Project Notes:

Project Title: Quantifying the Effect of Neuromodulation on the relationship between Calcium Dynamics and Electrical Stimulation in P.*americana*

Name: Moulik, Adrika

Note Well: There are NO SHORT-cuts to reading journal articles and taking notes from them. Comprehension is paramount. You will most likely need to read it several times, so set aside enough time in your schedule.

Contents:

Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
Are there glutamate receptors and is it the primary neurotransmitter in cockroaches?	October break	https://doi.org/10.1242/jeb.64.3.665	10/16/2025
Does glutamate have an effect on the neuromuscular junction of cockroaches?	October break	https://doi.org/10.1016/0006-8993(74)90723-9	10/18/2025
Does glutamate have an effect on the Ach release in the NMJ of cockroaches?	October break	https://doi.org/10.1242/jeb.148.1.501	10/20/2025
Do cockroaches have the protein EAAT2 that cleans up excess glutamate?	Thanksgiving break	https://doi.org/10.1016/S0014-5793(98)01695-0	11/23/2025
Is there a precise translation or ratio between humans and cockroaches?	Thanksgiving break	https://askabiologist.asu.edu/insect-and-human-biology?utm_source=chatgpt.com	11/25/2025
Do cockroaches have all the necessary functions to model an ALS environment?	Thanksgiving break	https://doi.org/10.1186/s12868-024-00890-z	11/29/2025

Literature Search Parameters:

These searches were performed between (9/15/2025) and XX/XX/2019.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
Google Scholar	Glutamate, neuromuscular junction, neuromodulation	Found articles that showed how glutamate effected neuromodulation and how it was found in the NMJ
Google Scholar	Acetylcholine, nerve regeneration, innervation	Found articles about how acetycholine is the primary neurotransmitter in the NMJ and how it can lead to reinnervation in some regions with minimal neuron activity
Google Scholar	Denervation, Parkinson's disease, neuroprosthetics	Found articles about how glutamate is connected with Parkinson’s disease as well as information on how neuroprosthetics can use glutamate.
Google Scholar	ALS, neurodegenerative disease, excitotoxicity	Found articles focused around the background of ALS and what happens in the disease in relation to excitiotoxicity and glutamate.

Tags:

Tag Name	
#glutamate	
#drosophilamelanogaster	

#acetylcholine	
#neuromuscularjunction	

Article #1 Notes (TEMPLATE): Title

Article notes should be on separate sheets

KEEP THIS BLANK AND USE AS A TEMPLATE

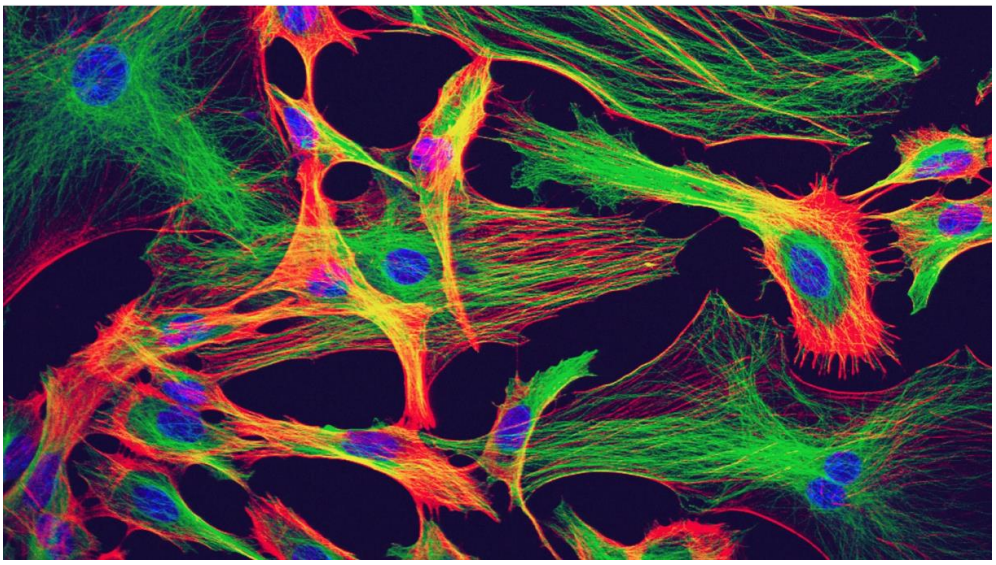
Source Title	
Source citation (APA Format)	
Original URL	
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #1 Notes: These Cells Spark Electricity in the Brain. They're Not Neurons.

Article notes should be on separate sheets

Source Title	These Cells Spark Electricity in the Brain. They're Not Neurons.
Source citation (APA Format)	Dattaro, L. (2024, January 4). These cells spark electricity in the brain. They're not neurons. *Quanta Magazine.* https://www.quantamagazine.org/these-cells-spark-electricity-in-the-brain-theyre-not-neurons-20231018/
Original URL	https://www.quantamagazine.org/these-cells-spark-electricity-in-the-brain-theyre-not-neurons-20231018/
Source type	Science Magazine Article
Keywords	Astrocytes, glutamate, glia, Drosophila melanogaster, synapses
#Tags	#glutamate #drosophilamelanogaster
Summary of key points + notes (include methodology)	The article, "These Cells Spark Electricity in the Brain. They're Not Neurons." by Laura Dattaro explains how astrocytes–a type of cell that is apart of the Central Nervous system and plays a role in maintaining internal balance in the brain and supporting neuron function–is involved in the electrical conversation as it responds to glutamate, a neurotransmitter. Inside the brain there are neurons, which communicate with each other using neurotransmitters by exchanging pulses of electricity. When this process of communication is repeated a multitude of times, thoughts, ideas, and memories are created from the interchanged chemicals. However, since there are over 86 million neurons, often some other chemical reactions/processes go undetected. Scientists in the field of neurology majorly agreed that neurons were the only type of brain cell that played a part in spreading electrical signals for most of the 1900s. Other brain cells, named glia, could not spread electrical signals and were only there for support. However, in the late 1900s, researchers discovered an astrocyte, which is a type of glia, that responded to glutamate-which can create electrical activity. In the following years since the discovery, there has been evidence of astrocytes that do respond to glutamate, but also astrocytes that do not respond to glutamate,

	creating conflicting results. There was a research paper published in September of 2023 in Nature by Andrea Volterra, and it clearly depicts images of glutamate flowing out of astrocytes, and genetic data that astrocytes have the capability and necessary cellular materials to utilize glutamate similar to how neurons do. The study also showed how there are specific astrocytes that can respond to glutamate and use it in the same way as neurons, meaning that not all astrocytes are capable of these abilities. Scientists originally assumed glia–all cells besides neurons–were simply there to hold the neurons together, and had little to no other purpose. However, upon further discoveries, scientists have observed that they have glutamate receptors which are used to clean up unused neurotransmitters. Scientists are unaware if astrocytes can generate an electrical signal by themselves using glutamate. There have been many studies done in which astrocytes have been stimulated, which resulted in close-by neurons responding and showed astrocytes potential to communicate with each other through synapses. Astrocytes have been observed to form a huge web-like shape over the brain and influence neuron activity using their constituent parts. By regulating glutamate effectively, it has been seen in mice that some neurons can enter a sleep-like state. Voltarra was able to find genetic evidence of the astrocytes involved in neurotransmitter storage, but he still needed to find evidence of the signaling occurring. METHODS: He used slices of mouse brain and simulated a neuronal signal, which resulted in some–not all– astrocytes responding with glutamate. He concluded that there are some select astrocytes that are able to release glutamate, but not all. This is related to my project because I plan to test if the stimulation of glutamate in neuronal cells can repair and strengthen the signals of neurons in Drosophila melanogaster, leading to parts of the muscles becoming more active. This article showed how cells that contain glutamate and respond to it are able to send electrical signals, though there are only specific cells with this ability, it is still possible that stimulating glutamate can send electrical signals, strengthening weak neurons.
Research Question/Problem/ Need	How are astrocytes involved in the electrical conversation?

Important Figures	 This shows the astrocytes described in this paper. Electrical activity was found in the astrocytes.
VOCAB: (w/definition)	astrocytes–a type of cell that is apart of the Central Nervous system and plays a role in maintaining internal balance in the brain and supporting neuron function glia–all cells besides neurons Glutamate-a neurotransmitter Synapses- the place where two neurons communicate
Cited references to follow up on	Renken, E. (2020, January 27). Glial brain cells, long in neurons' shadow, reveal hidden powers. *Quanta Magazine*. https://www.quantamagazine.org/glial-brain-cells-long-in-neurons-shadow-reveal-hidden-powers-20200127/ Renken, E. (2021, July 7). Neurons unexpectedly encode information in the timing of their firing. *Quanta Magazine*. https://www.quantamagazine.org/a-new-kind-of-information-coding-seen-in-the-human-brain-20210707/ Saplakoglu, Y. (2023, May 24). Is it real or imagined? How your brain tells the difference. *Quanta Magazine*. https://www.quantamagazine.org/is-it-real-or-imagined-how-your-brain-tells-the-difference-20230524/
Follow up Questions	1. What role does glutamate release by astrocytes play in brain neuron signaling? 2. How/can astrocytes contribute to memory in ways that were previously unknown? 3. What are limitations in detecting astrocyte electrical activity?

Notes:

- Investigate whether astrocytes, traditionally thought to support neurons, can generate electrical signals themselves.
- Astrocytes can release glutamate and have machinery to propagate electrical signals.
- Some astrocytes exhibit neuron-like activity, blurring the line between glial cells and neurons.
- Electrical signaling by astrocytes may influence neuronal activity and network communication.
- Challenges the traditional view that neurons are the only electrically active brain cells.
- Suggests astrocytes may play active roles in brain computation, learning, and plasticity.
- Opens potential avenues for therapies targeting astrocyte signaling in neurological disorders.

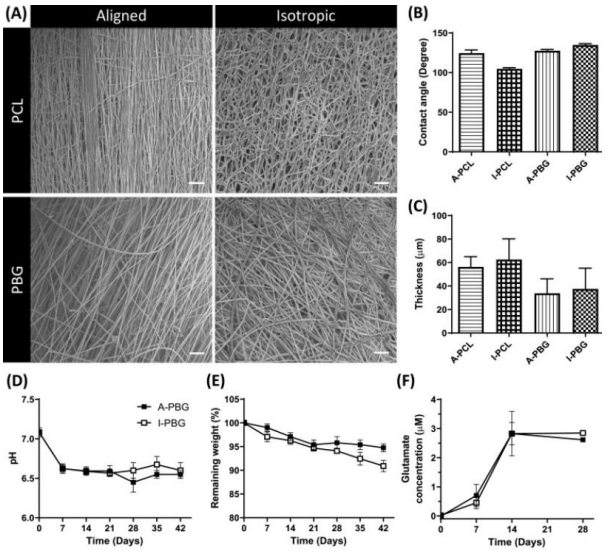
Article #2 Notes: Bioengineering strategy to promote CNS nerve growth and regeneration via chronic glutamate signaling

Article notes should be on separate sheets

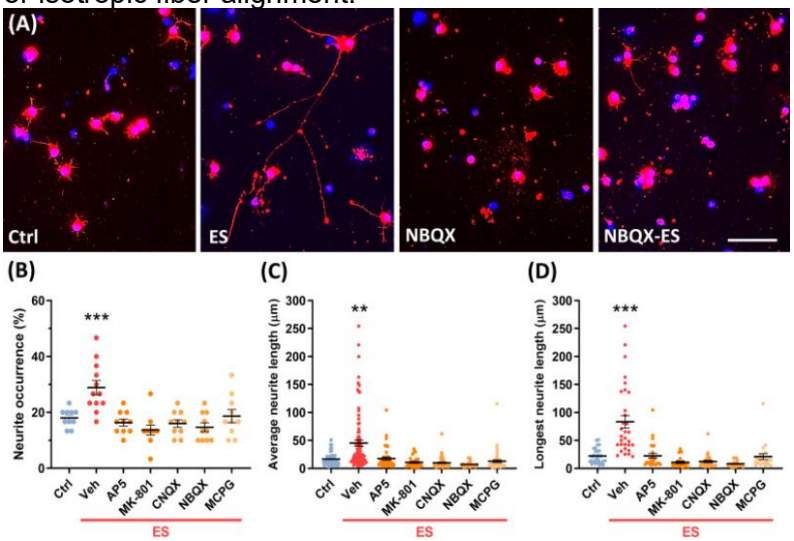
Source Title	Bioengineering strategy to promote CNS nerve growth and regeneration via chronic glutamate signaling
Source citation (APA Format)	Chang, K., Wu, J., Ma, T., Hsu, S., Cho, K., Yu, Z., Lennikov, A., Ashok, A., Rajagopalan, A., Chen, M., Su, W., Utheim, T. P., & Chen, D. F. (2024). Bioengineering strategy to promote CNS nerve growth and regeneration via chronic glutamate signaling. *Acta Biomaterialia,* [Advance online publication]. https://doi.org/10.1016/j.actbio.2024.10.023
Original URL	https://doi.org/10.1016/j.actbio.2024.10.023
Source type	Journal Article
Keywords	Electrical stimulation; Glutamate; Nerve regeneration; Retinal ganglion cells; Tissue engineering
#Tags	#glutamate #nerveregeneration
Summary of key points + notes (include methodology)	The research paper, "Bioengineering strategy to promote CNS nerve growth and regeneration via chronic glutamate signaling" is about how PBG scaffolds–which allow you to control the release of glutamate– can be used to regenerate the axons of the optic nerve. RGC's, which are retinal ganglion cells that form the optic nerve, aren't able to regenerate once they have reached the mature adult stage. This means that adults with vision loss have little to no chance of regaining their vision after they suffer from optic nerve diseases or injuries. Studies have shown that electrical stimulation has the capability to aid in regeneration of retinal neurons and can help preserve them. Glutamate, which is the primary neurotransmitter for retinal ganglion cells, can be toxic if the concentrations are extremely elevated, and can even cause RGC degeneration and lead to neurodegenerative diseases such as Alzheimer's. However, if the glutamate concentration is just enough that it has an impact but lower than toxicity levels, it can help in nerve growth and differentiation. The study wanted to investigate if electrical stimulation (ES) can regenerate retinal ganglion cell axons in the adult stage using glutamate signaling.

