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VOLUME 24    ISSUE 1    VERSION 1.0



GLOBAL JOURNAL OF MEDICAL RESEARCH: A  
NEUROLOGY AND NERVOUS SYSTEM

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GLOBAL JOURNAL OF MEDICAL RESEARCH: A  
NEUROLOGY AND NERVOUS SYSTEM  
VOLUME 24 ISSUE 1 (VER. 1.0)

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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

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**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*



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# Performing the Garcia Score for Accuracy and Reliability

## Performing the Garcia Score

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**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.

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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.

### 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\\_oHy5lcKGASFCdqiV/view?usp=sharing](https://drive.google.com/file/d/1-UEtnk7twwyEz0_oHy5lcKGASFCdqiV/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

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## ACKNOWLEDGEMENTS

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

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

	<b>Spontaneous Activity</b>	<b>Symmetry in the Four Limbs</b>	<b>Forepaw Outstretching</b>	<b>Climbing</b>	<b>Body Proprioception</b>	<b>Response to Vibrissae Touch</b>
<b>3</b>	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
<b>2</b>	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
<b>1</b>	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
<b>0</b>	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*



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# 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.*

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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.

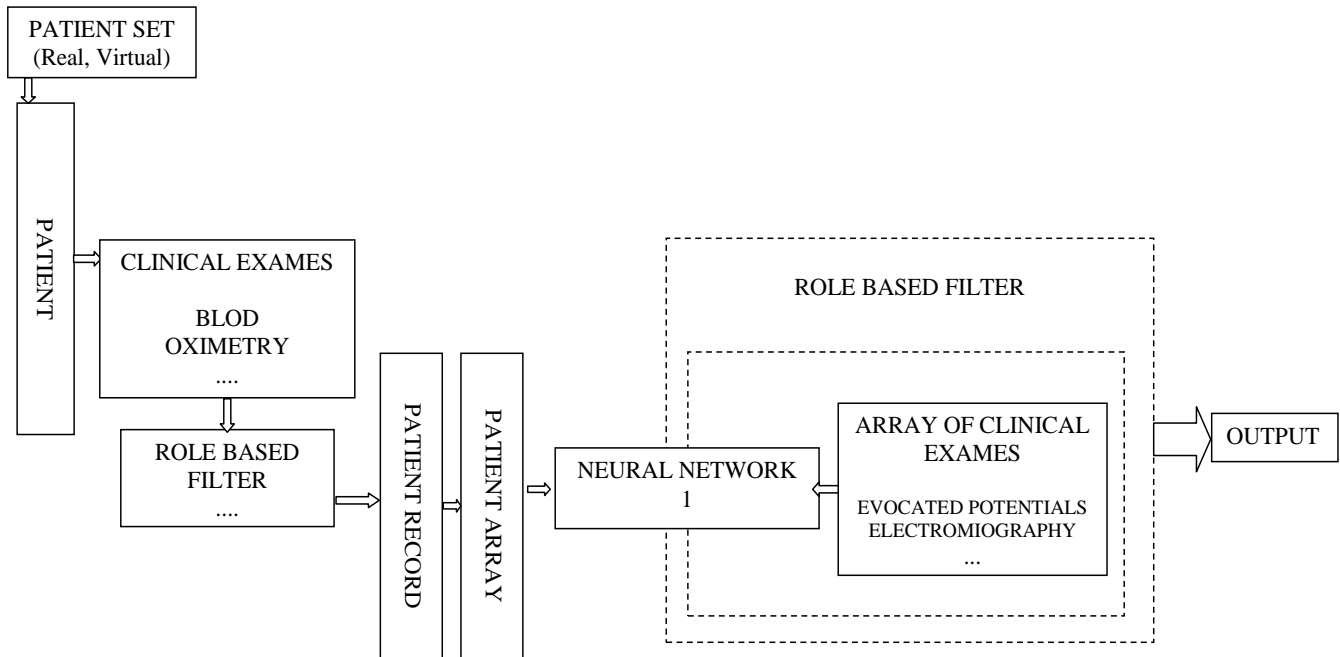
Then, let's say that the training must be done on "healthy" patients? validation on "sick" patients? And the test on "sick but not too sick" patients. A schift must be made on the inputs because they are very many so there is a risk of either over-fitting the net or not fishing well given the small number 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.

