological switchboard that primarily relays electrical signals between the mind and the body. If this were the case, it would logically redirect our attention to the mind as the chief source of psychopathology. However, that would raise the question of what, from a psychological standpoint, psychopathology really is.

At its most fundamental level, "psycho," meaning psychological, and "pathology," meaning disease, refers to unhealthy ways of thinking. But that raises the question of what one means by "unhealthy." To answer that question, we must delve into the purpose of life because the true health of the psyche involves an actualization of who we really are and where we are ultimately going. Of course, there are countless different philosophies about this, but there is only one that fully resonates with the human spirit. That is the belief that life is eternal because the idea of permanent annihilation is contrary to the eternal concepts of faith, hope, and love. In addition, if there were no life after death, it would render all pain, sorrow, and suffering in this life meaningless. It would also render all of our relationships meaningless, all of our accomplishments meaningless, and the whole dying process meaningless. On the other hand, if life continued after death, everything that we experience in this life would have eternal value. Being kind to others would have eternal value because our relationships would continue forever; working hard would have eternal value because it would teach us the value of eternal rest; the experience of suffering would have eternal value because it would create gratitude for eternal happiness; and the dying process would have eternal value because it would help us truly appreciate all that we have been given. Therefore, one form of

unhealthy thinking would be to assume a nihilistic attitude—a belief that life is limited to this world and that, contrary to the eternal concepts of faith, hope, and love, there is nothing to look forward to beyond the grave. In its most pathological form, this would include a lack of respect for the moral code or the dignity of other human beings.

Until recently, there had been little scientific evidence that life continued after death. However, as previously discussed, the rapidly growing body of NDEs is beginning to change all that. NDEers consistently affirm that consciousness, memories, and relationships continue even after the mind leaves the body. These reports provide evidence that is even stronger than traditional scientific investigation because most of them, though being independent of one another, concur with one another, even across diverse languages, cultures, and religions. Moreover, the observers typically have nothing to gain. On the contrary, they often have much to lose because their testimonies tend to be frowned upon and discounted by those who have never had such experiences. This contrasts sharply with traditional scientific research, which is often reported by only one observer (or handful of observers), is generally accepted by others based on faith in the scientific process, and is typically supported by monetary grants. Another observation that supports the validity of NDEs is that the impressions made on experiencers almost universally affirm what theologians have been saying for thousands of years…that the purpose of life on earth is to grow in closeness to God and one's fellow human beings.

A duality of mind and brain makes this spiritual growth possible because it implies that the mind has two natures; it has a carnal nature that overseas the physical body, and it has a moral nature that aligns with moral precepts. The carnal mind would relate to Freud's concept of the "id," and the moral mind would relate to Freud's concept of the "superego." However, just as the carnal mind would be troubled if the body's sense organs were to convey painful signals to it, the moral mind would be troubled if it were to violate its moral obligations. This can create intrapsychic conflict because we cannot always satisfy our moral obligations without incurring some degree of carnal discomfort. According to Freud, this intrapsychic conflict could be repressed and then re-emerge as various behavioral and somatic symptoms that he referred to as "neuroses." The problem with this theory, however, is that intrapsychic conflict is a normal consequence of our functional anatomy. It would be abnormal not to feel conflicted at times. That raises the question of whether Freud's patients had some overlapping problem that was driving their symptoms.

V. The Multi-Circuit Neuronal Hyperexcitability Hypothesis of Psychiatric Disorders

An emerging hypothesis contends that a pathological hyper-reactivity or "hyperexcitability" of the neurological system could abnormally amplify and perpetuate the thoughts and emotions with which the mind is grappling. According to the Multi-Circuit Neuronal Hyperexcitability (MCNH) hypothesis [23], psychiatric symptoms are the consequence of a pathological hyperactivity of the brain circuits that correspond to them. Thus, for example, abnormally-elevated and persistent feelings of anxiety would be the consequence of pathological hyperactivity in anxiety circuits; abnormally-elevated and persistent feelings of depression would be the consequence of pathological hyperactivity in depressive circuits; abnormally-elevated and persistent feelings of anger would be the consequence of pathological hyperactivity in irritability circuits; etc… Beyond causing mental and emotional symptoms, pathological hyperactivity in specific brain circuits could cause somatic symptoms, such as migraine headaches, musculoskeletal pain, and irritable bowel [24]. Without any knowledge of this, and in the absence of any demonstrable end-organ disease, it would have been logical for Freud to assume that such symptoms were purely psychosomatic in nature.

It is also noteworthy that the term "neurosis" did not originate with Freud. Rather, it originated with Sir William Cullen about a century earlier [25]. Cullen, a Scottish physician, coined the term to describe a "general affection of the nervous system." Cullen hypothesized that this abnormality was rooted in a sickness (*osis)* of a nerve, (*neuron)*; hence the term *neurosis.* Cullen divided neuroses into four basic types: melancholia (depressive), mania (euphoric), dementia (schizophrenic), and idiotism (deranged) [25]. These would roughly correspond to the different degrees and manifestations of psychopathology caused by different levels of neuronal hyperexcitability [20].

An associated finding that can likewise be explained by neuronal hyperexcitability is the tendency for psychiatric symptoms to cycle. Because the neurological system is highly interconnected, the chances that one hyperexcitable circuit would, like a short-circuit in a wired electrical system, aberrantly fuel hyperactivity in another circuit would tend to increase as the level of excitation in the system increased. This could help explain why the frequency of symptom-cycling tends to increase as one's level of stress and, over time, the frequency of psychiatric episodes increases due to the kindling effect of persistent stress on untreated neuronal hyperexcitability [26]. However, due to synaptic pruning, the number of neuron-to-neuron connections decreases dramatically from early

childhood to early adulthood [27]. This could help explain why children who develop psychiatric symptoms tend not to show any symptom-cycling until adolescence or early adulthood. On the other hand, childhood is generally a time of relatively low stress; hence, even in children who inherit the genes for neuronal hyperexcitability, there is often not enough excitation in the system to precipitate any symptom-cycling (or perhaps any psychiatric symptoms) until the child's stress levels begin to rise appreciably. This could help explain why most children who inherit the genes for neuronal hyperexcitability do not become symptomatic until adolescence, when the stress of transitioning from childhood to adulthood begins to fan the flames of neuronal hyperexcitability.

Beyond these observations, the aberrant circuit-induction hypothesis could help explain why different persons tend to have their own characteristic cycling frequency. The average adult brain has between 73 and 89 billion neurons [28], and with each neuron forming connections with up to 15,000 other neurons [29] there is a lot of room for variance in the number of neuron-to-neuron connections that any individual's brain can have. Persons with a greater number of neuron-to-neuron connections would be expected to cycle more rapidly than persons with fewer connections, and those with the fewest connections would be expected to cycle the least rapidly or perhaps not at all [30].

The idea that most of the common psychiatric disorders are rooted in a hyperexcitability of the neurological system is also supported by the effect that neuronal hyperexcitability would have on the mind-brain dynamic and, consequently, one's thoughts, emotions, and behavior. Stress in the mind would tend to overstimulate the brain. The hyperactive brain would then further stimulate the mind, thus creating a vicious cycle of mutual overstimulation between the mind and the brain. As the emotional tension continued to ramp up, various cognitive-emotional states would be experienced, and various defense mechanisms would be employed. The specific constellation of thoughts, emotions, defense mechanisms, and willful choices would determine which specific psychiatric syndrome (or syndromes) a particular individual would have [24]. In some cases, the level of neuronal hyperexcitability could be so high that various psychiatric symptoms could be induced even in the absence of any significant environmental stress or intrapsychic conflict. Of course, the pattern of neuronal firing could change at any time, thus explaining why a given individual could be diagnosed with one psychiatric syndrome at one point in time, and a different psychiatric syndrome at another point in time.

Although William Cullen did not elaborate on neurosis in this much detail, he recognized that it could cause a wide range of psychopathology from major depressive disorder to bipolar disorder and from generalized anxiety disorder to schizophrenia. In contrast, Freud divided psychopathology into two basic types: neurosis, in which he hypothesized that "the ego suppresses part of the id out of allegiance to reality," and psychosis, in which the ego "lets itself be carried away by the id and detached from part of reality." From the perspective of the MCNH hypothesis in conjunction with a mind-brain duality of the cognitive-emotional system, this would translate to the mildly hyperexcitable brain allowing the mind to remain partially in control of one's thoughts and emotions (i.e., neurosis), and the severely hyperexcitable brain completely usurping the mind's willful thoughts and emotions (i.e., psychosis).