	METHODS: The experimental design consisted of two groups of cultured primary mouse RGCs in which one group was treated with the electrical stimulation. The ES treated group saw more neuron regeneration as there were longer neurites–the thread-like extensions that sprout off the neurons–and more neurites compared to the control group. This shows how electrical stimulation promotes the regrowth of neurites on RGCs using glutamate. They also tested to see if the effects of electrical stimulation used glutamate, by using glutamate receptor blockers. They found that without the glutamate receptors, the ability of ES to grow more neurites/longer neurites on the RGCs was much more limited, however the RGCs did not die without the glutamate. In conclusion, though ES using glutamate signaling was able to regenerate retinal cells, it does not mean the new regenerated cells will be able to survive and integrate into the host retina without complications. The paper was successfully able to show how ES promoted RGC neurite outgrowth using glutamate signaling as glutamate acted on axon terminals and influenced neurogenesis and neuron growth. Glutamate is closely involved with neurotrophic factors–a family of proteins that support the survival, development, and function of neurons–and together they are able to regulate neuron development and plasticity. This research paper is related to my project as I wanted to test to see if increased glutamate activity in neurons could lead to weaker neurons becoming stronger and able to send signals to muscles. This study showed how electrical stimulation promoted the growth of neurites–threads attached to neurons that receive electrical impulses from other neurons and sensory organs–on retinal ganglion cells using glutamate signaling as glutamate plays a major factor in transmitting messages between neurons. If I can also grow longer neurites on the weak neurons in my organism using ES and glutamate receptors, then I can increase their activity and increase neuro-muscular communication.
Research Question/Problem/ Need	How can PBG scaffolds be used to regenerate the axons of the optic nerve?

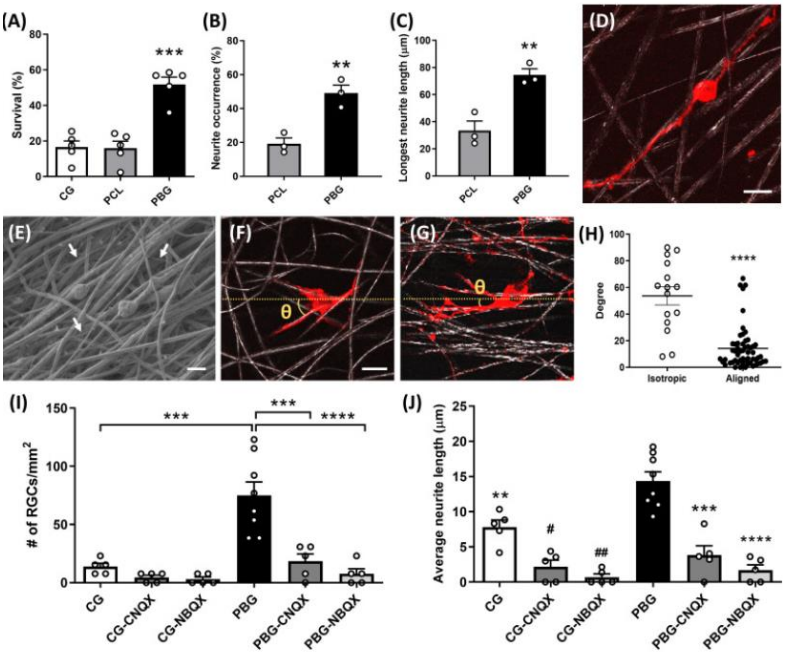
Important Figures



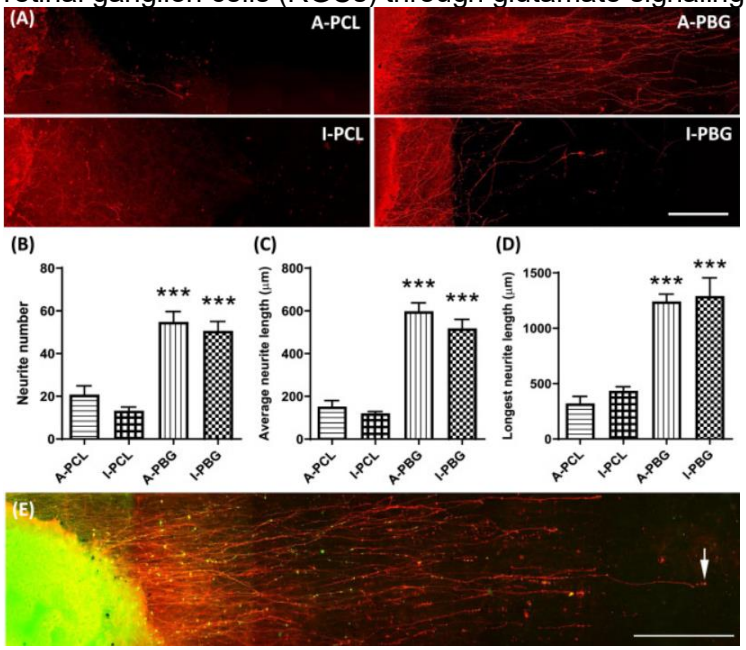
Design and characteristics of the electrospun polymer scaffolds of polycaprolactone (PCL) and poly(γ-benzyl-L-glutamate) (PBG) with aligned or isotropic fiber alignment.



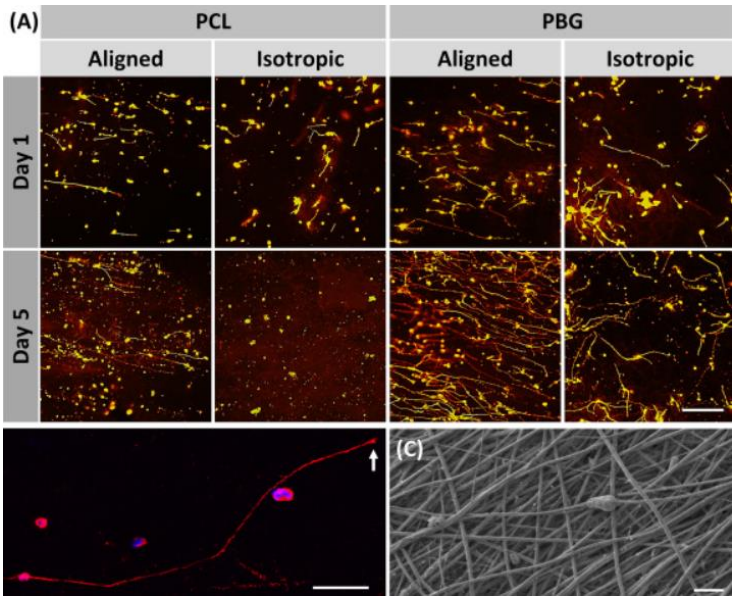
Electrical stimulation promotes neurite outgrowth of retinal ganglion cells through glutamate



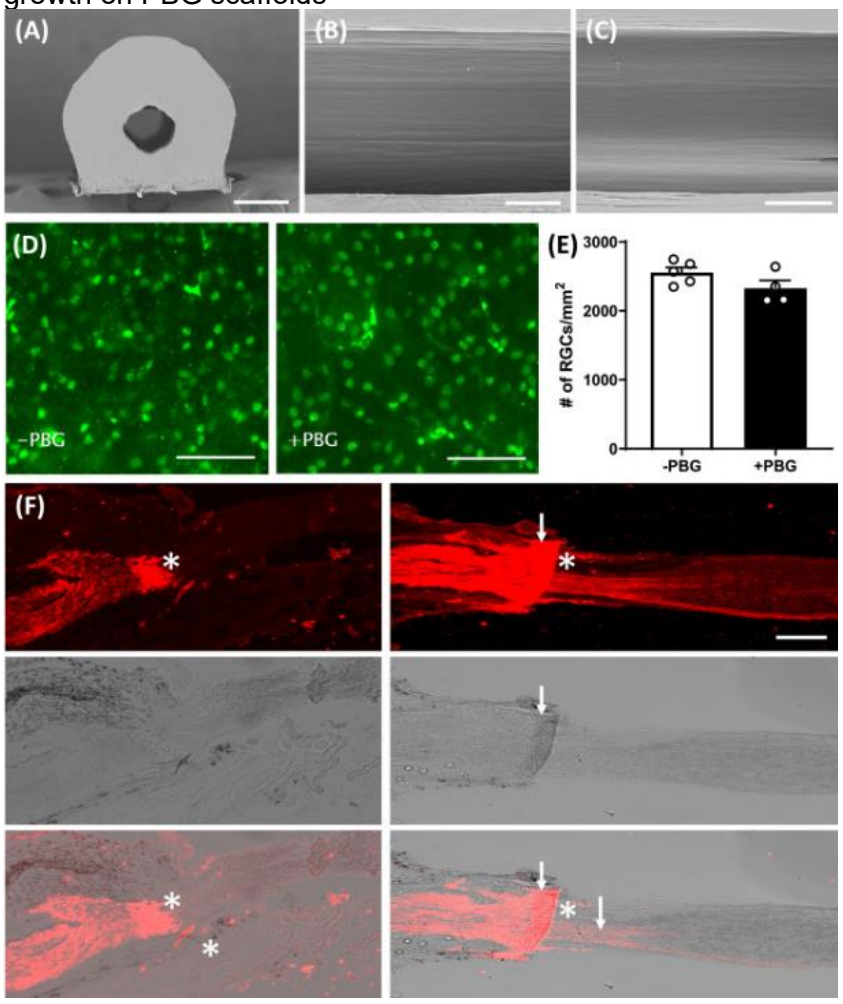
PBG scaffolds support survival and stimulate neurite growth of primary retinal ganglion cells (RGCs) through glutamate signaling.



Retinal explants develop long and robust neurites aligned with the direction of PBG fibers



Retinal ganglion cell progenitors (RGCPs) exhibit long and robust neurite growth on PBG scaffolds



PBG-coated 3D conduit exhibits the potential for optic nerve repair and

	regeneration
VOCAB: (w/definition)	• CNS (Central Nervous System) : brain and spinal cord; includes the optic nerve in this study. • Retinal Ganglion Cells (RGCs) :neurons in the eye that send visual signals to the brain; the ones researchers want to regrow. • Axon / Axonal growth :long nerve fiber of a neuron; axon growth = regrowth or extension after injury. • Neurite outgrowth: new projections (axons/dendrites) sprouting from neurons. • Glutamate signaling :chemical communication using the neurotransmitter glutamate; key to promoting growth in this paper. • Electrical stimulation (ES) :applying small electrical currents to boost nerve growth. • Scaffold: 3D structure used to support and guide neuron growth (like a framework). • Poly-γ-benzyl-L-glutamate (PBG) : special scaffold material here that slowly releases glutamate. • Differentiation :process where immature cells (progenitors) become specialized, like turning into RGCs. • Optic nerve regeneration : repairing and regrowing the optic nerve after it's damaged.
Cited references to follow up on	Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. *The Journal of Nutrition*, *130*(4S Suppl), 1007S–1015S. https://doi.org/10.1093/jn/130.4.1007S Ashok, A., Tai, W. L., Lennikov, A., Chang, K., Chen, J., Li, B., Cho, K.-S., Utheim, T. P., & Chen, D. F. (2023). Electrical stimulation alters DNA methylation and promotes neurite outgrowth. *Journal of Cellular Biochemistry*, *124*(10), 1530–1545. https://doi.org/10.1002/jcb.30462 Lewerenz, J., & Maher, P. (2015). Chronic glutamate toxicity in neurodegenerative diseases—What is the evidence? *Frontiers in Neuroscience*, *9*, Article 469. https://doi.org/10.3389/fnins.2015.00469
Follow up Questions	1. Why did the researchers use retinal ganglion cells as their model to study nerve regeneration? 2. How does glutamate signaling promote axon growth compared to other neurotransmitters? 3. Do the regenerated axons actually restore vision? Could this be tested in the future?

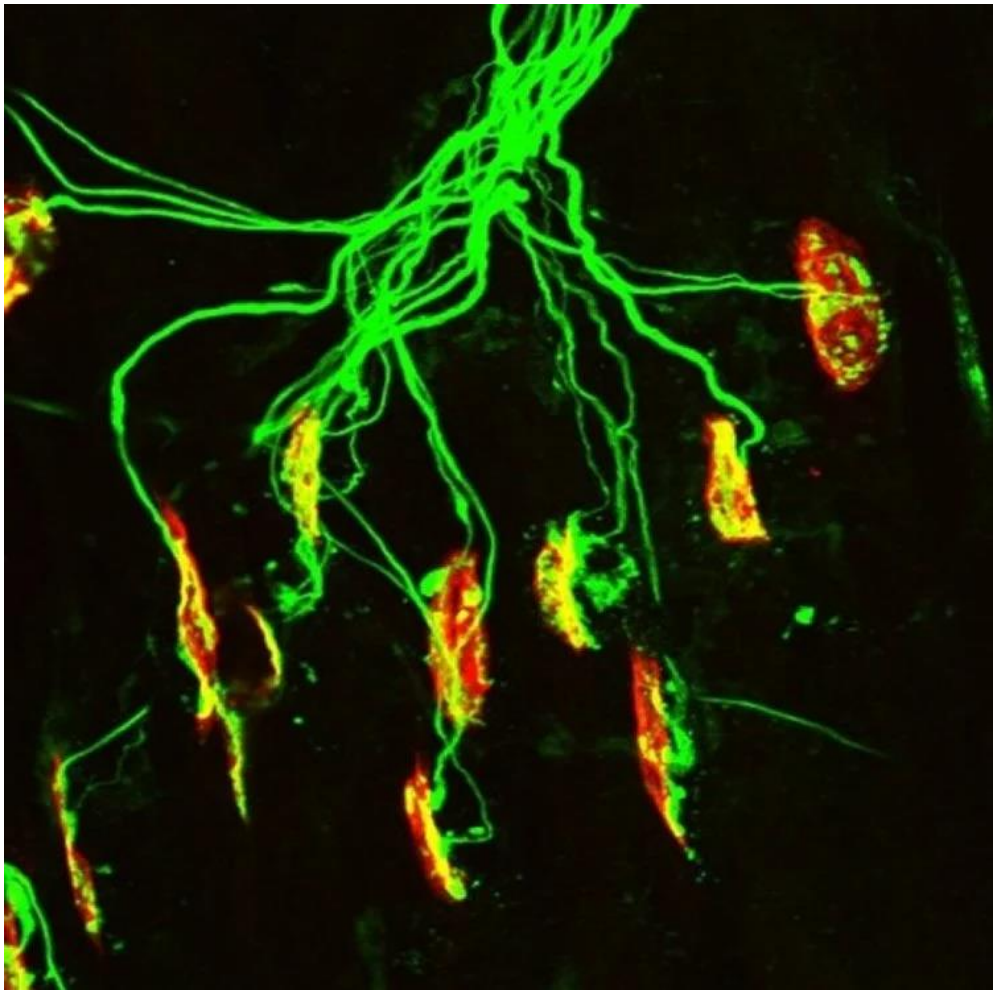
Notes:

- Test if chronic glutamate signaling can promote optic nerve regeneration.
- Use bioengineered scaffolds to support RGC survival, growth, and guidance
- Electrical stimulation (ES): showed RGC neurite growth depends on glutamate signaling.
- Built PBG scaffolds (release glutamate) vs. PCL scaffolds (no glutamate).
- Tested with:
    - Retinal explants
    - RGC cultures
    - Stem-cell derived RGC progenitors
- In vivo: optic nerve transection in mice, implanted PBG-coated conduits
- ES increased neurite growth via glutamate receptors.
- PBG scaffolds improved RGC survival, long axon growth, and progenitor differentiation.
- Aligned scaffolds guided axons in a specific direction.
- In mice, PBG scaffolds led to robust optic nerve regeneration.
- New strategy for CNS repair: scaffold + glutamate release mimics benefits of ES.
- Potential therapy for optic nerve injuries (e.g., glaucoma, trauma).
- Risk of excess glutamate (excitotoxicity).
- Unclear if regeneration = functional vision recovery.
- Needs testing for long-term safety and scaling to humans.