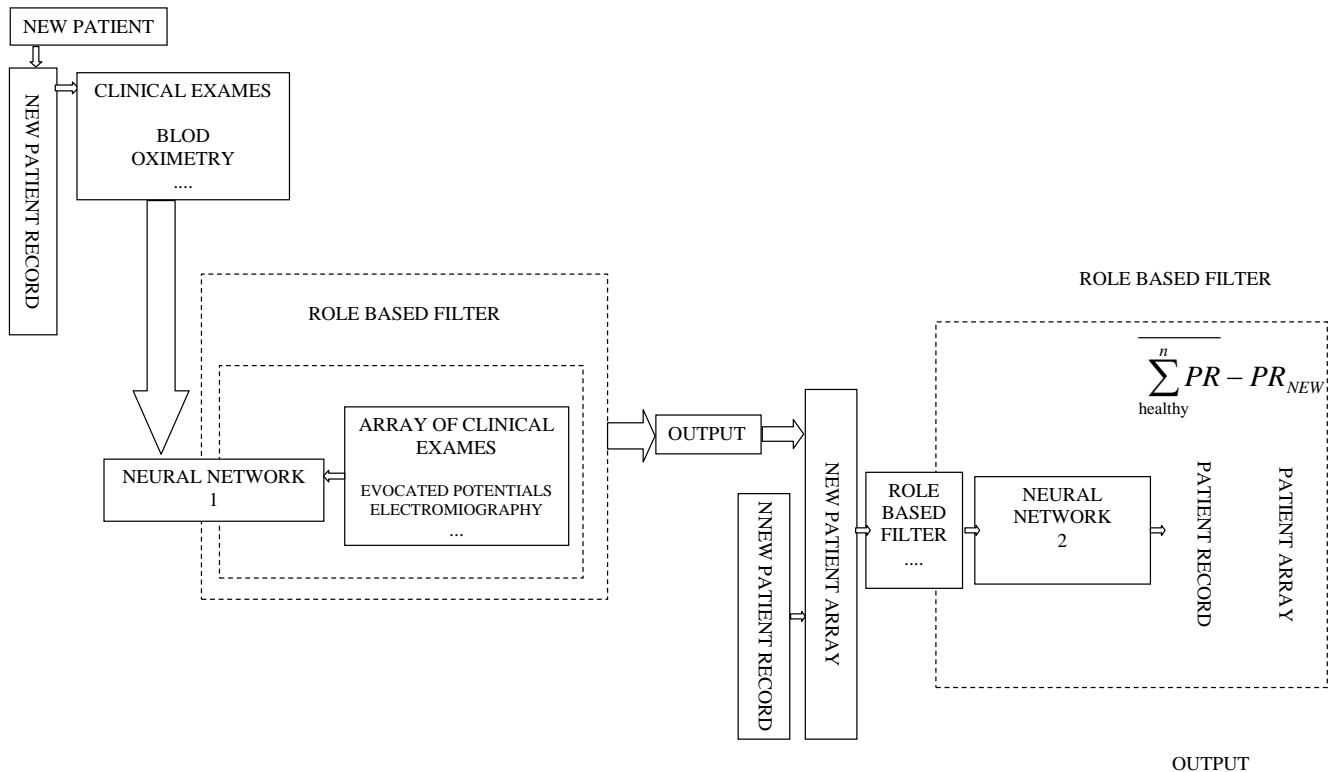
For evoked potentials and electromyography, a filter based on output rules will be used, hoping to limit the number of inputs, and since they are numerous, it will be necessary to ensure that the NN [12]-[44] has a variable and selectable range for the inputs both in terms of position and as breadth; 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 shift 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.

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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

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**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



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**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.

### 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%<sup>1</sup> and 6-month functional independence of only 20%.<sup>2</sup> 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.<sup>3,4</sup> 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.<sup>5,6,7</sup>

Multiple preclinical ICH models have been developed over time, including cortical vessel avulsion,<sup>8</sup> microballoon,<sup>9</sup> bacterial collagenase<sup>10</sup> and several variations of an autologous blood injection,<sup>11-14</sup> 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.<sup>15</sup> Cortical vessel avulsion demonstrated a mixed pathology of ischemia and hemorrhage, whereas, bacterial collagenase elicited a more intense and prolonged inflammatory reaction.<sup>15</sup>

Various modifications of a single-injection technique include blood injection via a permanently implanted needle into the basal ganglia,<sup>11</sup> bolus injection

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through a stereotaxically-placed intracerebral needle,<sup>16</sup> direct circuit from an intra-arterial femoral catheter to simulate hemorrhage induced at arterial pressure,<sup>12</sup> and utilization of a microinfusion pump to deliver a consistent volume of blood at a specified rate.<sup>13</sup> 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.<sup>17,18</sup>

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.<sup>14</sup> 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.<sup>14</sup> Femoral artery sampling does allow for fresh blood to be delivered, but requires an extended duration of anesthesia, which may confound ICH outcomes<sup>20</sup> and impairs post-surgical behavioral evaluation<sup>21</sup> critical to ICH studies.<sup>22</sup>

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<sup>24</sup> 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<sup>23</sup> 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<sup>14</sup> 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  $\mu$ L/min, injection volume of 100  $\mu$ L, 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 of the posterior-most aspect of the bregma intersecting where the periosteum remains attached at its lateral aspect, approximately 4.5mm in females and 5mm in 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  $\mu$ L at 10  $\mu$ L/min. \*\*\*Note: To ensure successful injection, note the starting volume of blood in the syringe and monitor for progression at a rate of 10  $\mu$ L/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 Turn 1-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  $\mu$ L 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 part by 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.