Although any mental, emotional, or biological factor that increases neuronal excitability could, in theory, precipitate psychiatric symptoms, most of the candidate genes that have been linked to the major psychiatric disorders code for proteins that are involved in the regulation of neuronal firing [31-43]. This suggests that a constitutional hyperexcitability of the neurological system is, in the vast majority of cases, the underlying driver of psychiatric symptoms and that that constitutional abnormality is the consequence of gene variants whose protein products fail to adequately regulate the firing of neurons.

VI. Structural and Functional Integration of Psychopathology

The difference between the MCNH classification of psychopathology and the Freudian classification of psychopathology is that the former integrates psychological and neurological function in conceptualizing the pathology. Accordingly, it divides psychopathology into two functionally distinct groups: psychosocial (due to extreme self-centeredness, immorality, and lack of respect for the dignity of others); and neuropsychiatric (due to neuronal hyperexcitability). Some of those in the first group may actually have *hypo*-excitable neurological systems [30], thus causing them to be relatively unemotional, matter-of-fact, and insensitive to the feelings of others [44, 45]. Such persons have been referred to clinically as "primary psychopaths" [45].

In contrast, persons in the second group are constantly hounded by recurrent signals from their hyperexcitable brains, thus causing their thoughts and emotions to keep replaying like a broken record. This may include physical restlessness, emotional hypersensitivity, persistent or cyclic anxiety, depression, irritability, euphoria, distractibility, impulsivity, insomnia, energy changes, persistent grief, substance misuse, somatic symptoms, or any other symptom that characterizes the common psychiatric disorders. If the neuronal hyperexcitability were in the mild-to-moderate range, the affected person would manifest what Freud called "neurosis." If the neuronal hyperexcitability were

in the moderate-to-severe range, the affected person would manifest what Freud called "psychosis." Hallucinations would occur when neuronal signaling in the associated sensory pathways became so high that the input was perceived as coming from the environment. Similarly, delusional thinking would occur when the intensity of internally-driven thoughts and emotions, which is normally lower than that of environmentally-driven thoughts and emotions, became so high that it was thought to reflect external rather than internal reality.

Of course, psychosocial and neuropsychiatric forms of psychopathology are by no means mutually exclusive. A person who is morally approbate could also have a hyperexcitable neurological system. In such cases, the neuronal hyperexcitability trait would tend to accentuate any psychopathic tendencies, especially as the individual began to encounter the stress of adolescence. However, due to the activating effect of neuronal hyperexcitability, such individuals, who have been referred to clinically as "secondary psychopaths," would tend to be highly reactive, anxious, and impulsive in comparison to the cold, callous, and calculating nature of primary psychopaths [44-46]. Yet because of the aforementioned differences in age-of-onset and emotional temperament, it can appear as though primary psychopathy is more genetically-based, whereas secondary psychopathy is more environmentally-based. What is more likely, however, is that both are equally genetically-based but with neurophysiological traits that are at opposite ends of the neuronal excitability spectrum: *hypo*-excitable neurons in the primary psychopath, and *hyper*-excitable neurons in the secondary psychopath [30]. This would allow primary psychopathy to grow out of a hedonistic disrespect for others in comparison to secondary psychopathy, which would grow out of a defensive disrespect for others. Primary psychopathy would be an emotional under-reactivity that was relatively unaffected by environmental stress, whereas secondary psychopathy would be an emotional hyper-reactivity that was highly affected by environmental stress.

Acquiring a better understanding of these two disorder-types is important because primary psychopathy, being an emotional deficit, would not be very amenable to pharmacotherapy, whereas secondary psychopathy, being an emotional disturbance, would be highly amenable to brain-calming drugs. The other reason that distinguishing between these two disorder-types is important is that a hyperexcitable brain, irrespective of a person's moral disposition or reason for seeking treatment, tends to both instigate and exacerbate intrapsychic tension [21]. Hence, attempting to psychotherapeutically treat psychopathology that is fueled by neuronal hyperexcitability can further stimulate circuits that are already pathologically hyperactive, thus placing affected persons at risk for regression. This risk would be greatest in those patients with the highest levels of neuronal excitability, irrespective of their symptom-based diagnosis.

Although the neurophysiological underpinnings of psychopathology were unclear to Freud, he learned clinically that he should limit his psychoanalytic practice to neurotic-range patients (i.e., those who would have had only mild or moderate levels of neuronal hyperexcitability). In such patients, he assumed that intrapsychic conflict was the primary driver of the symptoms; hence, he believed that their symptoms could be reduced if they could resolve their intrapsychic conflicts. Although this may be true, intrapsychic conflict is a normal part of life and, therefore, should not in itself be considered pathological. What creates the psychiatric symptoms is the abnormal amplifying effect that neuronal hyperexcitability has on cognitive-emotional processes.

VII. How to Identify the Neuronal Hyperexcitability Trait

That raises an important question: is there some objective way to determine whether a person's neurological system is hyperexcitable? If so, correcting that abnormality as a first-line intervention in affected persons would not only streamline treatment, but it would also reduce the risk of regression in the event that complementary psychotherapy were needed.

Until recently, there had been no objective way to identify the neuronal hyperexcitability trait. However, an explosion of recent studies has identified a link between resting vital-sign measurements and the later development of various psychiatric and general medical conditions. In a longitudinal study involving more than one million men in Sweden, Latvala et al. [47] found that subtle elevations in resting heart rate (RHR) were predictive of the later development of generalized anxiety disorder, obsessive-compulsive disorder, and schizophrenia. Similarly, Blom et al. [48] found that adolescent girls with emotional disorders had increased resting respiratory rates (RRR) in comparison to healthy controls. Persons with higher resting heart and respiratory rates have also been found to be at increased risk of developing a wide range of chronic medical conditions, including diabetes [49-52], high blood pressure [53-55], cardiovascular disease [56-61], cerebrovascular disease [62-64], cancer [64-66], dementia [67], and all-cause mortality [64, 68]. The subtle vital-sign elevations with which these conditions are associated are thought to be the consequence of a tonic elevation in basal neurological activity in those persons who inherit the genes for neuronal hyperexcitability [69]. This is the MCNH explanation for why the lifespan of persons with severe mental illness tends to be much shorter than the general population

[69]. The reason that psychiatric symptoms tend to precede the development of diagnosable physical abnormalities is that the cognitive-emotional system is more expressive of neuronal excitation than other organs and systems of the body [70]. The physical consequences tend to be delayed because they express the gradual erosive effects of neuronal hyperexcitability, which can take years or even decades to develop.

Thus, there is mounting evidence that the neuronal hyperexcitability trait can be identified objectively [30, 69]. It has been estimated that, in the absence of any significant cardiorespiratory disease, confounding medications, or substances of abuse, an RHR above 75 beats/min or an RRR above 15 breaths/min is indicative of the neuronal hyperexcitability trait. Notably, in the more than 300 consecutive outpatients that I have studied thus far, resting heart and respiratory-rate measurements have proven to be more sensitive in detecting the neuronal hyperexcitability trait than formal clinical assessments. Then again, any person who presents for treatment is likely to be a carrier of the neuronal hyperexcitability trait, else he or she would probably not be presenting for treatment. This is also important to understand from a medical standpoint because, as previously discussed, the neuronal hyperexcitability trait increases one's vulnerability to developing any of a wide range of general medical conditions. Hence, treating the abnormality early in life may be as important medically as it is psychiatrically.

VIII. Discussion

In the hope of streamlining treatment and improving clinical outcomes, the goal of this article was to differentiate the various forms of psychopathology as opposed to the different diagnostic categories that are described in the Diagnostic and Statistical Manual of Mental Disorders. What was discovered was that re-evaluating psychopathology from an anatomical and functional perspective yielded a very simple dichotomy of illness-types; namely, 1) psychosocial pathology due to extreme self-centeredness, immorality, and lack of respect for the dignity of others; and 2) neuropsychiatric pathology due to a hyperexcitability of the neurological system. This is based on a mind-brain duality of the cognitive-emotional system and the emerging hypothesis that psychiatric symptoms are driven by a pathological elevation in the activity of the neuronal circuits that correspond to them.

A major barrier to effective treatment in behavioral healthcare continues to be the application of various treatment techniques without a clear understanding of how those techniques confer their therapeutic effects or even what pathological process they are treating. Many patients who are being treated with psychotherapy alone should also be treated with medication, and many patients who are being treated with medication should be treated with different kinds of medication than they are currently being prescribed. This not only continues to delay clinical improvement but it also drains clinical resources and continues to perpetuate a lack of public confidence in the behavioral healthcare system. Moreover, when psychotherapy targets psychological symptoms that are actually rooted in neuropathology, the therapist and the patient may never get around to addressing the unhealthy attitudes and dysfunctional core beliefs that should be the primary focus of psychotherapy. This underscores the need to more accurately distinguish symptoms that are rooted in psychology from those that are rooted in biology.