Article #3 Notes: Glutamate plays previously unknown role in neuromuscular development Article notes should be on separate sheets

Source Title	Glutamate plays previously unknown role in neuromuscular development
Source citation (APA Format)	University at Buffalo. (2016, September 19). Glutamate plays previously unknown role in neuromuscular development. ScienceDaily. https://www.sciencedaily.com/releases/2016/09/160919094528.htm
Original URL	https://www.sciencedaily.com/releases/2016/09/160919094528.htm
Source type	Online Source
Keywords	Glutamate, Neurotransmitter, Acetylcholine, Neuromuscular system / neuromuscular development, Motor neuron, Synapse elimination / pruning, NMDA receptors (N-methyl-D-aspartate receptors), Innervation, Peripheral nerve trauma / injury, BCIs
#Tags	#glutamate #neurotransmitter #acetylcholine #motorneuron
Summary of key points + notes (include methodology)	The article, "Glutamate plays previously unknown role in neuromuscular development" written by David J. Hill explains how Glutamate–the most common neurotransmitter in the brain, which means it is a chemical messenger that sends signals between neurons and other cells such as muscle cells–was discovered to be involved in controlling how neurons and muscles are connected together while developing. In a fully developed human being, there are many muscle fibers that are each contacted by a single motor neuron. In contrast, during the developmental stage, such as in a newborn, each muscle fiber is contacted by around 10 motor neurons. Previous studies assumed that the release of acetylcholine, another neurotransmitter, caused the release of the other neurons and left one neuron behind. Instead, glutamate is created by the conversion of a molecule released from the motor neurons, and then glutamate activates receptors, also known as NMDA (N-methyl-D-aspartate) receptors that react to glutamate and play a huge role in controlling brain development, learning, and synaptic plasticity–the ability of neurons to modify the strength of their connections–but they seemed unrelated to nerve distribution on the muscles. Research showed that the glutamate receptor controls how the neuromuscular system develops, and that the muscles decrease in response to glutamate over time, as at birth the response is more

	extreme and eventually disappears. In conclusion, though acetylcholine was thought to be the only neurotransmitter associated with the development of the neuromuscular system, studies showed that acetylcholine was not associated, and instead, glutamate played an important role in the development by using NMDA receptors. This article relates to my project idea, as I was thinking of researching if stimulating muscle activity using glutamate could strengthen neuron activity, which would improve the results of neuroprosthetics for people with paralysis. The article explains how glutamate uses NMDA receptors to control the development of the neuromuscular system, which means that in a body with paralysis or weaker neurons, increased amounts of glutamate could cause neurons to fire up and communicate more, as glutamate controls the neuromuscular system, potentially leading to weaker neurons increasing in strength and responding better to neuroprosthetics such as BCIs. METHODS: - Studied mouse models during early neuromuscular development. - Manipulated NMDA receptor activity using blockers and activators. - Applied exogenous glutamate and NMDA to muscle tissue. - Used immunolabeling to visualize neuromuscular junctions. - Measured calcium signaling changes in muscle fibers. - Conducted genetic knockdown of NMDA receptor subunits in muscle. - Analyzed synapse elimination across developmental time points.
Research Question/Problem/ Need	How do glutamate and other neurotransmitters play a role in how motor neurons and other muscles connect and refine their synapses during neuromuscular development?

Important Figures	 This image is a fluorescence microscopy image showing nerve fibers (green) innervating hair follicles (red/orange) in the skin.
VOCAB: (w/definition)	• Glutamate :A common neurotransmitter in the brain; here discovered to be involved also in how muscles and nerves are wired during development • Neurotransmitter :Chemical used by neurons to communicate with other neurons or muscles. • Acetylcholine :The neurotransmitter long thought to be the only one controlling nerve-muscle wiring (innervation) during development. • Neuromuscular system / neuromuscular development : The system involving nerves and muscles; development refers to how connections (synapses) form, mature, or are pruned during growth. • Motor neuron : A type of nerve cell that controls muscle activity (makes muscles contract). • Synapse elimination / pruning : The process by which extra or less-used neural connections are withdrawn or removed, leaving the strongest / most used ones. • NMDA receptors (N-methyl-D-aspartate receptors) :A specific kind of

	glutamate receptor; known in brain development and plasticity, now found to play a role in muscle innervation during development. • Innervation: The supply or formation of nerve connections to a muscle or organ. • Peripheral nerve trauma / injury : Damage to nerves outside the brain or spinal cord; relevant because repair after such injury may involve processes similar to those in development. • BCI: Brain computer interface
Cited references to follow up on	Personius, K. E., Slusher, B. S., & Udin, S. B. (2016). Neuromuscular NMDA receptors modulate developmental synapse elimination. *The Journal of Neuroscience*, *36*(34), 8783–8794. https://doi.org/10.1523/JNEUROSCI.1181-16.2016
Follow up Questions	1. How does glutamate's role in muscular development differ from acetylcholine's? 2. How does glutamate signaling decide which neurons are kept vs. Eliminated? 3. What are the possible risks of altering glutamate activity? What can these risks lead to?

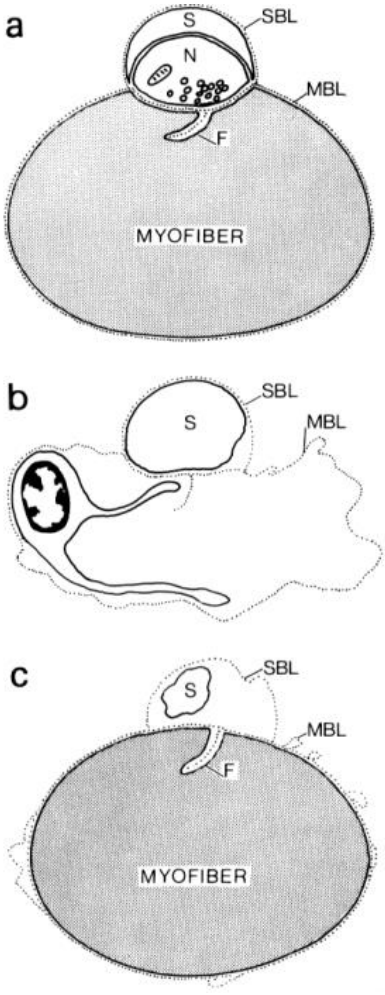
Notes:

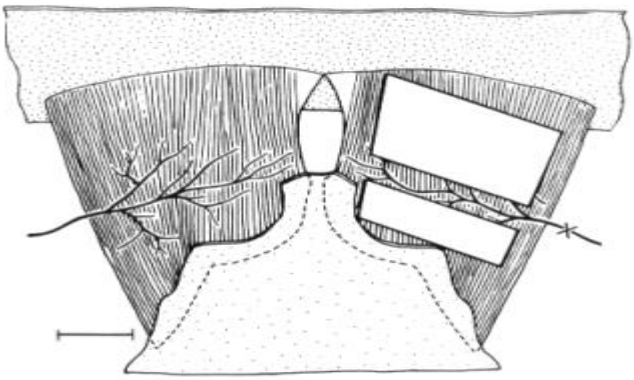
- Investigate whether **glutamate** plays a role in neuromuscular development, in addition to acetylcholine.
- At birth, muscle fibers are contacted by multiple motor neurons; over time, excess connections are **pruned**.
- Glutamate released from motor neurons **activates NMDA receptors** on muscles, guiding which connections are kept.
- Role of glutamate is **strong at birth**, decreases with maturation.
- Challenges the idea that acetylcholine is the only neurotransmitter controlling muscle innervation.
- Could inform therapies for **nerve injury repair** in adults.

Article #4 Notes: Acetylcholine receptors in regenerating muscle accumulate at original synaptic sites in the absence of the nerve

Article notes should be on separate sheets

Source Title	**Acetylcholine receptors in regenerating muscle accumulate at original synaptic sites in the absence of the nerve**
Source citation (APA Format)	Burden, S. J., Sargent, P. B., & McMahan, U. J. (1979). Acetylcholine receptors in regenerating muscle accumulate at original synaptic sites in the absence of the nerve. *The Journal of Cell Biology*, *82*(2), 412–425. https://doi.org/10.1083/jcb.82.2.412
Original URL	https://doi.org/10.1083/jcb.82.2.412
Source type	Journal Article
Keywords	Neuromuscular junctions, basal lamina, Schwann cell, junctional folds, a bungarotoxin
#Tags	#neuromuscularjunction #nmj #acetylcholine
Summary of key points + notes (include methodology)	The article, "Acetylcholine Receptors in Regenerating Muscle Accumulate at Original Synaptic Sites in the Absence of the Nerve" by Steven J. Burden, Peter B. Sargent, and U. J. McMahan is about the way neuron terminals affect how acetylcholine receptors are distributed on new regenerating muscle fibers. Usually after muscle fibers are damaged, they are ingested and they degenerate, but their thin layer of extracellular matrix or basal lamina sheath survives. The new single muscle cells form on the basal lamina sheaths and supply the area with nerves around the original nerve sites. METHODS: In this study, the researchers purposely damaged and removed the nerve supply of muscle cells and allowed new single muscle cells to grow on the basal lamina sheath, however they didn't allow them to resupply the area with nerves. How and where the acetylcholine receptors were placed on the single muscle cells was determined using histological procedures–a series of procedures used to prepare tissue samples for further examination–and they identified the original nerve sites on the basal lamina sheaths using a cholinesterase strain. Highlighting the original nerve sites allowed for the researchers to gauge whether the new acetylcholine receptors

	were located around that area post testing. After a time period of one month, the researchers observed that the acetylcholine receptors on the new single muscle cells located on the basal lamina sheath were centered around the original nerve sites. The number of receptors was like regular neuromuscular junction amounts. There were also folds on the single muscle cells that were similar to the folds in regular NMJ (neuromuscular junction) folds around nerve sites even when there were no nerve terminals present which is significant. This study overall showed how the way nerves and receptors on the basal lamina sheath are organized in regenerating muscle cells is determined by structures located on the original nerve sites after the removal of the original nerve.
Research Question/Problem/ Need	How do nerve terminals affect how acetylcholine receptors are organized on new muscle cells?
Important Figures	 Steps in degeneration and regeneration of myofibers in denervated bridges

	 FIGURE 1 Normal and denervated, damaged cutaneous pectoris muscles. The left muscle and its nerve are intact. Slabs have been removed from the right muscle; a bridge of damaged myofiber segments extends between intact myofibers at the muscle's medial and lateral borders. The nerve has been severed (X) near the lateral border. Bar, 3 mm. Normal and denervated, damaged cutaneous pectoris muscles.
VOCAB: (w/definition)	Phagocytized: ingested basal lamina sheaths: thin layers of extracellular skin matrix myofibers: single muscle cells innervated: supply with nerves junctional folds: specialized folds in the muscle cell membrane of the neuromuscular junction bungarotoxin: group of toxins found in the venom of snakes Schwann cell: a type of glial cell in the peripheral nervous system that plays a significant role in the support of nerve health and insulation
Cited references to follow up on	Dennis, M. J., & Miledi, R. (1974). Characteristics of transmitter release at regenerating frog neuromuscular junctions. *The Journal of Physiology*, *239*(3), 571–594. https://doi.org/10.1113/jphysiol.1974.sp010583 Birks, R., Katz, B., & Miledi, R. (1960). Physiological and structural changes at the amphibian myoneural junction, in the course of nerve degeneration. *The Journal of Physiology*, *150*(1), 145–168. https://doi.org/10.1113/jphysiol.1960.sp006379 Anderson, M. J., Cohen, M. W., & Zorychta, E. (1977). Effects of innervation on the distribution of acetylcholine receptors on cultured muscle cells. *The Journal of Physiology*, *268*(3), 731–756. https://doi.org/10.1113/jphysiol.1977.sp011879
Follow up Questions	1. How did the researchers constrict the new muscle cells from innervating the area on the basal lamina sheath? Instead of completely restricting them from innervating, could they only be partially restricted? Could this blocking process be controlled?

	2. Why did the researchers wait one month before observing the results? If they observed the results after a longer period of time, say 1 year, would the results be any different? Would the amount of acetylcholine receptors increase or stray away from the original nerve sites, maybe be more distributed? 3. Why/How is it that when damaged muscle cells are ingested and degenerated their thin layers of extracellular matrix stay? If parts of the basal lamina sheath were damaged and removed would this process still work?

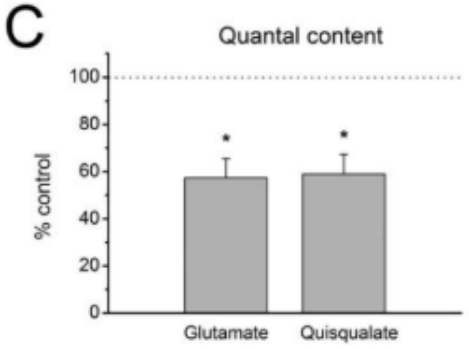
Notes:

- Glutamate can modulate cholinergic transmission at the neuromuscular junction, not just act as a CNS neurotransmitter.
- Activation of Group I mGluRs inhibits spontaneous and evoked ACh release at frog motor nerve terminals.
- NMDA receptors at the NMJ influence the timing (synchrony) of quantal ACh release.
- The effects of glutamate/quisqualate on ACh release appear mediated via metabotropic (mGluR) mechanisms rather than ionotropic ones in this preparation.
- Immunohistochemistry confirms localization of mGluR1a/5 and NMDA receptor subunits in the muscle endplate region.