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**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.

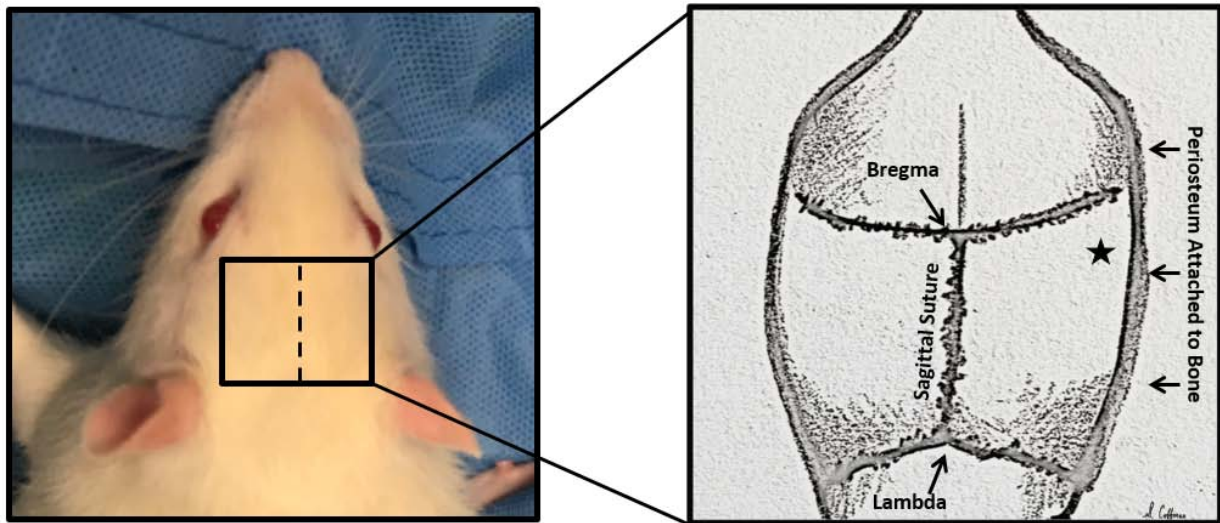


Figure 2: The incision (dashed line, left image) is approximately 2 cm long, midline on the scalp, from the back of the eyes to the lambda. After the incision is made, the periosteum is dissected and the bregma located, with the burr hole (star, right image) placed at the posterior-most point of the 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.

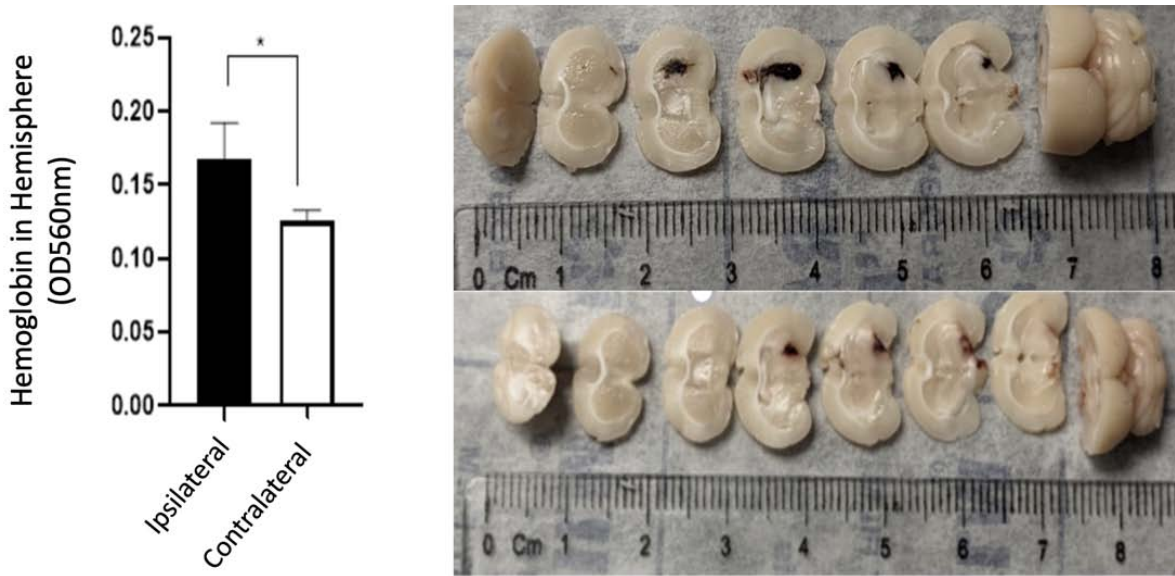


Figure 3: Graph (left) demonstrates mean total hemoglobin concentration in the ipsilateral hemisphere after induced autologous-ICH, demonstrating consistent hematoma volume and images (right) display serial brain sections demonstrating consistent basal ganglia hematoma.