As previously discussed, the vast majority of patients who present for psychotherapy are actually suffering from a neurologically-based abnormality; namely, neuronal hyperexcitability. Yet some of these patients may not be open to the idea that their symptoms are neurologically-based. Also, some of them may fear the stigma of taking medication for what they perceive to be a purely psychological problem. For such patients, resting vital-sign measurements may provide the kind of objective evidence that they need to believe that medical intervention is appropriate. These measurements may also help clinicians either validate or invalidate their subjective clinical impressions.

For those patients whose symptoms are primarily rooted in neuronal hyperexcitability, both natural and pharmacological interventions should be discussed. Natural interventions include stress-reduction, establishment of an early sleep schedule, moderate exercise, avoidance of caffeine and other psychostimulants, minimization of refined sugar, and meditative practices. For patients with low-to-moderate-range neuronal hyperexcitability, these interventions may be adequate. However, for those with higher levels of neuronal hyperexcitability, natural interventions may neither be sufficient nor practically doable because of the disruptive effect that higher levels of neuronal hyperexcitability have on self-discipline. Also, the majority of these patients have chronic insomnia, which robs their brains of their primary way to reduce their excitability. For all of these reasons, pharmaceutical agents that reduce neuronal excitability will usually be needed in such patients. These drugs, which in neurology are known as "anticonvulsants" but in psychiatry are known as "mood stabilizers," are fast-acting, safe, and non-addictive. Also, unlike antidepressants, mood stabilizers can easily be stopped and started, and they are generally effective in long-term use without the need for further dosage adjustment or medication changes.

Although mood stabilizers, which could more aptly be called "neuroregulators" [71] in light of their

neurophysiological effects, have been in psychiatric use for more than fifty years, they have been sorely underutilized due to the traditional practice of symptom-based treatment. Fortuitously, however, the increasing acceptance of the "bipolar spectrum" as a dimensional diagnostic classification is helping to identify more patients who could benefit from neuroregulator therapy [72]. Note, however, that this is still a symptom-based treatment approach. What is desperately needed in psychiatry is a deeper understanding of mental illness that would allow a shift from symptom-based treatment to pathology-based treatment. The MCNH hypothesis in conjunction with a mind-brain duality of the cognitive-emotional system offers such a paradigm shift because it identifies the core biological abnormality that drives the symptoms not just of bipolar spectrum disorders but of virtually all of the common psychiatric disorders. Moreover, because the MCNH approach to treatment focuses on correcting the electrical abnormally that underlies the chemical imbalances in psychiatric disorders, neuroregulators can safely be combined with one another in a technique called "focused neuroregulation" [73]. Unlike with other psychotropic drugs, combining neuroregulators caries little risk of creating new chemical imbalances because it simply normalizes brain function. This far exceeds the safety, tolerability, and long-term effectiveness of other classes of psychotropic drugs, such as antidepressants, antipsychotics, and psychostimulants, which attempt to reduce symptoms by correcting chemical imbalances in specific neuronal circuits. Finally, all of the neuroregulators that have demonstrated benefit in reducing neuronal excitability are now available in generic form, thus helping to make neuroregulation more affordable than any other treatment approach. Never has there been such an opportunity to save lives, reduce costs, and resolve the mental health crisis.

IX. Directions for Future Research

Urgently needed are controlled studies to evaluate the effectiveness of neuroregulator therapy in comparison to symptom-based treatment for a variety of psychiatric disorders, including unipolar depressive disorders. These studies should include an evaluation of the sensitivity and specificity of resting heart and respiratory-rate measurements in identifying which patients would benefit most from neuroregulator therapy. Also needed are family, twin, and adoption studies aimed at identifying the familial distribution of neuronal hyperexcitability, a trait that may, in some carriers, manifest only as soft signs of psychiatric illness or upper-end-of-normal resting vital signs. If confirmed by these studies, the informal clinical observations that have thus far identified an autosomal dominant distribution of the neuronal hyperexcitability trait could have enormous implications for genetic engineering as a means of correcting the gene abnormalities that, based on a classic Mendelian distribution, appear to be isolated single nucleotide polymorphisms [74].

X. Conclusion

Based on the MCNH hypothesis in conjunction with a mind-brain duality of the cognitive-emotional system, the assessment, referral, and treatment of psychiatric disorders could potentially be streamlined, as the new paradigm divides psychopathology into just two groups: those who have psychosocial pathology, and those who have neuropsychiatric pathology. It also provides the first objective way to identify what is believed to be the core neurophysiological abnormality in the vast majority of patients who present for psychiatric treatment. Along with this, it identifies the wide-ranging utility of a sorely underutilized class of generic drugs that are known to be faster acting, safer, and more continuously effect than any other class of psychotropic medications. The clinical application of these insights could markedly simplify treatment, more rapidly reduce symptoms, and potentially change the face of modern psychiatry.

Conflicts of Interest

The author declares that he has no competing interests.

References Références Referencias

1. Voineskos D, Daskalakas, ZJ, Blumberger, DM. Management of Treatment-Resistant Depression: Challenges and Strategies. Neuropsychiatric Disease and Treatment 2020; 16: 221-234.
2. Rush AJ, Trivedi MH, Wisniewski SR, et al. Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. American J. Psychiatry 2006. 163 (11): 1905-1917.
3. Mental Health Project Rapid Report. Mental health has a bigger challenge than stigma. Mental Health Million Project 2021. https://mentalstateoftheworld.report/wp-content/uploads/2021/05/Rapid-Report-2021-Help-Seeking.pdf.
4. Parnia S, Spearpoint K, de Vos G, et al. AWARE — AWAreness during Resuscitation—A prospective study. Resuscitation 2014; 85: 1799-1805.
5. Greyson B. After: A doctor explores what near-death experiences reveal about life and beyond. St. Martin's Essentials. New York, NY 2021.
6. Van Lommel P. Consciousness beyond life: The science of the near-death experience. Harper-Collins Publishers. New York, NY 2010.
7. Moody RA. Life after life. Mockingbird Books 1975.
8. Fenwick P, Fenwick E. The art of dying. Continuum Books. New York, NY 2008.
9. Greyson B, Kelly EF, Dunseath WJR. Surge of neurophysiological activity in the dying brain.