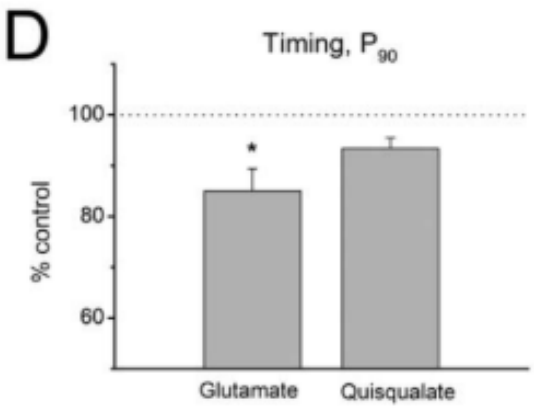
Article #5 Notes: Metabotropic and ionotropic glutamate receptors mediate the modulation of acetylcholine release at the frog neuromuscular junction

Article notes should be on separate sheets

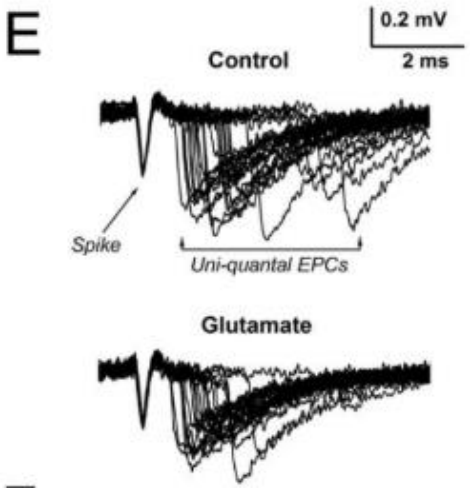
Source Title	Metabotropic and ionotropic glutamate receptors mediate the modulation of acetylcholine release at the frog neuromuscular junction
Source citation (APA Format)	Tsentsevitsky, A., Nurullin, L., Nikolsky, E., & Malomouzh, A. (2016). Metabotropic and ionotropic glutamate receptors mediate the modulation of acetylcholine release at the frog neuromuscular junction. *Journal of Neuroscience Research*, *95*(7), 1391–1401. https://doi.org/10.1002/jnr.23977
Original URL	https://doi.org/10.1002/jnr.23977
Source type	Journal Article
Keywords	cholinergic synapse, neurotransmitter Release, NMDA receptor, neuromodulation, metabotropic Glu receptors
#Tags	#neuromodulation #neurotransmitter
Summary of key points + notes (include methodology)	• There is evidence that glutamate is a signaling molecule at the NMJ where ach is a neurotransmitter • In this study frogs were used • Glutamate receptors that modulate ach release were analyzed • Electrophysiological experiments showed that glutamate reduces both spontaneous (when ach naturally releases) and electrically stimulated release of ach • Glu also makes it more synchronized and timed with electrical stimulation • Quisqualate, is a compound that activates glutamate receptors, it decreases/prevents the release of ach, however, does not affect the timing of the release, it does not prevent if a group 1 metabotropic (initiates a sequence of metabolic steps, does not directly open ionic channels but instead activates internal signaling pathways) glutamate blocker is present, and if there

	is a plc (phospholipase) inhibitor present. Group 1 mGlu receptors and plc signaling are involved in quisqualate 's effect • NMDA (glutamate receptor antagonist (reduces neuronal damage caused by excess glutamate) increases the synchrony of ach release, but doesn't mean it reduces total amount of ach released • Group 1 mglu receptors and nmda receptors are present at the neuromuscular synapse • The glutamate bonds to mglu, then plc is triggered and this is a key part of the signaling cascade METHODS: • Connective tissue was placed in a 3ml translucent chamber and superfused • Flowed through at a rate of 3 ml/min • Drugs applied via superfusing solution • measurements started 15-20 mins after drug application • EPCs recorded extracellularly with micropipettes (filled with Ringer solution, with a resistance of 2–4 MX) • Data acquired 10-15 min after positioning of recording microelectrodes • Up to 2,500 stimuli were applied to the motor nerve before and after drug application • Muscle samples fixed with paraformaldehyde, then washed, and permeabilized detergent • Muscles blocked to reduce non-specific binding and incubated overnight with primary antibodies • Samples incubated with fluorescent secondary antibody • Ach visualized by labeling with fluorescent α-bungarotoxin
Research Question/Problem/ Need	How do types of Glutamate receptors modulate quantal and non-quantal Acetylcholine release and synchrony in the NMJ?
Important Figures	 The effects of glutamate (50 mM) and quisqualate (50 mM) on the

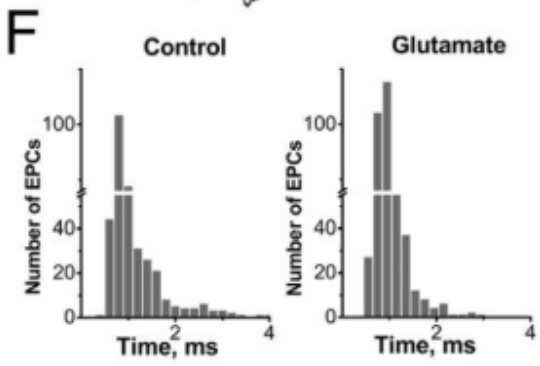
quantal content of endplate currents



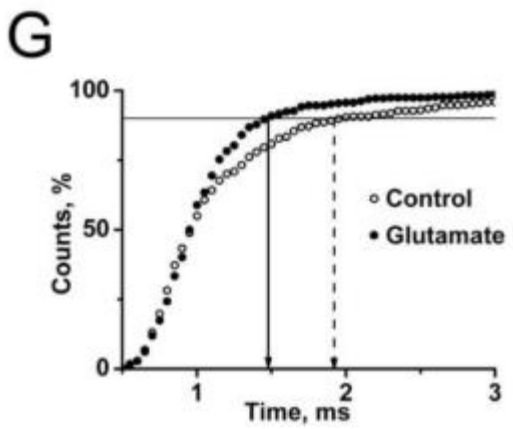
The effects of glutamate (50 mM) and quisqualate (50 mM) at the frog neuromuscular junction.



This image shows the effects of glutamate on the synchrony of uniquantal EPCs currents.



This image shows the amount of time it takes for EPC release to happen with glutamate vs in the control.

	 This image shows the time distribution of EPC current with glutamate vs in the control.
VOCAB: (w/definition)	Cholinergic synapse – synapse where Ach is released Neurotransmitter – chemical that communicates with neurons NMDA receptor – inotropic (opens ion channels) glutamate receptor Neuromodulation – signals from neurons are controlled Metabotropic glu receptors – internal pathways to generate a action potential when glutamate is bonded
Cited references to follow up on	Boulland JL, Qureshi T, Seal RP, Rafiki A, Gundersen V, Bergersen LH, Fremeau RT Jr, Edwards RH, Storm-Mathisen J, Chaudhry FA. 2004. Expression of the vesicular glutamate transporters during development indicates the widespread corelease of multiple neurotransmitters. *J Comp Neurol* **480**: 264–280. Bray JJ, Forrest JW, Hubbard JI. 1982. Evidence for the role of non-quantal acetylcholine in the maintenance of the membrane potential of rat skeletal muscle. *J Physiol* **326**: 285–296. Fu WM, Liou HC, Chen YH, Wang SM. 1998. Coexistence of glutamate and acetylcholine in the developing motoneurons. *Chin J Physiol* **41**: 127–132.
Follow up Questions	1. What is the effect of glutamate on Ach release in mammalian organsims? 2. Why Glu receptors inhibit Ach release in mature frog NMJs, but facilitate Ach release in early stages? 3. What specific types of metabotropic Glu receptors mediate cholinergic transmission in the frog NMJ?

Notes:

Glutamate & Ontogeny(development)

- When amphibians are small, glu facilitates release of ach by activating ionotropic receptors
- But in adult stages, it inhibits ach release, effect is eliminated by blocking metabotropic glu receptors

Ionotropic - directly opens ion pore to bond to neurotransmitter
Metabotropic - intercellular pathways and metabolic events triggered while binding to a neurotransmitter
Activate Group I mGluRs, especially when they want to differentiate metabotropic vs. ionotropic effects
Induce excitotoxicity in neurons (high concentrations can mimic pathological glutamate release)
QUANTAL = INDUCED EPCS
SEPCS - SPONTAENOUS, NONQUANTAL
Methods:
Epc micropipette data acquired 10 to 15 min after positioning of recording microelectrodes to achieve a steady-state level of spontaneous and evoked ACh
Secretions.
Electrophysiology - To obtain reliable EPC measurements, in each experiment up to 2,500 stimuli were applied to the motor nerve before and after drug application. We recorded 250 to 400 nerve ending currents and uniquantal postsynaptic responses.
Figures:

- A, B - Therefore, quisqualate exerts an inhibitory action similar to that of Glu on the spontaneous ACh quantal release of sEPCs and EPCs. Quisqualate reduces the frequency of sEPCs more than Glutamate
- C: glutamate reduces quantal content more than quisqualate, they both decrease
- D: glutamate reduces time that 90% of quantal release occurs by a lot! Quisqualate also decreases time but less reduction
- E: Motor nerve spikes (arrow) and uniquantal endplate currents (EPCs) in a glutamate-free control and in the presence of glutamate. Twenty-seven to thirty signals are superposed. With glutamate, the synaptic delays are more synchronized and less distributed compared to the control
- F: The distribution of synaptic delays in a control and after the application of glutamate recorded from a single synapse. The bin width is 0.2 msec. More EPCs in shorter amount of time with glutamate added compared to the control.
- G: Cumulative plots of synaptic delays of the uniquantal EPCs in a control (open circles) and after application of glutamate (filed circles). The bin width is 0.1 msec. Glutamate has less synaptic delays than the control
- Vertical lines indicate the times at which 90% of the quanta had been released(P90).
- Asterisk is significant based on p value greater than 0.05
- A: The effect of quisqualate (Quis, 50 mM) on the frequency of spontaneous endplate currents and on the quantal content of endplate currents. Quis reduces frequency of sEPCs with the blocker + glutamate is not reduced anymore, and quis + blocker is reduced the most, greater than the control
- B: The effect of quisqualate (Quis, 50 mM) on the frequency of spontaneous endplate currents in the absence or presence of a selective blocker of mGluR1a (LY 367385, 100 mM). Only quis reduces ach content, just blocker is closer to regular quantal release, and quis + blocker is much closer to regular content release, reduction is countered by blocker
- Table - In all our experiments, the amplitude, rise time, and decay time constant of evoked EPCs and sEPCs did not change following Glu or quisqualate treatment (Table II). Thus, quisqualate, like Glu, exerts presynaptic action by reducing both spontaneous and evoked release of ACh quanta. However, unlike Glu, quisqualate does not affect the time course of transmitter quanta secretion. Therefore, these data suggest that the effect of Glu on neuromuscular transmission may be mediated, not by one, but by several types of Glu receptors, whose activation triggers a variety of regulatory mechanisms. Group I mGlu receptors are apparently one of these types of receptors.
- Color lines - first A - metabotropic glu receptors look like this
- B- more ach with mGlu compared to control
- C - distribution of ach with glu recep compared to control

Analyzation:

Why they used Group 1 mGlu quisqualate
Glu was shown to reduce the level of spontaneous and evoked quantal ACh release in amphibian synapses (Pinard et al., 2003; Pinard and Robitaille, 2008; Adamek
et al., 2010); this depressing effect was eliminated by a nonselective Group I/Group II mGlu receptor antagonist. That is why we used the Group 1 mGlu agonist quisqualate to identify the role of specific groups of receptors in the effects of Glu. At the initial stage of our investigation, it was necessary to establish whether, and to what degree, quisqualate reproduces the effects of Glu.
Results tested using Wilcoxon test is a non-parametric statistical test
General - In all experiments in this section, the amplitude–
time parameters of recorded postsynaptic responses remained unchanged (Table II), as did the value of parameter P90,
Key findings slide - Their activation by quisqualate leads to a reduction of both spontaneous and induced quantal secretion of ACh but does not affect the timing of secretion.
Why?- Quisqualate is a known agonist of Group I mGluRs

- It activates mGluR1 and mGluR5, which are G-protein-coupled receptors involved in modulating synaptic transmission.
- These receptors can influence neurotransmitter release indirectly (unlike fast, ionotropic receptors).

100% release is hard to define precisely due to baseline noise, recording artifacts, or asymptotic tailing in the release curve.

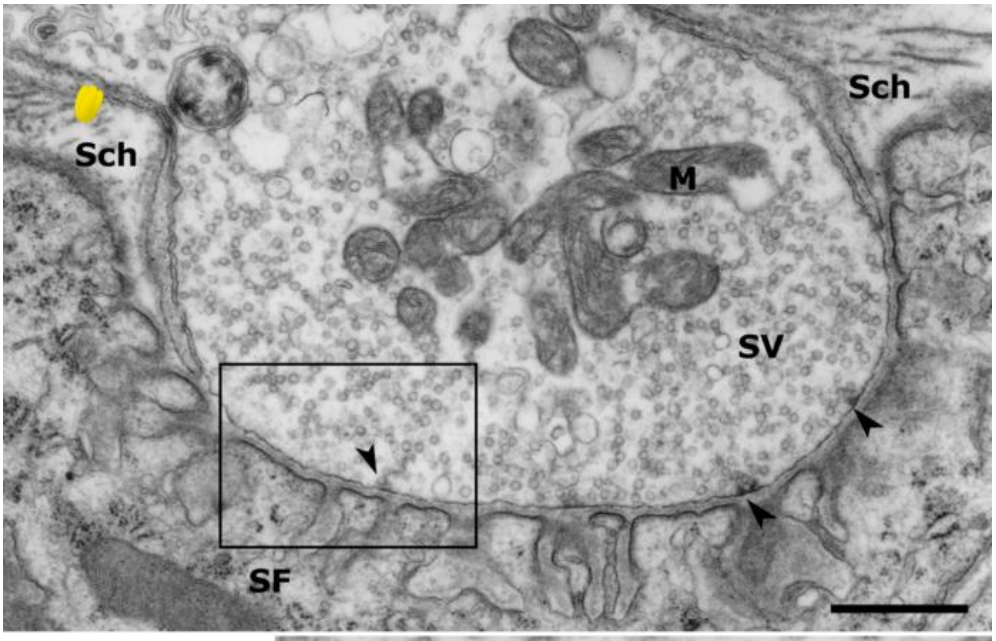
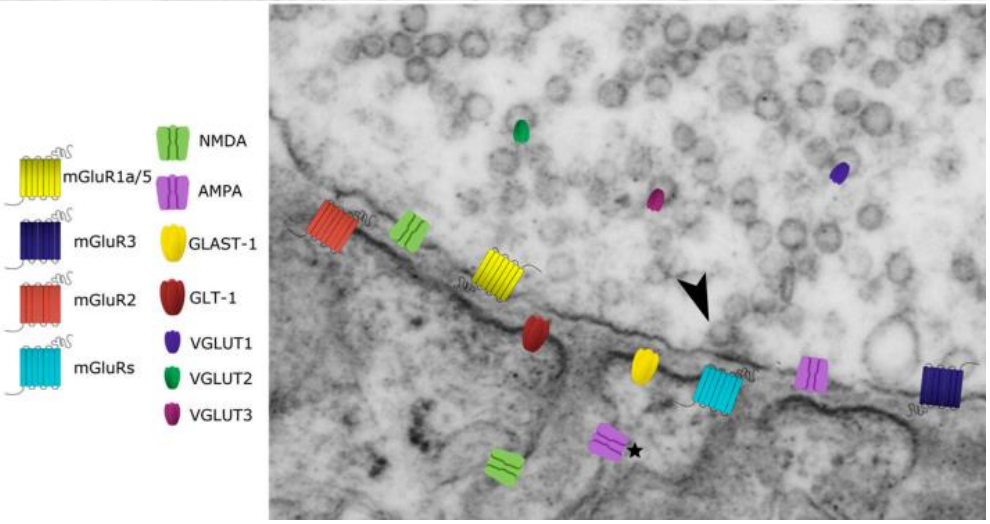
90% gives a reliable cutoff that still includes almost all physiologically relevant release.