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	$\chi^2$	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.

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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

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**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*



*Strictly as per the compliance and regulations of:*



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

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**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  $\gamma$ -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.  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type (AMPA-type) glutamate receptors (AMPARs) play a crucial role in synaptic plasticity

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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

I onotropic 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)  $\alpha$ -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].

$\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type (AMPA-type) glutamate receptors (AMPA receptors) 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 (AMPA receptors), 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 (AMPA receptor), 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 DNP 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  $\gamma$ -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- $\gamma$ -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<sup>-/-</sup> and GluA1<sup>-/+</sup>. exosomes ( $P < 0.05$ ) at similar levels ( $P > 0.05$  between genotypes). SNSGluA2<sup>-/-</sup> mice did not differ from their GluA1<sup>-/+</sup> 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% CO<sub>2</sub> 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 nocifensive 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-AMPA receptors from synapses is common in other systems. For example, VTA dopamine neurons express CP-AMPA 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-AMPA receptors, 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-AMPA 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-AMPA receptors, 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-AMPA receptors. Otherwise, cell viability is reduced 24 hours after injury in cultured neurons, making it difficult to assess surface or synaptic CP-AMPA expression at later time points. In addition, GluA2 mRNA levels begin to decline 6 hours after ischemic stroke in vivo, promoting CP-AMPA expression after this time. Although transport of specific subunits triggers a switch to CP-AMPA 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,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA-type) glutamate receptors (AMPA receptors) have emerged as important mediators of synaptic plasticity in the brain. Unlike NMDA-type glutamate receptors, which always mediate  $Ca^{2+}$  influx when activated, AMPARs are an activity-dependent switch that controls glutamate-induced  $Ca^{2+}$  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  $Ca^{2+}$  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  $\gamma$ -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. The results showed that exosome derivatives containing GluA1, 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. While exosome SNS-GluA1<sup>-/-</sup>+ 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. AMPARs 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 AMPARs are located on the postsynaptic surface. When bound to glutamate, they are permeable to Na and K ions, but usually not to Ca<sup>2+</sup> 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. 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 Ca<sup>2+</sup> 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 Ca<sup>2+</sup> influx they can also activate Ca<sup>2+</sup>-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<sup>2+</sup> 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<sup>2+</sup>-permeable AMPARs act as surrogates for NMDA receptors to mediate central pain sensitization. Additional evidence for the accumulation of Ca<sup>2+</sup>-permeable AMPARs during pain conditions comes from studies focusing on chronic pain. Ca<sup>2+</sup>-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<sup>2+</sup> 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<sup>2+</sup> permeable AMPARs in the cell membrane. Finally, administration of Ca<sup>2+</sup>-permeable AMPARs allows for increased Ca<sup>2+</sup> 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 model impairing 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 Ca<sup>2+</sup>-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  $\gamma$ 8 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 floccs 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 biotinylation, 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-AMPA receptors 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-AMPA receptors 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-AMPA receptors. 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-AMPA transport immediately following LTP induction and perhaps other forms of plasticity that also include the CP-AMPA insert [124-127]. Internalization of GluA2, which is part of CP-AMPA expression, may share mechanisms with the induction of LTD (which does not involve synaptic CP-AMPA expression). It will be important to see how signaling pathways upstream of AMPAR subunits and the accessory proteins are specific for CP-AMPA 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.

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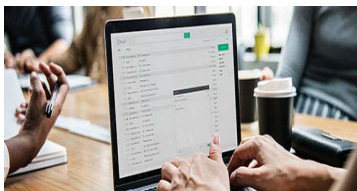
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Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is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

Career

Credibility

Financial

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### EARLY INVITATIONS TO ALL THE SYMPOSIUMS, SEMINARS, CONFERENCES

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Exclusive





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Exclusive

Financial

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Financial

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ASSOCIATE	FELLOW	RESEARCH GROUP	BASIC
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# 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](mailto:submit@globaljournals.org) or get in touch with [chiefeditor@globaljournals.org](mailto: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

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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.

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### 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 through 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:**

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<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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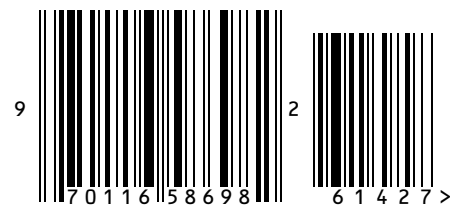


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ISSN 9755896



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