Proceedings of the National Academy of Sciences 2013; 110 (47) E4405.
10. Parnia S, Post SG, Lee MT, et al. Guidelines and standards for the study of death and recalled experiences of death—a multidisciplinary consensus statement and proposed future directions. Annals of the New York Academy of Sciences 2022; 1511 (1): 5-21.
11. Cerf M, Thiruvengadam N, Mormann F, et al. On-line, voluntary control of human temporal lobe neurons. Nature 2010; 467: 1104-1108.
12. Penfield W. Epilepsy and surgical therapy. Archives of Neurology and Psychiatry 1936; 36 (3): 449-484.
13. Aravanis AM, Wang L-P, Zhang F, et al. An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. Journal of Neural Engineering 2007; 4 (3).
14. Boyden ES, Zang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. Nature Neuroscience 2005; 8: 1263-1268.
15. Crick F. Function of the thalamic reticular complex: The searchlight hypothesis. Proceedings of the National Academy of Sciences 1984; 81: 4586-4590.
16. Kwasniak J. Looking at the thalamic reticular nucleus. http://charbonniers.org/2013/02/13/looking at-the-thalamic-reticular-nucleus/. (Accessed 5/17/ 18).
17. Llinas R, Jahnsen H. Electrophysiology of mammalian thalamic neurons in vitro. Nature (London) 1982; 297 (5865): 406-408.
18. Jahnsen H, Llinas R. Electrophysiological properties of guinea-pig thalamic neurones: an in vitro study. J. Physiol 1984; 349 (1): 205-226.
19. Jahnsen H, Llinas R. Ionic basis for the electro-responsiveness and oscillatory properties of guinea-pig thalamic neurones in vitro. J Physiol 1984; 349: 227-247.
20. Binder MR. The racing mind: Brave new insights untangle the ancient mystery of mental illness. Lightningsource Publishing, 2024, p.13.
21. Binder MR. Mind-brain dynamics in the pathophysiology of psychiatric disorders. Am J Psychiatry and Neurosci 2022; 10 (2): 48-62.
22. Dimkov P. Large-scale Brain Networks and Freudian Ego. ResearchGate 2018 https://www.researchgate. net/ publication/326468259.
23. Binder MR. The multi-circuit neuronal hyperexcitability hypothesis of psychiatric disorders. AJCEM 2019; 7 (1): 12-30.
24. Binder MR. Neuronal hyperexcitability: Significance, cause, and diversity of clinical expression. AJCEM 2021; 9 (5); 157-167. https://en.wikipedia.org/wiki/ Neurosis
25. Post RM. Kindling and sensitization as models for affective episode recurrence, cyclicity, and tolerance phenomena. Neuroscience & Biobehavioral Reviews 2007; 31 (6): 858-873.
26. Zdravko Petanjek, Miloš Judaš, Goran Šimić, et al. Extraordinary neoteny of synaptic spines in the human prefrontal cortex. PNAS 2011; 108 (32): 13281-13286.
27. Azevedo FA, Carvalho LR, Herculano-Houzel S, et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol 2009; 10; 513 (5): 532-541.
28. Nguyen T. Total number of synapses in the adult human neocortex. Undergraduate Journal of Mathematical Modeling: One + Two 2010; 3 (1): Article 14.
29. Binder MR. The neuronal excitability spectrum: A new paradigm in the diagnosis, treatment, and prevention of mental illness and its relation to chronic disease. AJCEM 2022; 10 (1): 1-7.
30. Ferreira, MAR, O'Donovan MC, and Sklar P. (2008) Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet. 40 (9): 1056-1058.
31. Yuan A, Yi Z, Wang Q, et al. (2012) ANK3 as a risk gene for schizophrenia: new data in Han Chinese and meta analysis. Am J Med Genet B Neuropsychiatr Genet. 159B (8): 997-1005.
32. Lopez AY, Wang X, Xu M, et al. (2017) Ankyrin-G isoform imbalance and interneuronopathy link epilepsy and bipolar disorder. Mol Psychiatry. 22 (10): 1464–1472.
33. Green EK, Grozeva D, Jones I, et al., Wellcome Trust Case Control Consortium, Holmans PA, Owen MJ, O'Donovan MC, Craddock N. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry 2010; 15 (10): 1016-1022.
34. Liu Y, Blackwood DH, Caesar S, et al. Meta-analysis of genome-wide association data of bipolar disorder and major depressive disorder. Mol Psychiatry 2011; 16 (1).
35. Iqbal Z, Vandeweyer G, van der Voet M, et al. Homozygous and heterozygous disruptions of ANK3: at the crossroads of neurodevelopmental and psychiatric disorders. Human Molecular Genetics 2013; 22: 1960-1970.
36. Subramanian J, Dye L, and Morozov, A. Rap1 Signaling Prevents L-Type Calcium Channel-Dependent Neurotransmitter Release. Journal of Neuroscience 2013; 33 (17): 7245.
37. Santos M, D'Amico D, Spadoni O, et al. Hippocampal hyperexcitability underlies enhanced fear memories in TgNTRK3, a panic disorder mouse

model. Journal of Neuroscience 2013; 33 (38): 15259-15271.
38. Contractor A, Klyachko VA, and Portera-Cailliau C. Altered neuronal and circuit excitability in Fragile X syndrome. Neuron 2015; 87 (4): 699-715.
39. O'Brien NL, Way MJ, Kandaswamy R, et al. The functional GRM3 Kozak sequence variant rs148754219 affects the risk of schizophrenia and alcohol dependence as well as bipolar disorder. Psychiatric Genetics 2014; 24: 277–278.
40. Schizophrenia Working Group of the Psychiatric Genomics Consortium: Ripke S, Neale BM, and O'Donovan MC. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014; 511 (7510): 421-427.
41. Freedman R, Coon H, Myles-Worsley M, et al. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. PNAS 1997; 94 (2): 587–592.
42. Pizzarelli R and Cherubini E. Alterations of GABAergic signaling in autism spectrum disorders. Neural Plast 2011; 1011: 157193.
43. Hawes DJ, Brennan J, Dadds MR. Cortisol, callous-unemotional traits, and pathways to antisocial behavior. Curr Opin Psychiatry 2009; 22 (4): 357-362.
44. Skeem J, Johansson P, Andershed H, Kerr M, Louden JE. Two subtypes of psychopathic violent offenders that parallel primary and secondary variants. J Abnorm Psychol 2007; 116 (2): 395-409.
45. Lykken DT. A study of anxiety in the sociopathic personality. J Abnorm Soc Psych 1957; 55 (1): 6–10.
46. Latvala A, Kuja-Halkola R, Rick C, et al. Association of resting heart rate and blood pressure in late adolescence with subsequent mental disorders: A longitudinal population study of more than 1 million men in Sweden. JAMA Psychiatry 2016; 73 (12): 1268-1275.
47. Blom EH, Serlachius E, Chesney MA, Olsson EMG. Adolescent girls with emotional disorders have a lower end-tidal CO2 and increased respiratory rate compared with healthy controls. Psychophysiology 2014; 51 (5): 412-418.
48. Colangelo LA, Yano Y, Jacobs Jr DR, Lloyd-Jones DM. Association of resting heart rate with blood pressure and incident hypertension over 30 years in black and white adults: The CARDIA study. Hypertension 2020; 76 (3): 692-698.
49. Shi Y, Zhou W, Liu S, et al. Resting heart rate and the risk of hypertension and heart failure: a dose-response meta- analysis of prospective studies. J Hypertens 2018; 36 (5): 995-1004.
50. Shen L, Wang Y, Jiang X, et al. Dose-response association of resting heart rate and hypertension in adults: A systematic review and meta-analysis of cohort studies. Medicine (Baltimore) 2020; 99 (10): e19401.
51. Dalal J, Dasbiswas A, Sathyamurthy I, et al. Heart rate in hypertension: Review and expert opinion. International Journal of Hypertension 2019; 2019.
52. Lee DH, de Rezende LFM, Hu FB, Jeon JY, Giovannucci EL. Resting heart rate and risk of type 2 diabetes: a prospective cohort study and meta-analysis. Diabetes Metab Res Rev 2019; 35 (2): e3095.
53. Aune D, o'Hartaigh B, Vatten LJ. Resting heart rate and the risk of type 2 diabetes: A systematic review and dose-response meta-analysis of cohort studies. Nutr Metab Cardiovasc Dis 2015; 25 (6): 526-534.
54. Nagaya T, Yoshida H, Takahashi H, Kawai M. Resting heart rate and blood pressure, independent of each other, proportionally raise the risk for type-2 diabetes mellitus. Int J Epidemiol 2010; 39 (1): 215-222.
55. Kannel W, Kannel C, Paffenbarger R, Cupples A. Heart rate and cardiovascular mortality: The Framingham study. Am Heart J 1987; 113: 1489-1494.
56. Gillum R, Makuc D, Feldman J. Pulse rate, coronary heart disease, and death: The NHANES I epidemiologic follow-up study. Am Heart J 1991; 121: 172-177.
57. Cooney MT, Vartiainen E, Laatikainen T, et al. Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. Am Heart J 2010; 159 (4): 612-619.
58. Khan H, Kunutsor S, Kalogeropoulos AP, et al. Resting heart rate and risk of incident heart failure: three prospective cohort studies and a systematic meta-analysis. J Am Heart Assoc 2015; 4 (1): e001364.
59. Alhalabi L, Singleton MJ, Oseni AO, et al. Relation of higher resting heart rate to risk of cardiovascular versus noncardiovascular death. Am J Cardiol 2017; 119 (7): 1003-1007.
60. Zhang D, Shen X, Qi X. Resting heart rate and all-cause and cardiovascular mortality in the general population: a meta-analysis. CMAJ 2016; 188 (3): E53-E63.
61. Yu J, Dai L, Zhao Q. Association of cumulative exposure to resting heart rate with risk of stroke in general population: The Kailuan Cohort Study. Journal of Stroke and Cardiovascular Diseases 2017; (26): 11: 2501-2509.
62. Huang Y-Q, Shen G, Huang J-Y, Zhang B, Feng Y-Q. A nonlinear association between resting heart rate and ischemic stroke among community elderly hypertensive patients. Postgrad Med 2020; 132 (2): 215-219.
63. Aune D, Sen A, o'Hartaigh B, et al. Resting heart rate and the risk of cardiovascular disease, total