Article #6 Notes: Glutamate at the Vertebrate Neuromuscular Junction: From Modulation to Neurotransmission

Article notes should be on separate sheets

Source Title	Glutamate at the Vertebrate Neuromuscular Junction: From Modulation to Neurotransmission
Source citation (APA Format)	Colombo, M. N., & Francolini, M. (2019). Glutamate at the vertebrate neuromuscular junction: from modulation to neurotransmission. *Cells*, *8*(9), 996. https://doi.org/10.3390/cells8090996

Original URL	https://doi.org/10.3390/cells8090996
Source type	Literature Review
Keywords	neuromuscular junction, glutamate, acetylcholine, neurotransmitter, receptor, transporter
#Tags	#glutamate #neuromuscularjunction #neurotransmitter
Summary of key points + notes (include methodology)	Acetylcholine is the major neurotransmitter at the neuromuscular junction (place where muscles and neurons interact), however glutamate is an important part in controlling how acetylcholine is released and controlling what happens with neuronal connections. In a neuron, the region that is presynaptic (meaning it begins the formation of the synapse) releases glutamate and contain it which contributes to the regulation of neurotransmission at the synapses through how it interacts with the pre and post synaptic receptors, which activates lower pathways that fix the communication at synapses and neuronal connections. While vertebrates are developing, their neurotransmitter at the NMJ can be experimentally shifted by regulating the calcium balances in motor neurons, in this case from acetylcholine to glutamate. This causes the muscle fibers to adapt and form new receptors for their new mediator. Also, in adult rodents, by diverting glutamate releasing nerves to a muscle with no nerve connection, nerve regeneration can occur and communication can be restored because of the formation of new NMJS. This creates an NMJ that is more brain-like, as glutamate is found in the brain, and less classic. They are trying to support glutamates' role as a neurotransmitter at synapses in the NMJ, focusing on the glutamate activated signaling pathways that are already present. Methods: • They analyzed existing research on glutamate at vertebrate neuromuscular junctions (NMJs). • The scientists combined and analyzed data from electrophysiology, immunohistochemistry, molecular biology, and pharmacology. • The scientists focused on evidence for glutamate receptors, transporters, and signaling at NMJs in development, injury, and modulation environments.
Research Question/Problem/ Need	How does glutamate affect Acetylcholine release and modulation in the neuromuscular junction?

Important Figures	  Glutamate signaling machinery at the vertebrate neuromuscular junction.
VOCAB: (w/definition)	• Neuromuscular junction: connection between a motor neuron and a muscle fiber where nerve signals trigger muscle contraction. • Glutamate: major excitatory neurotransmitter in the brain; important for learning and memory. • Acetylcholine: neurotransmitter used at neuromuscular junctions and in the brain; involved in muscle movement and attention. • Neurotransmitter: chemical messenger that transmits signals between neurons or from neurons to muscles. • Receptor: protein that binds to neurotransmitters and triggers a response in the cell. • Transporter: protein that moves neurotransmitters across cell

	membranes, often for reuptake or recycling.
Cited references to follow up on	Waerhaug, O.; Ottersen, O.P. Demonstration of glutamate-like immunoreactivity at rat neuromuscular junctions by quantitative electron microscopic immunocytochemistry. Anat. Embryol. (Berl.) 1993, 188, 501–513. [CrossRef] Boulland, J.L.; Qureshi, T.; Seal, R.P.; Rafiki, A.; Gundersen, V.; Bergersen, L.H.; Fremeau, R.T.; Edwards, R.H.; Storm-Mathisen, J.; Chaudhry, F.A. Expression of the vesicular glutamate transporters during development indicates the widespread corelease of multiple neurotransmitters. J. Comp. Neurol. 2004, 480, 264–280. [CrossRef] Nishimaru, H.; Restrepo, C.E.; Ryge, J.; Yanagawa, Y.; Kiehn, O. Mammalian motor neurons corelease glutamate and acetylcholine at central synapses. Proc. Natl. Acad. Sci. USA 2005, 102, 5245–5249. [CrossRef]
Follow up Questions	1. Does glutamate release scale with stimulation frequency? 2. Is induced glutamatergic transmission beneficial or detrimental in diseases of the NMJ? 3. How does activity-dependent plasticity at NMJs change when glutamate signaling is enhanced/blocked?

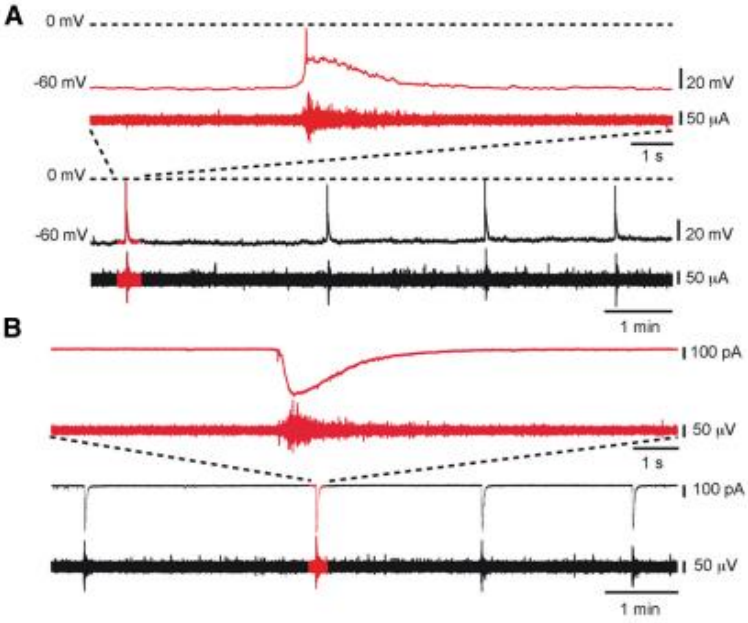
Article #7 Notes: Acetylcholine Controls GABA-, Glutamate-, and Glycine-Dependent Giant Depolarizing Potentials that Govern Spontaneous Motoneuron Activity at the Onset of Synaptogenesis in the Mouse Embryonic Spinal Cord

Article notes should be on separate sheets

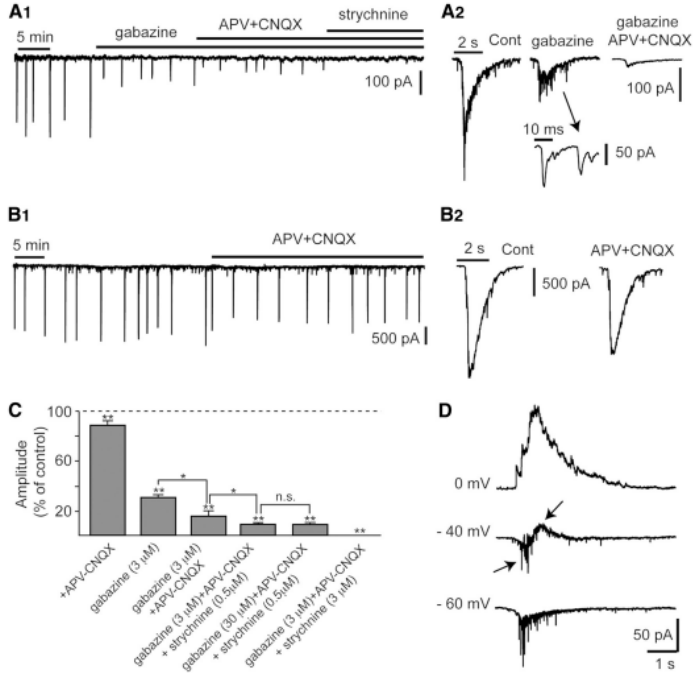
Source Title	Acetylcholine Controls GABA-, Glutamate-, and Glycine-Dependent Giant Depolarizing Potentials that Govern Spontaneous Motoneuron Activity at the Onset of Synaptogenesis in the Mouse Embryonic Spinal Cord
Source citation (APA Format)	Czarnecki, A., Corronc, H. L., Rigato, C., Bras, B. L., Couraud, F., Scain, A., Allain, A., Mouffle, C., Bullier, E., Mangin, J., Branchereau, P., & Legendre, P. (2014). Acetylcholine Controls GABA-, Glutamate-, and Glycine-Dependent Giant Depolarizing Potentials that Govern Spontaneous Motoneuron Activity at the Onset of Synaptogenesis in the Mouse Embryonic Spinal Cord. *Journal of Neuroscience*, *34*(18), 6389–6404. https://doi.org/10.1523/jneurosci.2664-13.2014

Original URL	https://doi.org/10.1523/JNEUROSCI.2664-13.2014
Source type	Journal Article
Keywords	embryo; giant depolarizing potential; motoneuron; mouse; spinal cord; synaptogenesis
#Tags	#motoneuron #synapse
Summary of key points + notes (include methodology)	• Giant depolarizing potentials facilitate spontaneous motoneuron activity in the embryonic mouse spinal cord • GABA-, glutamate, and glycine all cause giant depolarizing potentials to form • Motoneurons aren't directly triggered by Ach, Ach controls giant depolarizing potentials by increasing the amount of neurotransmitter release from the interneurons inside the central nervous system • If GABA-, glutamate and glycine are all blocked, giant depolarizing potentials wouldn't exist • Ach is active in the presynaptic region of the motoneuron, so it facilitates early network activity needed for the synapse to fully develop Methodology • A whole cell patch clamp for motoneurons was used to measure spontaneous and evoked motoneuron activity • Receptor blockers and Ach modulators were used to figure out which neurotransmitters were causing the giant depolarizing potentials in motoneurons • The scientists immuno-stained to detect neurotransmitter vesicle markers in the spinal cord • The evoked motoneuron release was tested with high K+ and calcium dependence • Conductance modeling (measure of how easily ions can flow through a membrane) was used to estimate the contribution of each receptor type to the giant depolarizing potentials
Research Question/Problem/ Need	What is the effect of acetylcholine on the generation of giant depolarizing potentials that govern motoneuron activity, and what roles do GABA-, glutamate, and glycine have in controlling those giant depolarizing potentials in the spinal cord?

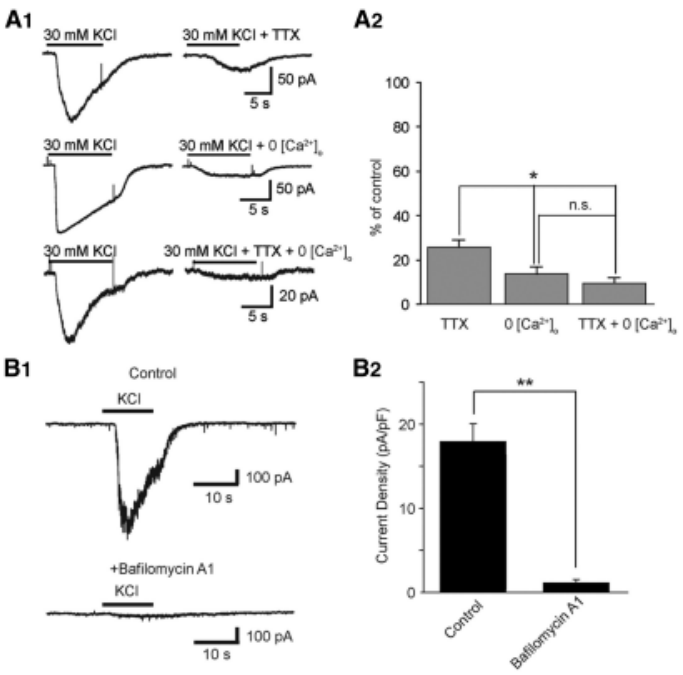
Important Figures



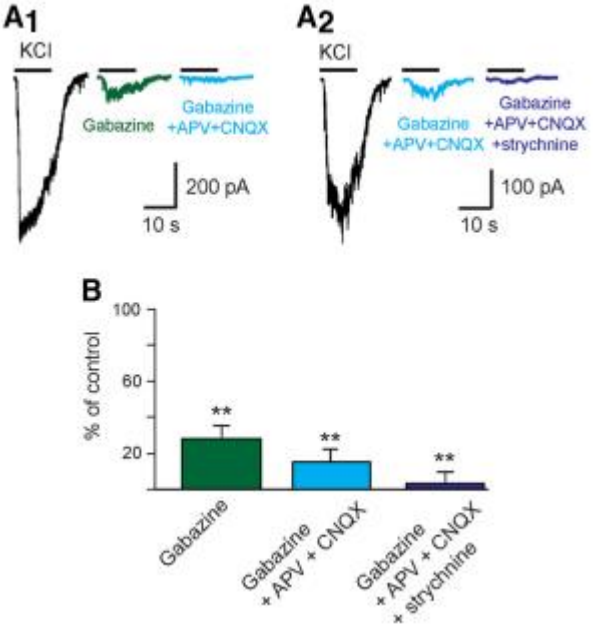
Spontaneous electrical activity recorded from MNs in the embryonic spinal cord.



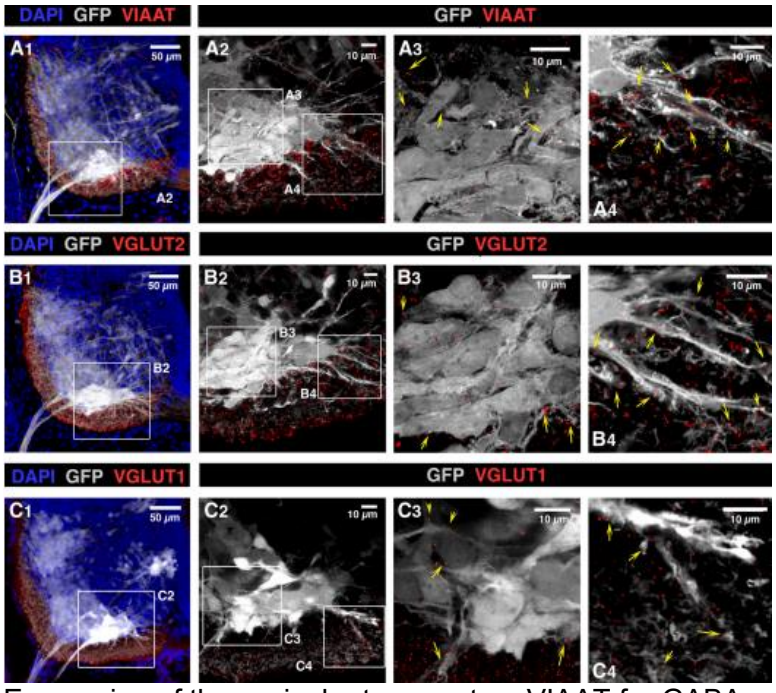
Simultaneous activation of GABA-, glutamate, and glycine receptors is involved in the generation of spontaneous GICs.



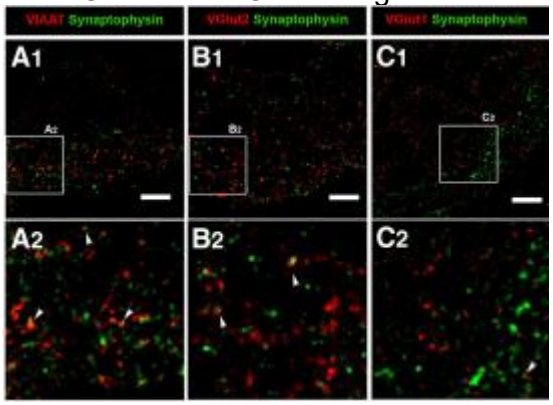
GICs evoked by neuronal network depolarization are dependent on vesicular release of neurotransmitters.



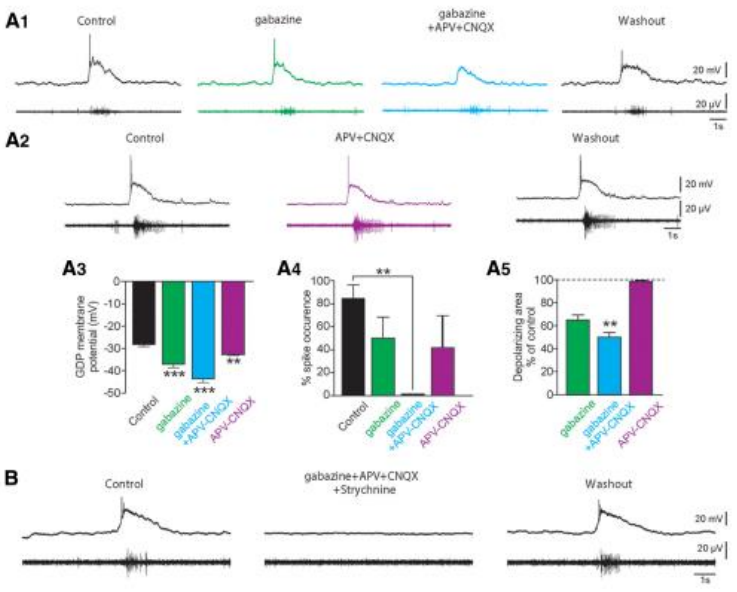
Evoked GICs reflect simultaneous release of GABA-, glutamate, and glycine.



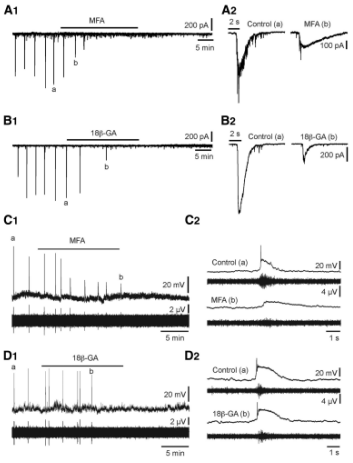
Expression of the vesicular transporters VIAAT for GABA- and glycine and VGluT2 and VGluT1 for glutamate in the ventral horn



Double immunostaining using VIAAT, VGluT2, VGluT1, and synaptophysin in the ventral horn.

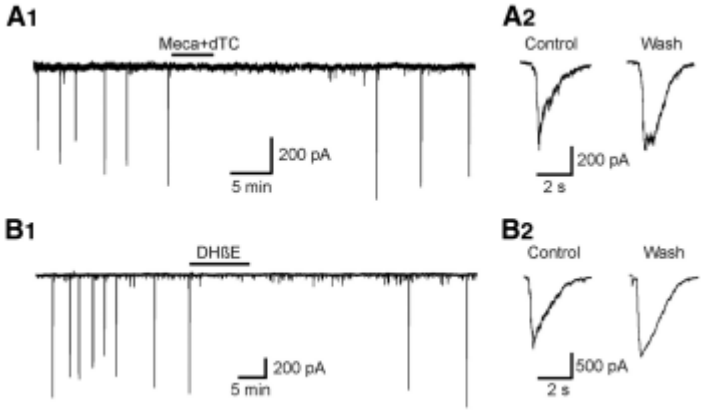


A simultaneous block of GABAA, glutamate, and glycine receptors is required to fully inhibit both the SC local network activity and GDPs on

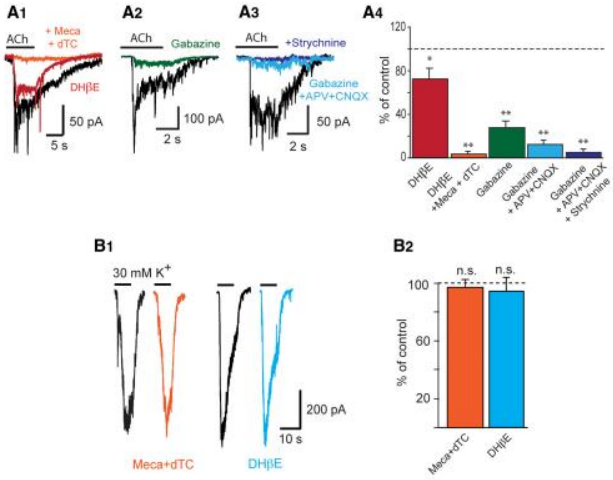


MNs.

GAP junctions participate in the generation of sGICs and sGDPs. Application of the GAP junction blockers MFA (50 µm).



Spontaneous nAChR activation controls the spontaneous occurrence of

	GICs  nAChR activation evoked GABA, glutamate, and glycine release.
VOCAB: (w/definition)	• Embryo: early stage of development after fertilization, before birth or hatching. • Giant depolarizing potential: spontaneous, synchronized bursts of electrical activity in immature neural circuits, especially during early development. • Motoneuron: nerve cell that sends signals from the spinal cord to muscles, causing them to contract. • Mouse: small mammal widely used as a model organism in biomedical and neuroscience research. • Spinal cord: part of the central nervous system running through the spine; transmits signals between the brain and body. • Synaptogenesis: the process by which neurons form new synapses (connections) with other neurons or target cells.
Cited references to follow up on	Alvarez FJ, Villalba RM, Zerda R, Schneider SP (2004) Vesicular glutamate transporters in the spinal cord, with special reference to sensory primary afferent synapses. J Comp Neurol 472:257–280. CrossRef Medline Chub N, O'Donovan MJ (1998) Blockade and recovery of spontaneous rhythmic activity after application of neurotransmitter antagonists to spinal networks of the chick embryo. J Neurosci 18:294 –306. Medline Gao BX, Ziskind-Conhaim L (1998) Development of ionic currents underlying changes in action potential waveforms in rat spinal motoneurons. J

	Neurophysiol 80:3047–3061. Medline
Follow up Questions	1. Does disrupting acetylcholine signaling during early development affect long-term motoneuron connectivity or function? 2. What specific interneuron types are activated by acetylcholine to trigger GABA-, glutamate, and glycine release? 3. How does the timing of acetylcholine's influence on GDPs change across development?

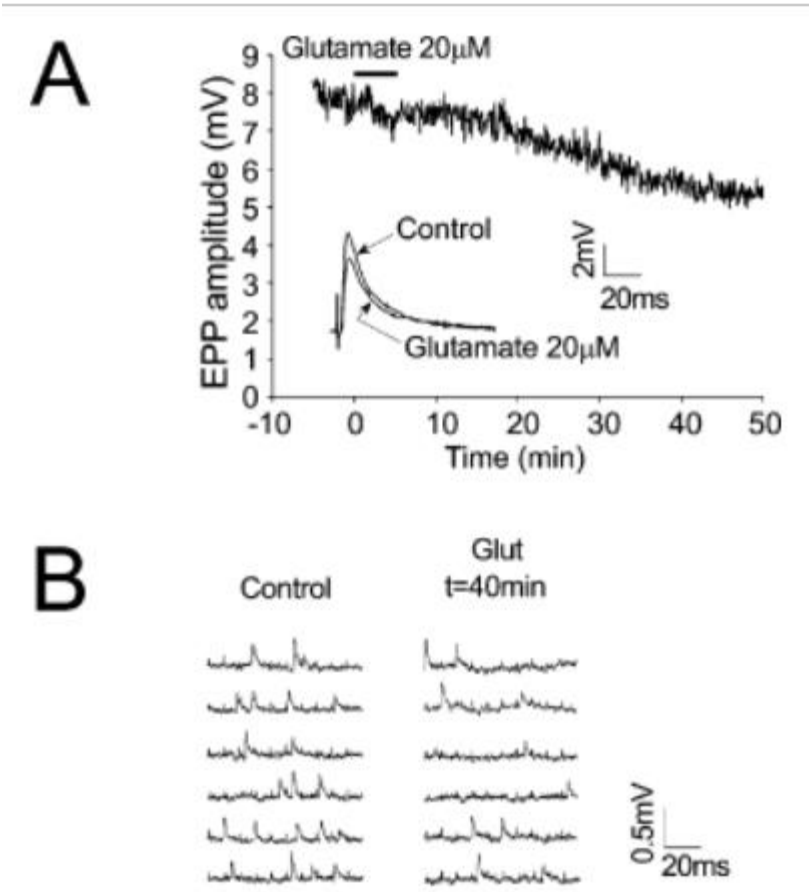
Article #8 Notes: Glutamatergic modulation of synaptic plasticity at a PNS vertebrate cholinergic synapse

Article notes should be on separate sheets

Source Title	Glutamatergic modulation of synaptic plasticity at a PNS vertebrate cholinergic synapse
Source citation (APA Format)	Pinard, A., Lévesque, S., Vallée, J., & Robitaille, R. (2003). Glutamatergic modulation of synaptic plasticity at a PNS vertebrate cholinergic synapse. *European Journal of Neuroscience*, *18*(12), 3241–3250. https://doi.org/10.1111/j.1460-9568.2003.03028.x
Original URL	https://doi.org/10.1111/j.1460-9568.2003.03028.x
Source type	Journal Article

Keywords	Glutamatergic regulation, Frog neuromuscular junction (NMJ), Cholinergic synapse, Transmitter release, (1S,3R)-aminocyclopentanedicarboxylic acid (ACPD), (S)-alpha-methyl-4-carboxyphenylglycine (MCPG), Metabotropic glutamate receptors (mGluRs)
#Tags	#glutamate #neuromuscularjunction #cholinergicsynapse #neurotransmitter #neuromodulation
Summary of key points + notes (include methodology)	• Glutamate, acetylcholine (ACh) = prominent excitatory neurotransmitters in vertebrate CNS, PNS • Neurotransmitters are co-released with neuromodulators and neuropeptides at same synapses • Proven that glutamate coexists with ACh at this synapse • Exogenous glutamate can modulate cholinergic synapses • increase of spontaneous activity at immature NMJs indicates this • Previous studies say how glutamate might be a neurotransmitter at cholinergic neuromuscular synapses • no direct evidence that endogenous glutamate regulates synaptic efficacy at cholinergic NMJs Methods: • Frogs anaesthetized using 3-aminobenzoic acid ethyl ester (Sigma) and were double-pithed • Cutaneus pectoris muscles + innervation --> dissected from Rana pipiens frogs • Motor nerve stimulated with suction electrode • EPP recordings taken with sharp glass microelectrodes • if Em depolarized by more than 15mV -->excluded from the study • constant perfusion performed with Ringer solution (room temperature) • muscle contractions prevented by partially + irreversibly blocking ACh receptors with 30-min closed bath application of alpha-bungarotoxin • Frog muscles incubated for 90min at room temperature • time course of fluorescence changes in PSCs monitored using 488nm line of argon ion laser for excitation • Muscles incubated for a period of 60min room temperature with primary antibody directed against mGluR group II and III • antibody also recognizes groupI mGluRs • followed by an incubation of 60min at room temperature with a secondary antibody • mGluR2/3 antibody revealed with streptavidin AlexaFluor • Alpha bungarotoxin double labelling with mGluR had the same steps as above however blockade solutionw was also used • Preparations observed with confocal microscope • Muscles fixed with 4% formaldehyde (10min), membranes permeabilized in 0.3% TritonX-100 solution (45min) • Secondary antibodies incubated for 60min each (room temperature) • Biotinylated guinea pig secondary antibody revealed with Streptavidin-

	Texas-Red • Western blot analysis used to perform specificity of GLAST and mGluR • Drugs such as l-glutamic acid, NMDA receptor antagonist, AMPA (non-selective), I/II mGluR antagonist, GLAST, GLAST inhibitor, were all obtained from various sources Results: • glutamate affects synaptic transmission • reduction of EPP amplitude by glutamate due to decrease in release of transmitter by nerve terminal • Glutamate does not affect overall presynaptic calcium levels • Glutamate does not affect calcium entry in nerve terminals • Exogenous glutamate decreases transmitter release • glutamate reduces transmitter release without affecting entire presynaptic calcium levels • endogenous glutamate doesn't act as neurotransmitter • results more consistent with glutamate being a modulator at frog NMJ • Glutamate acts through mGluR receptors • ionotropic glutamate receptors not involved • release of endogenous glutamate modulate synaptic efficacy + short-term plasticity at amphibian NMJ mainly through activation of mGluR receptors • mGluRs present at amphibian NMJ, predominantly clustered in the postsynaptic membrane • EPP amplitude evoked by nerve stimulation • Endogenous glutamate efficiently regulated by functional glutamate uptake machinery • glutamate transporter GLAST present at amphibian NMJ • large concentrations of glutamate required to induce small + occasional calcium responses • unlikely that glutamate efficiently activates PSCs during synaptic activity
Research Question/Problem/ Need	How does glutamate modulate synaptic plasticity at the peripheral nervous system cholinergic synapse?

Important Figures	 This image shows how exogenous glutamate decreases transmitter release compared to the control. Figure A shows the amplitude of EPPs before, during, and after glutamate application. Figure B shows the randomly selected recordings of MEPPs gotten after and before application of glutamate.