cancer, and all-cause mortality - A systematic review and dose-response meta-analysis of prospective studies. Nutr Metab Cardiovasc Dis 2017; 27 (6): 504-517.
64. Anker MS, Ebner N, Hildenbrandt B, et al. Resting heart rate is an independent predictor of death in patients with colorectal, pancreatic, and non-small cell lung cancer: results of a prospective cardiovascular long-term study. European Journal of Heart Failure 2016; 18 (12).
65. Park J, Kim JH, Park Y. Resting heart rate is an independent predictor of advanced colorectal adenoma recurrence. PLoS One 2018; 13 (3): e0193753.
66. Burke SL. Resting heart rate moderates the relationship between neuropsychiatric symptoms, MCI, and Alzheimer's disease. Innov Aging 2019; 3 (suppl 1): S641.
67. Jouven X, Empana J-P, Schwartz PJ, et al. Heart-rate profile during exercise as a predictor of sudden death. N Engl J Med 2005; 352: 1951-1958.
68. Binder MR. FLASH syndrome: tapping into the root of chronic illness. AJCEM 2020; 8 (6): 101-109.
69. Binder MR. Psychiatric and functional physical symptoms: the more telling "fifth" vital sign. AJCEM 2021; 9 (6): 233-237.
70. Binder MR. Introducing the term "Neuroregulator" in psychiatry. AJCEM 2019; 7 (3): 66-70.
71. Akiskal HS. The bipolar spectrum: new concepts in classification and diagnosis. In: Grinspoon L, editor. Psychiatry Update; The American Psychiatric Association Annual Review. Vol. 2. Washington DC: American Psychiatric Press 1983, pp. 271–292.
72. Binder MR. Focused neuroregulation in the treatment and prevention of mental and physical illness. AJCEM 2022; 10 (2): 49-58.
73. Binder MR. Exploring the potential to prevent human disease by genetically altering the excitability of the neurological system. Am J Psychiatry and Neurosci 2023; 11 (1): 22-29.

This page is intentionally left blank

Global Journal of Medical Research: a
Neurology & Nervous System
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Five-year Retrospective Study on Severe Traumatic Brain Injury in ICU Conditions of a Middle-Income Country

By Dragan Svraka, Anita Djurdjevic Svraka & Dragan Milosevic

University of Banja Luka

Abstract- *Background:* Traumatic injuries are the leading cause of death in the age group of 1 to 45 years in the United States of America and globally in the age group of 15 to 49 years. The World Health Organization (WHO) estimates that almost 90% of deaths caused by trauma occur in low- or middle-income countries, where 85% of the world's population. The primary aim is to assess the safety of barbiturate coma use in traumatic brain injury (TBI) patients without invasive intracranial pressure monitoring. The study aimed to investigate etiological factors, health outcomes, and the effects of barbiturate infusion on neurotrauma and describe the characteristics of the TBI population. Data from 55 patients with isolated severe TBI were analysed.

Keywords: *trauma brain injury, barbiturate coma, epidemiology of trauma.*

GJMR-A Classification: *NLMC: WL354*

Strictly as per the compliance and regulations of:

Five-year Retrospective Study on Severe Traumatic Brain Injury in ICU Conditions of a Middle-Income Country

Dragan Svraka [α], Anita Djurdjevic Svraka [σ] & Dragan Milosevic [ρ]

Abstract- Background: Traumatic injuries are the leading cause of death in the age group of 1 to 45 years in the United States of America and globally in the age group of 15 to 49 years. The World Health Organization (WHO) estimates that almost 90% of deaths caused by trauma occur in low- or middle-income countries, where 85% of the world's population. The primary aim is to assess the safety of barbiturate coma use in traumatic brain injury (TBI) patients without invasive intracranial pressure monitoring. The study aimed to investigate etiological factors, health outcomes, and the effects of barbiturate infusion on neurotrauma and describe the characteristics of the TBI population. Data from 55 patients with isolated severe TBI were analysed.

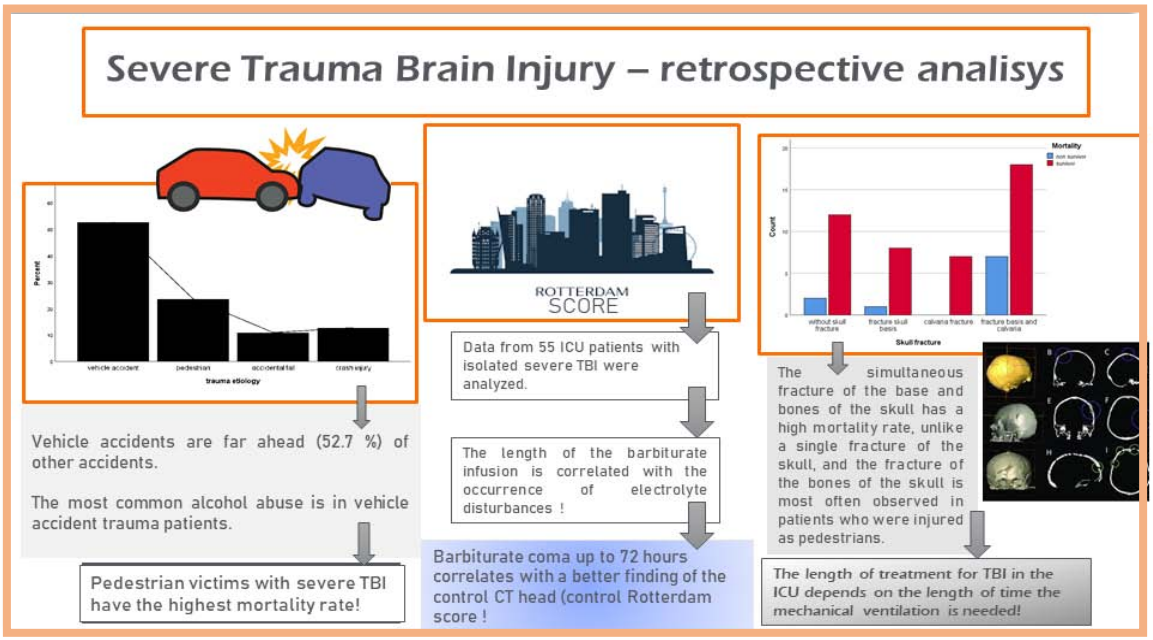
Methods: A retrospective observational study was conducted on 55 patients who suffered from severe traumatic brain injury (TBI) between the years 2017 and 2022. Before being admitted to the intensive care unit, all patients received clinical examination and care from an anesthesiologist and neurosurgeon, along with laboratory and radiological assessments. As per our clinic's protocol, all patients with severe TBI were treated with barbiturate coma. During this treatment, we monitored various parameters such as electrolyte disturbances, support for vasoactive therapy, the Rotterdam score at the beginning of barbiturate therapy, and the control Rotterdam score after 72 hours. The study also presents the general demographic characteristics of TBI patients, including gender, age, and etiological factors of trauma.

Results: Vehicle accidents are far ahead (52.7 %) of other accidents; the demographic with the highest prevalence of severe Traumatic Brain Injury (TBI) diagnoses is individuals in their fourth decade (31-40y). Patients treated with barbiturate infusion for barbiturate comma showed significant improvement in their computerised tomography head scan, with their Rotterdam score values shifting from the initial Rotterdam score I (4.06±0.12; mean±SD) to the control score values of Rotterdam score II (3.35±0.13; mean±SD) p *<0.001*.

Conclusion: The safety of a patient in a barbiturate coma is reflected in the absence of evidence that such treatment, without modern monitoring of intracranial pressure, has an impact on unwanted outcomes.

Keywords: trauma brain injury, barbiturate coma, epidemiology of trauma.

Graphical Abstract



Author α ρ: University Clinical Center of Republic of Srpska, Banja Luka, Bosnia and Herzegovina Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina. e-mails: svrale@yahoo.com, dragan.svraka@kc-bl.com, (draganmil48@gmail.com)
Author σ: General Hospital Gradiska, Gradiska, Bosnia and Herzegovina. Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina. e-mail: anita.djurdjevic@gmail.com

I. Background

Brain injuries are a leading cause of death and disability, particularly among young people, in both developed and developing countries. Approximately 1.19 million people die each year as a result of road traffic crashes. It's disheartening to know that road traffic injuries are the leading cause of death among children and young adults aged 5 to 29 years old. What's even more surprising is that low- and middle-income countries account for 92% of the world's fatalities on the roads despite having only 60% of the world's vehicles. It's important to note that pedestrians, cyclists, and motorcyclists are the most vulnerable road users and account for over half of all road traffic deaths. Additionally, road traffic crashes cost countries 3% of their gross domestic product worldwide. That's why the United Nations General Assembly has set an ambitious goal of reducing the number of deaths and injuries from road traffic crashes by 50% globally by 2030[1]. Traumatic brain injuries are typically categorised using scoring systems derived from clinical examinations of the patient (e.g. Glasgow coma scale) or radiological diagnostics (e.g. Rotterdam score). The goal of treating patients with severe neurotrauma in intensive care units is to prevent any secondary brain injuries while continuously monitoring vital functions. The choice of sedatives administered to reduce increased cerebral metabolic demands stemming from the injury is dependent on the experience of the physician and the adopted ICU treatment protocol for neurotrauma. The primary aim is to assess the safety of barbiturate coma use in TBI patients without invasive intracranial pressure monitoring. The secondary objective is to investigate the etiological factors and health outcomes and describe the characteristics of the population with traumatic brain injury.