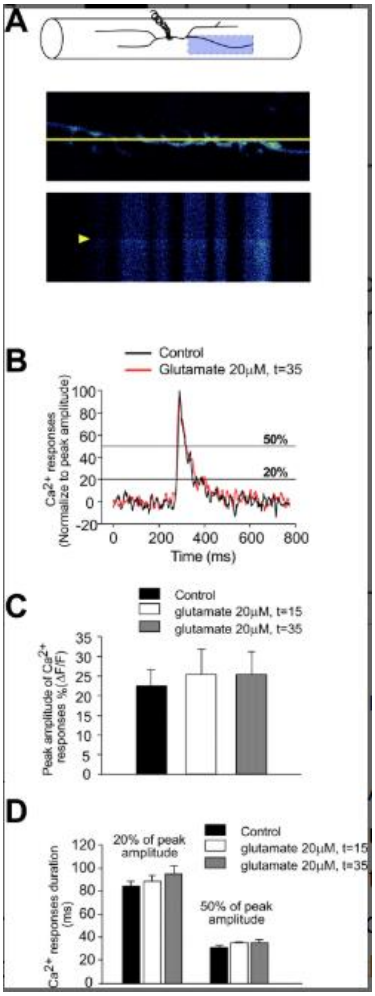
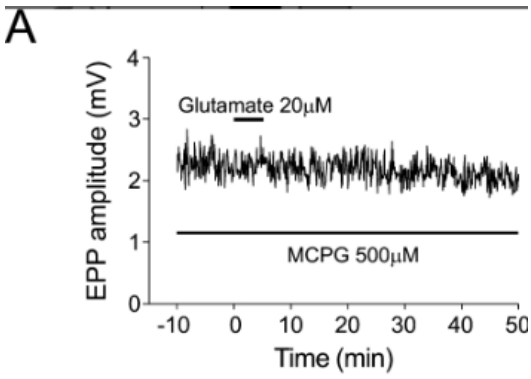
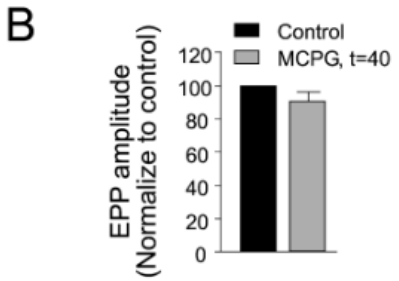


Figure A shows the NMJ at rest with a fluorescent indicator applied.
Figure B shows the relative change in fluorescence corresponding to calcium responses with glutamate vs in the control.
Figure C shows the peak amplitude of calcium responses in the control vs two glutamate levels.
Figure D shows the duration of calcium responses in the control vs two glutamate levels. It is distributed into 20% of peak amplititude and 50% of peak amplitude.





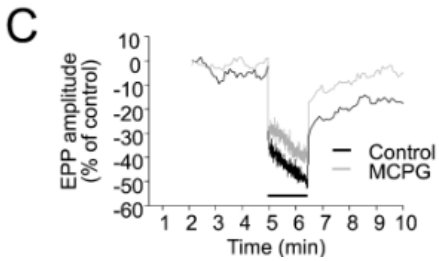
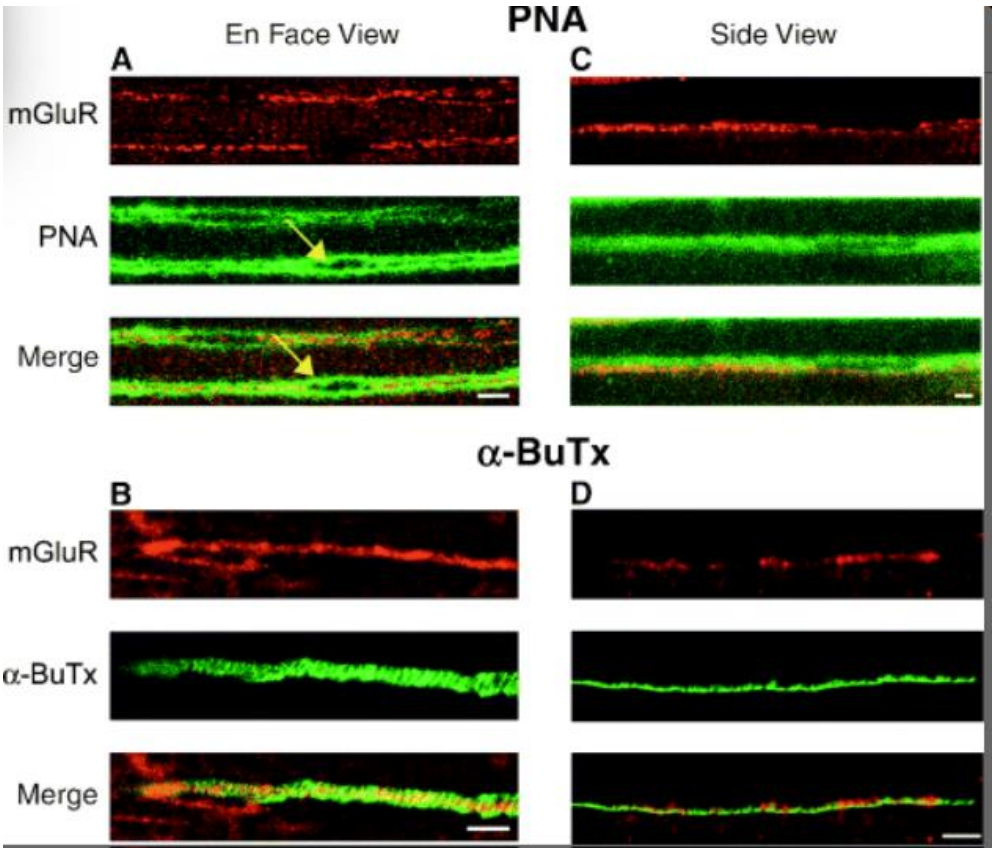
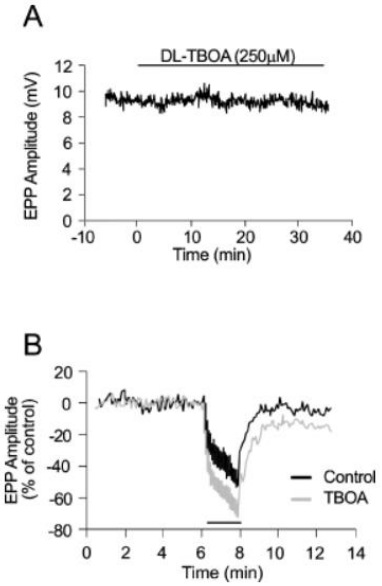


Figure A shows the EPP amplitude in millivolts during time durations with glutamate vs MCPG.
Figure B shows the EPP amplitude with MCPG vs the control.
Figure C shows the EPP amplitude (% of control) during time durations with the control vs MCPG.

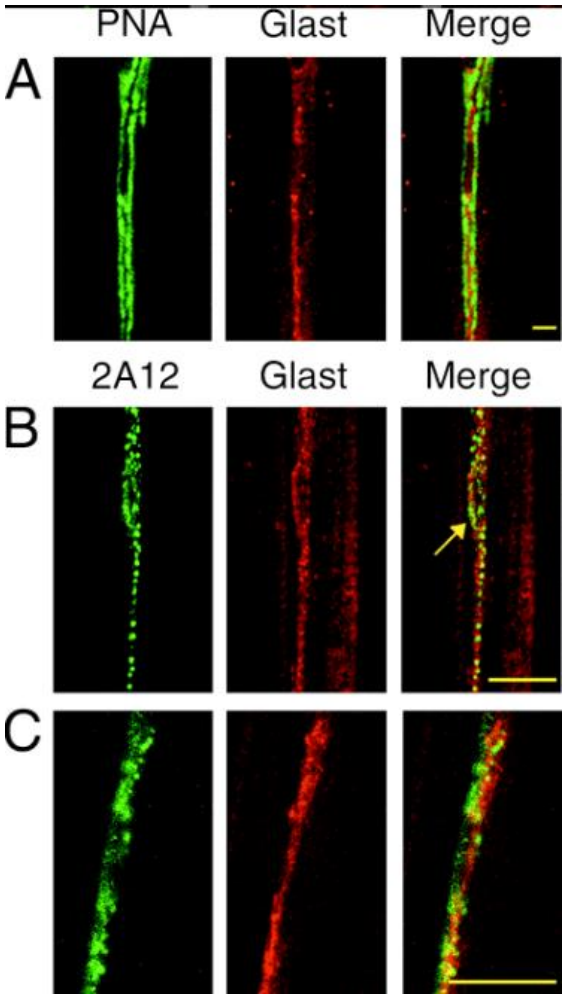


Localization of metabotropic glutamate receptors at the amphibian NMJ. Figure A shows the En Face view with mGluR, PNA, and Merge. It also shows the side view of mGluR, PNA, and Merge.
Figure B shows the en face view of mGluR, alpha BuTx, and Merge. It also shows the side view of these.

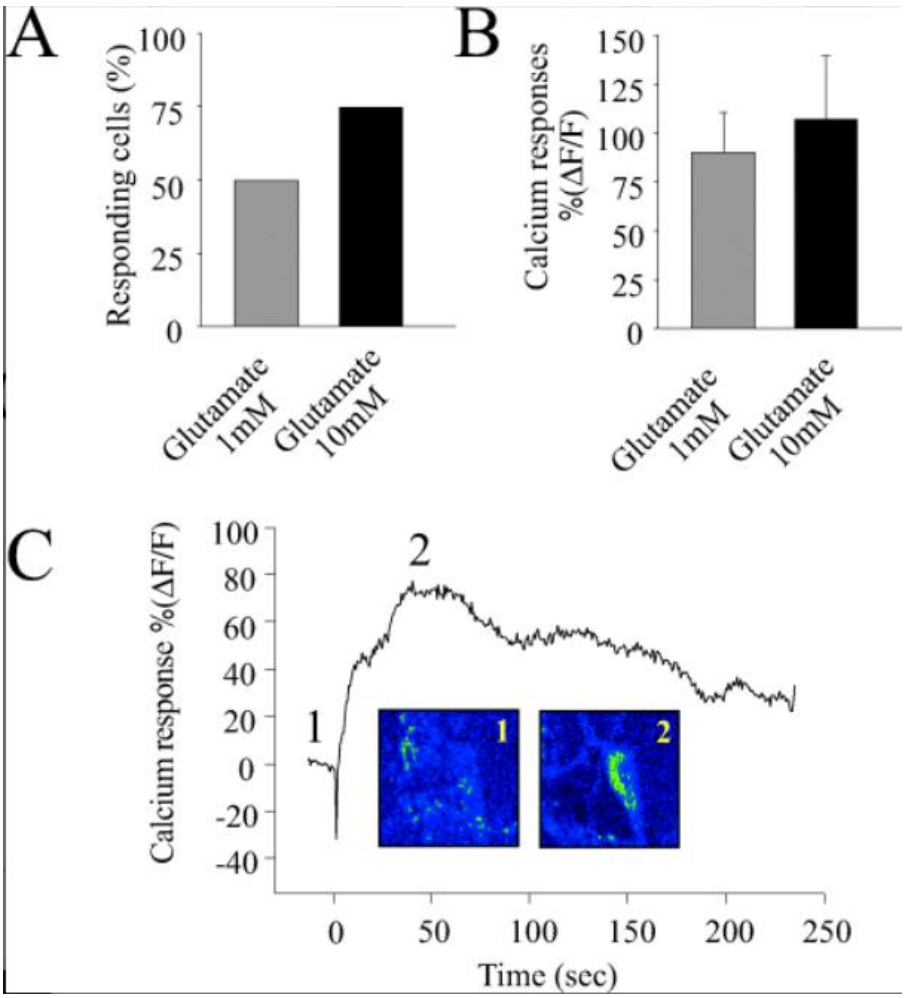


Functional glutamatergic uptake machinery at the amphibian NMJ. Figure A shows the EPP amplitude in millivolts with DL-TBOA in a time duration.

Figure B shows the EPP Amplitude (% of control) in a time duration with the control vs TBOA.



The glutamate transporter GLAST is present at the amphibian NMJ. Figure A shows the false color confocal images of the labelling of GLAST transporter in red and of the PNA lectin staining in green.
Figure B shows the false color confocal images of the labelling of the GLAST transporter in red and of the PSCs revealed with a 2A12 antibody in green.
Figure C shows the side view of a NMJ labelled with 2A12 antibody in green and for the GLAST transporter in red.

	 Large glutamate concentrations are required to induce Ca2+ responses in PSCs. Figure A shows the percentage of PSCs responding to local application of different amounts of glutamate (grey & black). Figure B shows the mean SEM relative changes in flourescence that the PSCs gave off wiht application of the two types of glutamate amounts (grey & black). Figure C shows the calcium responses in the PSCs induced by the local application of glutamate.
VOCAB: (w/definition)	• Glutamatergic regulation: Modulation of cell signaling or synaptic activity by the neurotransmitter glutamate. • Frog neuromuscular junction (NMJ): The synapse between a motor neuron and a muscle fiber in frogs, which uses acetylcholine (ACh) as the neurotransmitter. • Cholinergic synapse: A synapse where acetylcholine (ACh) is the primary neurotransmitter used for communication.

	• Transmitter release: The process of neurotransmitters being released from the presynaptic neuron into the synaptic cleft. • Presynaptic Ca^{2+} entry and handling: The entry of calcium ions into the presynaptic terminal to trigger neurotransmitter release and the subsequent regulation of those ions. • (1S,3R)-aminocyclopentanedicarboxylic acid (ACPD): A chemical that acts as an mGluR agonist, activating metabotropic glutamate receptors to mimic glutamate's effects. • (S)-alpha-methyl-4-carboxyphenylglycine (MCPG): A chemical that acts as an mGluR antagonist, blocking the action of glutamate on metabotropic glutamate receptors. • Metabotropic glutamate receptors (mGluRs): A class of glutamate receptors that are G-protein-coupled and produce slower, modulatory effects on synaptic transmission.
Cited references to follow up on	Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S. & Smith, S.J. (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science*, **247**, 470–473. Docherty, M., Bradford, H.F. & Wu, J.Y. (1987) Co-release of glutamate and aspartate from cholinergic and GABAergic synaptosomes. *Nature*, **330**, 64–66. Fu, W.M., Liou, J.C., Lee, Y.H. & Liou, H.C. (1995) Potentiation of neurotransmitter release by activation of presynaptic glutamate receptors at developing neuromuscular synapses of *Xenopus*. *J. Physiol.*, **489**, 813–823.
Follow up Questions	1. Why did local applications of glutamate on PSCs unreliability only evoke small calcium responses? 2. Why is there no evidence of endogenous glutamatergic regulation of synaptic transmission at a cholinergic NMJ? 3. Why can't it be ruled out that PSCs are activated by glutamate independently by calcium?