II. Methods

A retrospective observational study was conducted on patients with severe traumatic brain injury (TBI) at a five-year time point from 2017 to 2022.To conduct the study, the Ethics Committee of the University Clinical Center of the Republic of Srpska in Banja Luka (number: 01-19-126-2/22; date 26/04/2022) granted consent according to the Helsinki declaration for patients treated with barbiturate-induced coma due to traumatic brain injury. Patient information was obtained by reviewing printed and electronic medical records through the Clinical Information System (CIS) and the Radiological Information System (PACS).

The study aimed to investigate etiological factors, health outcomes, and the effects of barbiturate infusion on neurotrauma and describe the characteristics of the TBI population. Data from 55 ICU patients with isolated severe TBI were analysed.

a) *Sampling Strategy*

All patients included in the study (n 55) received clinical examination and care from an anesthesiologist and neurosurgeon, as well as laboratory and radiological assessments before being admitted to the intensive care unit.

The criteria for patient selection included a primary diagnosis of traumatic brain injury requiring continuous barbiturate infusion for deep sedation, admission to the ICU within 12 hours of the injury, availability of comprehensive clinical and laboratory records, and access to radiological CT scans before and after cessation of the barbiturate infusion.

There are several criteria for excluding patients who have sustained traumatic brain injury but do not have it as their primary injury. These factors include severe traumatic damage to other organs or organ systems, limited use of barbiturate infusion, a short duration of continuous barbiturate infusion (less than 72 hours), admission to the ICU more than 12 hours after the trauma occurred, the need for extensive transfusion, incomplete medical documentation, and an inability to obtain insight into radiological CT diagnostics.

b) *The Adopted ICU Treatment Protocol for Neurotrauma*

Due to the severity of the traumatic brain injuries, upon admission, we implemented a comprehensive treatment plan that included anti-edematous therapy with 20% mannitol at a dosage of 1g/kg. Additionally, we initiated continuous barbiturate therapy (barbiturate-induced deep sedation) at a rate of 4g/12h for adult patients, 12,5 mg/kg/h, and 3-5 mg/kg/h for pediatric patients. We continuously monitored hemodynamics invasively and non-invasive hemodynamic and respiratory parameters throughout the treatment process. A clinical neurological examination was conducted every six hours, and CT diagnostics were performed as necessary based on clinical assessments. Control CT diagnostics and suspension of the barbiturate infusion were determined by experienced anesthesiologists and neurosurgeons based on clinical and neurological stability achieved over 48 hours. Our team also evaluated the Rotterdam score, which was assessed by a radiologist who reviewed both the admission and control CT scans. Compliance with the adopted protocol for administering barbiturates is of paramount importance in the treatment of traumatic brain injuries (TBI), as the inability to measure intracranial pressure poses a significant challenge in monitoring therapy. This limitation undermines the ability to evaluate the treatment's efficacy, thus impeding optimal patient care.

To ensure stable hemodynamics and achieve the desired cerebral perfusion pressure (CPP), we maintain a sufficient mean arterial pressure (MAP) by administering vasoactive drugs and providing adequate

volume replacement. It is crucial to constantly monitor the MAP by placing an arterial line. For pain management, we prescribe opioid medication, and anticonvulsive drugs are initiated once barbiturate infusion is stopped.

c) Statistical and Data Processing

In conducting our observational studies, we followed the STROBE guidelines. We used the statistical program SPSS (IBM® Statistics, Version 20) to process our data. We reported the mean +/- standard deviation or the median (interquartile range) for continuous data. Categorical data was compared using the Chi-square test. To test the significance of individual parameters and correlations between variables, we employed T-tests and Spearman Correlation. P-values of $p \leq 0.05$ were considered to be statistically significant.

III. Results

Vehicle accidents are far ahead (52,7%) of other accidents that most often lead to isolated neurotrauma, surpassing pedestrian accidents (23,6 %), accidental falls (10,9 %), or crash injuries (12,7 %). – Figure1.

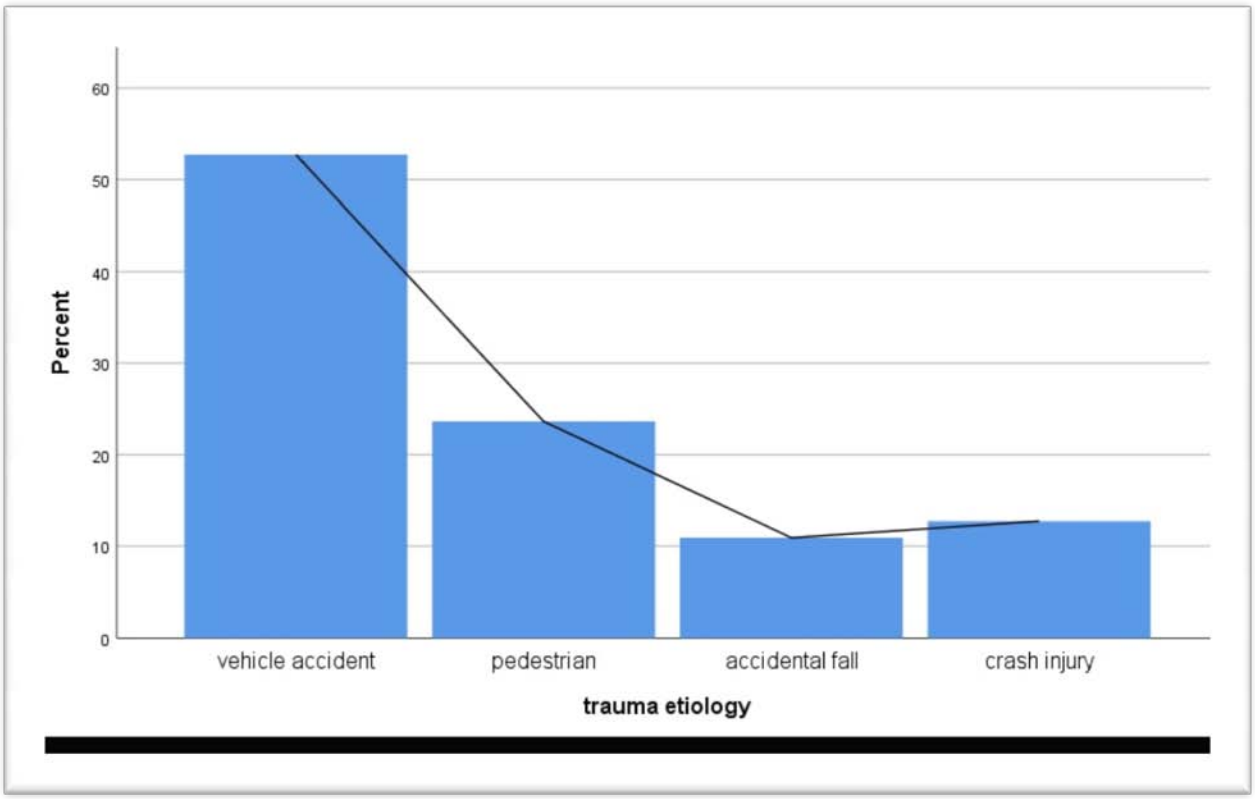


Figure 1: Bar chart of TBI aetiology by per cent of the examined patient

The demographic with the highest prevalence of severe Traumatic Brain Injury (TBI) diagnoses is individuals in their fourth decade (31-40y), followed by those in their third decade (21-30y). The average age of our patients is 39 years old, and children up to 16 years old represented 12.7% of our study. Males make up the majority of TBI patients across all age groups (74,6 %), except for the second decade (11 to 20 years old), where females are more commonly diagnosed.– Figure 2.

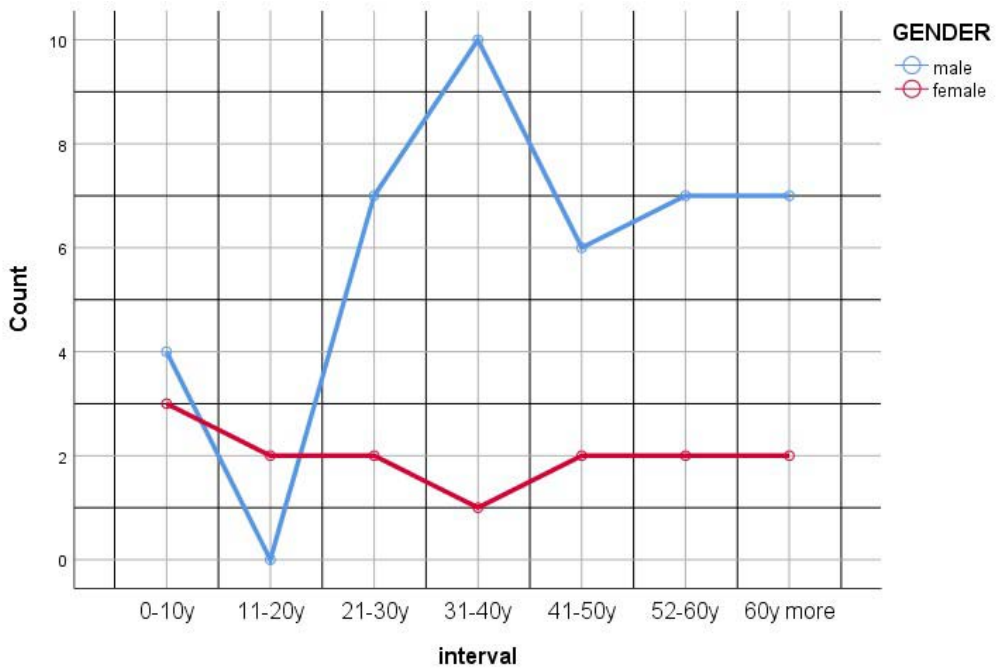


Figure 2: Gender distribution by age intervals in patients with severe TBI

After analysing the data, it was discovered that motor vehicle accidents are the leading cause of traumatic brain injuries. Thoracic trauma was found to be the most common type of injury (65%). Interestingly, cervical injuries were more significant for victims who were inside vehicles (p 0.03). On the other hand, pedestrians involved in accidents were more prone to skull bone fractures (84.6%). The study also revealed that a considerable proportion of patients admitted to the ICU due to vehicular accidents had consumed alcohol (15.8%). These findings are further supported by the data presented in Table 1.

Table 1: Categorical Data of Trauma Aetiology, Type of non-dominant injury with severe TBI patients, and mortality

	Vehicle accident (%)	p*	Pedestrian (%)	p*	Crash Injury (%)	p*	Mortality (%)	p*
Alcohol abuse	15.8%	0.7	7.7%	0.4	11.1%	0.6	%	0.1
Fracture of calvaria	76.4%	0.3	84.6%	0.7	22.2%	0.6	80%	0.3
Cervical injury	31.6%	0.03	23.1%	0.9	0%	-	30%	0.6
Toracic trauma	65.8%	0.08	53.8%	0.7	66.7%	0.4	70%	0.4
Mortality	18.2%	0.9	38.5%	0.03	0%	-	-	-

**Chi-square test for categorical data with sig P ≤ 0.05*

Patients treated with barbiturate coma showed significant improvement in their computerised tomography head scan, with their Rotterdam score values shifting from the initial Rotterdam score I (4.06±0.12; mean±SD) to the control score values of Rotterdam score II (3.35±0.13; mean±SD) p <*0.001*. Moreover, those who received neurosurgery within the first 24 hours, TBI patients with vasoactive support during barbiturate infusion, and surviving TBI patients displayed a significantly more favourable Rotterdam score II compared to their Rotterdam score I upon admission(p 0.001; p 0.001; p < 0.001).– Table 2.

Table 2: The initial Rotterdam score (Rotterdam score I) and the control Rotterdam score (Rotterdam score II) are based on the patient's hemodynamic stability at ICU admission, neurosurgery, vasopressors, duration of barbiturate infusion, and survival

	Rotterdam scor I (mean±SD)	Rotterdam scor II (mean±SD)	p*
Total (mean±SD)	4.06±0.12	3.35±0.13	*<0.001*
Hemodynamically stable on ICU admission (n 21, 38%)	4.1±0,8	3.4±1	0.2
Hemodynamically unstable on ICU admission (n 34, 62%)	4±1	3.2±0.9	0.2
The neurosurgical operation performed in the first 24 hours (n 16, 29%)	4.6±0.8	3.6±0.8	*0.001*
The neurosurgical operation was not performed in the first 24 hours (n 39, 71%)	3.8±0.9	3.3±1	*0.02*
There was no vasoactive support during the barbiturate coma (n 16, 29%)	4.6±0.9	4.1±1.1	0.2
There was vasoactive support during the barbiturate coma (n 39, 71%)	3.8±0.9	3.1±0.8	*0.001*
Surviving (n 45, 82%)	3.9±0.9	3.2±0.9	*<0.001*
Deceased (n 10, 18%)	4.6±0.7	4.3±1	0.4

**T-test (95% CI, Sig 0,05)*

It has been observed that patients who have severe traumatic brain injury and are receiving barbiturate infusion may be more likely to develop hypokalemia (29 cases) compared to hypernatremia (17 cases). The extent of electrolyte imbalances seems to be directly linked to the duration of the barbiturate infusion (p=0.007 for hypokalemia; p=0.006 for hypernatremia). The duration of mechanical ventilation and barbiturate infusion are crucial factors that can affect the length of treatment in the intensive care unit (p<0.001). However, these elements do not significantly impact the mortality rate (p=0.3; p=0.5). -Table 3.

Table 3: The impact of the duration of barbiturate infusion and mechanical ventilation on electrolyte disturbances and treatment length in the ICU for severe TBI patients

	Total (median±IQR)	Hypokalemia (n 29)	Normokalemia (n 26)	p*	Hypernatremia (n 17)	Normonatremia (n 38)	p*	ICU length p**	Mortality p**
Length of barbiturate infuseion by days (median±IQR)	4(6)	5(1)	3(4)	0.007	5(2)	3(2)	0.006	<0.001	0.3
Length of mechanical ventilation by days (median±IQR)	10(36)	10(7)	10.5(11)	0.2	10(7)	10(10)	0.6	<0.001	0.5

**Spearman Correlation (95% CI; ≤0.05)*

*** Chi-Square test (Sig ≤0.05)*

IV. Discussion

Traumatic brain injuries (TBI) are a significant public health concern as they affect a large number of people in their most productive years. The care and treatment of TBI are both long-term and costly, and the outcomes of treatment in intensive care units worldwide are uncertain and vary greatly. Often called the "silent epidemic," [2] TBI continues to be a growing public health issue, causing the highest impact on death and disability among all trauma-related injuries globally [3]. According to our study, TBI was more prevalent in men. This result is consistent with previous research on TBI by Majdan [4]. One of the reasons for this trend is that men tend to use seat belts less frequently while driving and protective equipment less often at work. Additionally, they are more likely to consume alcohol, which can increase the risk of trauma-related injuries.

The average age of our patients is 39 years old, and children up to 16 years old represented 12.7% of our study. These demographic findings are similar to those of a significant epidemiological cross-sectional analysis conducted by Majdan et al. on TBI among residents of European Union countries[5]. However, the study showed substantial differences in TBI incidence rates among different EU countries, partly attributed to the varying methodologies used to process and manage medical data. The same authors also studied TBI epidemiological data in Belgium for over ten years. Their findings indicated a slight increase in the incidence of TBI among individuals over 65 years old and a significant decrease in the incidence among those under 65 years old[6].

This kind of data is an increasingly frequent finding in TBI studies, and it is impossible to explain it only by an increase in the proportion of older people in the general population [7].

Our research provides a comprehensive overview of how vehicle accidents affect individuals. It includes detailed information about the percentage of pedestrian injuries, crash injuries, and mortality rates resulting from these accidents. Additionally, it highlights the percentage of cases involving alcohol abuse, fracture of skull bones, cervical injury, thoracic trauma, and mortality rates.

The study's findings varied depending on the region and were greatly influenced by living conditions and cultural factors. It is worth noting that over the past decade, incidents of traffic-related trauma have decreased in the USA, Europe, and Japan. However, incidents of TBI caused by falls among those over 65 years have increased significantly, as reported in studies [7, 8, 9].

Providing sufficient volume replacement and administering vasoactive drugs as necessary is essential to ensure the best possible outcome. It is crucial to continuously monitor the mean arterial pressure (MAP) by inserting an arterial line. Studies have shown that hypotension is associated with a twofold rise in mortality in traumatic brain injuries compared to controls [10].

Elevated intracranial pressure (ICP) is a fatal event associated with TBI. In treating patients with TBI, basic therapeutic measures must be implemented regardless of the availability of ICP monitoring [11]. According to our research, barbiturate infusion is a viable treatment option where invasive intracranial pressure monitoring is not feasible. Our findings indicate that patients who received this treatment exhibited noteworthy enhancements in their control head CT scans. All TBI patients with severe injuries experienced a marked improvement in their control Rotterdam score values compared to their initial Rotterdam score. Patients suffering from severe traumatic brain injury and undergoing barbiturate infusion treatment may be at a higher risk of developing hypokalemia, as opposed to hypernatremia. The severity of electrolyte imbalances is proportional to the duration of barbiturate infusion. The duration of mechanical ventilation and barbiturate infusion can influence the length of treatment within the intensive care unit. However, these factors do not seem to affect the mortality rate significantly.

The study has certain limitations due to the retrospective nature of the analysis. Despite a five-year duration, it is not appropriate to conduct this research in a single centre, mainly if it is carried out in a middle-income country that lacks a national cohort of trauma patients.

V. Conclusion

This study is valuable in helping to understand the nature of TBI and identify specific areas that require attention and intervention to reduce their occurrence and severity. By analysing this data, we can gain insight into the severity of trauma illnesses and develop effective therapies to improve treatment outcomes. The safety of a patient in a barbiturate coma is reflected in the absence of evidence that such treatment, without invasive monitoring of intracranial pressure, has an impact on unwanted outcomes.

The ongoing research in this area should emphasise neuroinflammation as a hidden metabolic parameter. Additionally, it should focus on developing medications such as growth factors and stem cells that promote neuronal growth and repair.

Abbreviations:

TBI – traumatic brain injury
ICU – intensive care unit
CT – computed tomography
MAP – mean arterial pressure
ICP – intracranial pressure

Declarations

Ethics Approval: for the study was obtained from the Ethics Committee of the University Clinical Center Republic of Srpska. The approval was granted under the number 01-19-126-2/22 on April 26, 2022. The Ethics Committee followed the ICH-GCP guidelines and the latest version of the Declaration of Helsinki.

Conflict of Interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authors' Contributions: Dragan Svraka and Anita Djurdjevic Svraka contributed to the study's conception and design. Anita Djurdjevic Svraka and Dragan Svraka performed material preparation, data collection, and analysis. Dragan Svraka wrote the first draft of the manuscript, and Anita Djurdjevic Svraka, Vlado Djajic, Miso Miskic and Marko Kantar read and approved the final manuscript.

Availability of Data and Materials: All research-related materials and data are stored at the University Clinical Center Republic of Srpska, Dvanaest Beba Street No1, Banjaluka 78000, Bosnia and Herzegovina.

Funding Information: The observational study did not require any additional costs. All examinations and analyses were performed as part of routine procedures on TBI ICU patients. The authors did not receive any financial resources or fees and declared that they did not receive any funds, grants, or other support during the preparation of this manuscript.

Consent for publication: Patients or patients' relatives signed informed consent regarding publishing their data.

References Références Referencias

1. WHO. Road traffic injuries. (2023) https://www.who.int/news-room/fact-sheets/detail/road-traffic-injuries Accessed 05.01.2024.
2. Rusnak M. Traumatic brain injury: Giving voice to a silent epidemic. Nature reviews. Neurology. 2013; 9(4), 186–187. https://doi.org/10.1038/nrneurol.2013.38
3. Dewan, MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, Agrawal A, Adeleye AO, Shrime MG, Rubiano AM, Rosenfeld JV, Park KB. Estimating the global incidence of traumatic brain injury. Journal of Neurosurgery. 2018; 130(4), 1080–1097. https://doi.org/10.3171/2017.10.JNS17352
4. Brazinova A, Rehorcikova V, Taylor MS, Buckova V, Majdan M, Psota M, Peeters W, Feigin V, Theadom A, Holkovic L,Synnot A. Epidemiology of Traumatic Brain Injury in Europe: A Living Systematic Review. Journal of neurotrauma. 2021; 38(10), 1411–1440. https://doi.org/10.1089/neu.2015.4126
5. Majdan M, Plancikova D, Brazinova A, Rusnak M, Nieboer D, Feigin V, Maas A. Epidemiology of traumatic brain injuries in Europe: a cross-sectional analysis. The Lancet. 2016; 1(2), e76–e83. https://doi.org/10.1016/S2468-2667(16)30017-2
6. Peeters W, Majdan M, Brazinova A, Nieboer D,Maas AIR. Changing Epidemiological Patterns in Traumatic Brain Injury: A Longitudinal Hospital-Based Study in Belgium. Neuroepidemiology. 2017; 48(1-2), 63–70. https://doi.org/10.1159/000471877
7. Iaccarino C, Carretta A, Nicolosi F, Morselli C. Epidemiology of severe traumatic brain injury. Journal of Neurosurgical Sciences. 2018; 62(5): 535-541. http://doi.org/10.23736/S0390-5616.18.04532-0
8. Hawryluk GW, Bullock MR. Past, Present, and Future of Traumatic Brain Injury Research. Neurosurgery clinics of North America. 2016; 27(4), 375–396. https://doi.org/10.1016/j.nec.2016.05.002
9. Shivaji T, Lee A, Dougall N, McMillan T, Stark C. The epidemiology of hospital-treated traumatic brain injury in Scotland. BMC neurology. 2014; 14, 2. https://doi.org/10.1186/1471-2377-14-2
10. LiJ, Murphy-Lavoie H, Bugas C, Martinez J, Preston C. Complications of emergency intubation with and without paralysis. The American journal of emergency medicine. 1999; 17(2), 141–143. https://doi.org/10.1016/s0735-6757(99)90046-3
11. Godoy DA, Rabinstein AA. How to manage traumatic brain injury without invasive monitoring? Current opinion in critical care. 2022; 28(2), 111–122. https://doi.org/10.1097/MCC.0000000000000914

Global Journals Guidelines Handbook 2024

www.GlobalJournals.org

Memberships

Fellows/Associates of Medical Research Council

FMRC/AMRC Memberships

Introduction

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals' mission to advance technology for humanity and the profession.

FMRC

Fellow of Medical Research Council

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.

Benefits

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get access to a closed network

A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

Certificate

Certificate, LoR and Laser-Momento

Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation

Designation

Get honored title of membership

Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

Career | Credibility | Exclusive | Reputation

Recognition on the Platform

Better visibility and citation

All the Fellow members of FMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

Career | Credibility | Reputation

Future Work

Get discounts on the future publications

Fellows receive discounts on the future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

GJ Internal Account

Unlimited forward of Emails

Fellows get secure and fast GJ work emails with unlimited storage of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

Premium Tools

Access to all the premium tools

To take future researches to the zenith, fellows receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

Conferences & Events

Organize seminar/conference

Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

Early Invitations

Early invitations to all the symposiums, seminars, conferences

All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive

Publishing Articles & Books

Earn 60% of sales proceeds

Fellows can publish articles (limited) without any fees. Also, they can earn up to 70% of sales proceeds from the sale of reference/review books/literature/publishing of research paper. The FMRC member can decide its price and we can help in making the right decision.

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

Access to Editorial Board

Become a member of the Editorial Board

Fellows and Associates may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer.

Career | Credibility | Exclusive | Reputation

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.

ASSOCIATE OF MEDICAL RESEARCH COUNCIL

ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

Benefits

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get access to a closed network

A AMRC member gets access to a closed network of Tier 2 researchers and scientists with direct communication channel through our website. Associates can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

Certificate

Certificate, LoR and Laser-Momento

Associates receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation

Designation

Get honored title of membership

Associates can use the honored title of membership. The “AMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., AMRC or William Walldroff, M.S., AMRC.

Career | Credibility | Exclusive | Reputation

Recognition on the Platform

Better visibility and citation

All the Associate members of AMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation.

Career | Credibility | Reputation

Future Work

Get discounts on the future publications

Associates receive discounts on future publications with Global Journals up to 30%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

GJ Account

Unlimited forward of Emails

Associates get secure and fast GJ work emails with 5GB forward of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

Premium Tools

Access to all the premium tools

To take future researches to the zenith, fellows receive access to almost all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

Conferences & Events

Organize seminar/conference

Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

Early Invitations

Early invitations to all the symposiums, seminars, conferences

All associates receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive

Publishing Articles & Books

Earn 60% of sales proceeds

Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

Associate	Fellow	Research Group	Basic
$4800 **lifetime designation**	$6800 **lifetime designation**	$12500.00 **organizational**	APC **per article**
Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors' research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

save our planet

Global Journal of Medical Research

Visit us on the Web at www.GlobalJournals.org | www.MedicalResearchJournal.org
or email us at helpdesk@globaljournals.org

ISSN 9755896