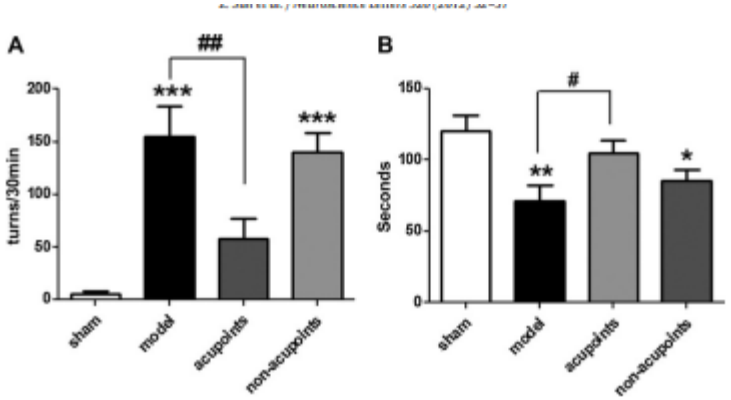
Article #9 Notes: Inhibition of glutamate and acetylcholine release in behavioral improvement induced by electroacupuncture in parkinsonian rats

Article notes should be on separate sheets

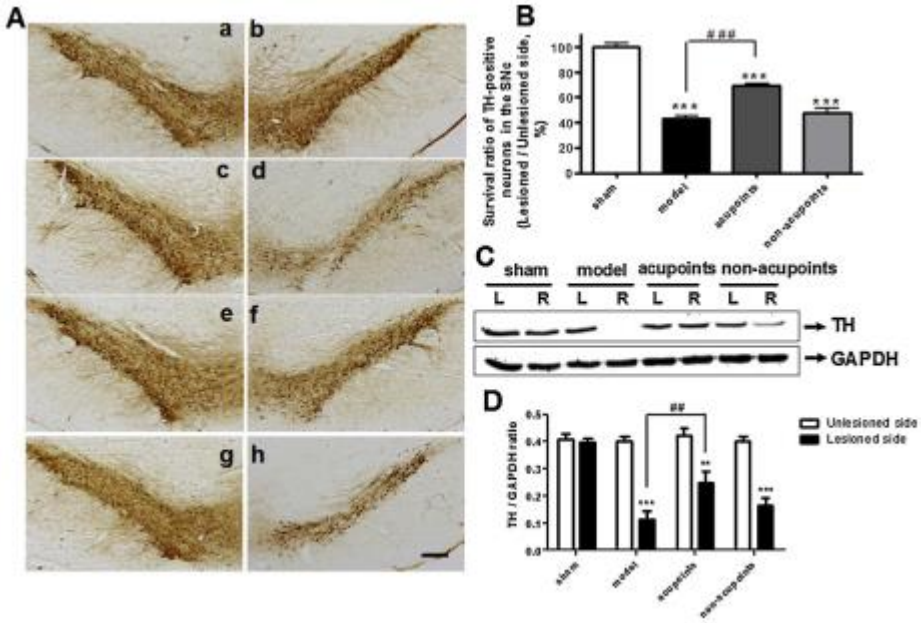
Source Title	Inhibition of glutamate and acetylcholine release in behavioral improvement induced by electroacupuncture in parkinsonian rats
Source citation (APA Format)	Sun, Z., Jia, J., Gong, X., Jia, Y., Deng, J., Wang, X., & Wang, X. (2012). Inhibition of glutamate and acetylcholine release in behavioral improvement induced by electroacupuncture in parkinsonian rats. *Neuroscience Letters*, *520*(1), 32–37. https://doi.org/10.1016/j.neulet.2012.05.021
Original URL	https://doi.org/10.1016/j.neulet.2012.05.021
Source type	Journal Article
Keywords	Parkinson's disease, Electroacupuncture,Dopamine, Glutamate, Acetylcholine
#Tags	#glutamate #acetylcholine
Summary of key points + notes (include methodology)	• The striatum is a large subcortical structure that mainly gets dopaminergic projections from the SN and glutamatergic projections from the cortex and thalamus (Sun). • Studies suggest that elevated neurotransmitters levels may be associated with systems of Parkinson's Disease • Studies showed that acupuncture lessened the symptoms of Parkinson's disease • Mechanisms underlying improvement are unstudied

	METHODS: • 48 Male Wistar rats were used weighing 180-200g each • They were anesthetized, lesions were performed, and used for a micro dialysis assay • A second hole was drilled at 1.0mm rostral to bregma and 2.5mm lateral to midline • 4 additional holes drawn for four flathead screws that hold the cannula in place • Rats randomly divided into 4 groups, received sham lesions, MFB lesions, electrical acupuncture stimulation on day 2, depending on what group they were in • The intensity of electrical acupuncture stimulation was increased step wise with multiple steps • Animals were relaxed during stimulation so no anesthetic needed • Behavioral tests assessed at the second day after 4 weeks of electrical acupuncture stimulation • All experiments were examined blind • After behavioral tests, rats implanted with guide cannulas were used for micro dialysis • Micro dialysis probe inserted and artificial cerebrospinal fluid was perfused • Samples collected every 15 min • Samples injected into high performance liquid chromatography system • Immunoblotting analysis and immunohistochemical analysis was performed to see the protective effect of electrical acupuncture on dopaminergic neurons • Rotational and rotarod test were used to measure motor-abilty of rats • Protein levels were measured to measure the effects of electrical acupuncture on TH expression in MFB lesioned rats • Decreased Glu and Ach levels, not dopamine levels, could play an important role in the improvement of abnormal rotation behavior and reduced treadmill capacity of high frequency electrical acupuncture stimulation in MFB lesioned rats. • Methods summary: vivo micro dialysis and high performance liquid chromatography (HPLC) was used to measure neurotransmitter levels in the striatum
Research Question/Problem/ Need	What is the effect of inhibiting glutamate and acetylcholine release in the behavioral development of parkinsonian rats?

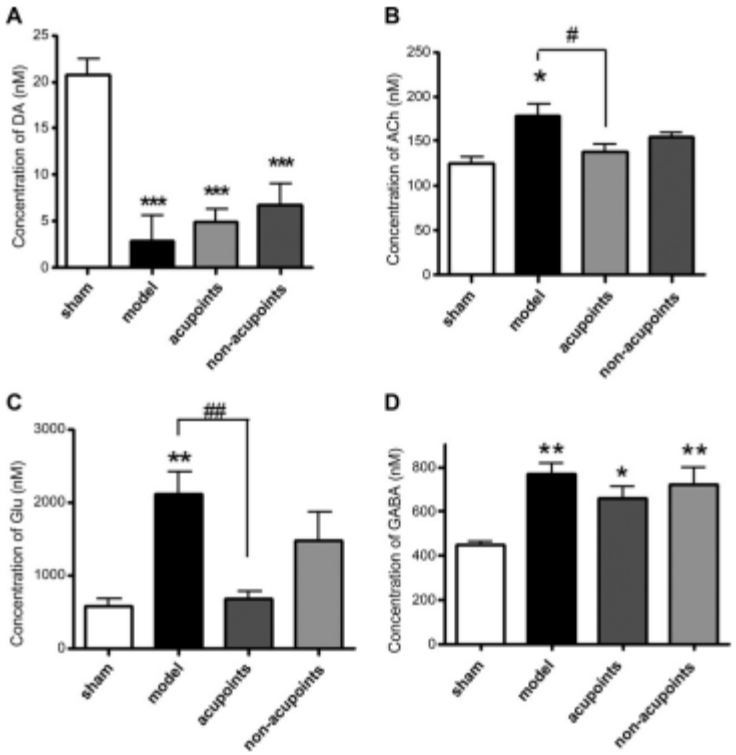
Important Figures	



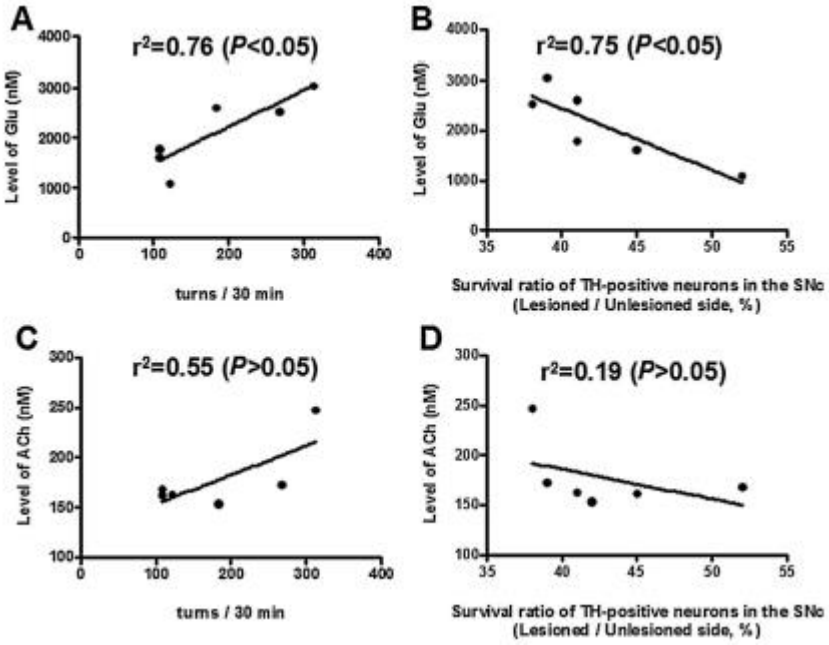
Effects of Electrical acupuncture on movement behavior at the end of 4 weeks after MFB axotomy.



Neuroprotective effects of Electrical acupuncture stimulation on TH-positive neurons and TH protein expression at 4 weeks after MFB axotomy.



Effects of electrical acupuncture on extracellular levels of dopamine.



Correlation between neurotransmitters levels and survival ratios of TH-positive neurons or rotational behavior induced by APO.

VOCAB: (w/definition)

- Glutamate: excitatory neurotransmitter commonly found in the central nervous system

	• Acetylcholine: primary neurotransmitter in the neuromuscular junction • Dopamine: a neurotransmitter that is associated with Parkinson's disease • Parkinson's Disease: a progressive neurological disorder that affects movement, balance, and coordination • Electroacupuncture: a form of acupuncture that combines traditional acupuncture needles with electrical stimulation
Cited references to follow up on	A. Dekundy, M. Pietraszek, D. Schaefer, M. Cenci, W. Danysz, Effects of group I metabotropic glutamate receptors blockade in experimental models of Parkinson's disease, Brain Research Bulletin 69 (2006) 318–326. G.J. Lee, C.S. Yin, S.K. Choi, S. Choi, J.S. Yang, H. Lee, H.K. Park, Acupuncture attenuates extracellular glutamate level inglobal ischemiamodel of rat, Neurological Research 32 (2010) S79–S83 A. Pisani, P. Bonsi, D. Centonze, P. Gubellini, G. Bernardi, P. Calabresi, Targeting striatal cholinergic interneurons in Parkinson's disease: Focus on metabotropic glutamate receptors, Neuropharmacology 45 (2003) 45–56.
Follow up Questions	1. How can these findings be generalized to humans? 2. How does electrical acupuncture stimulation downregulate neurotransmitters such as Glu and Ach? 3. What happens if we block or boost certain brain receptors—does it change how the neurons respond?

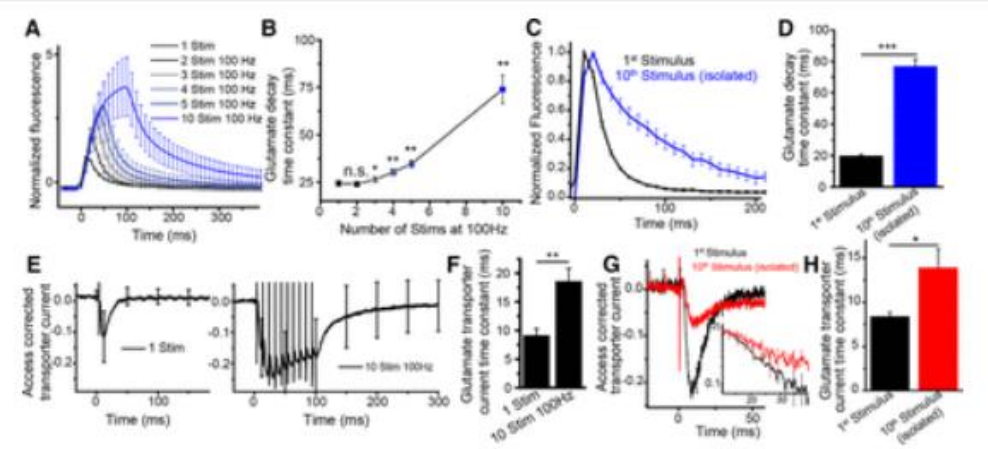
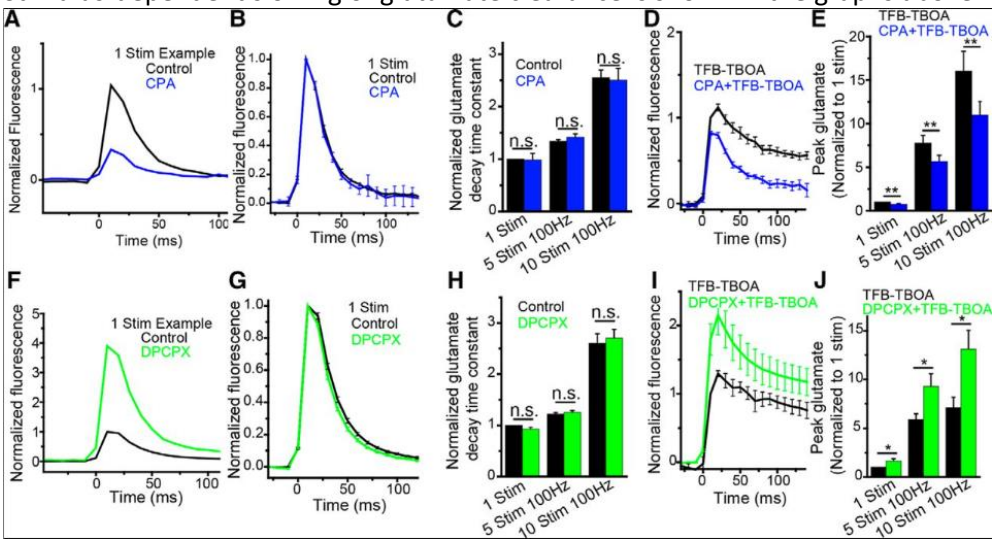
Article #10 Notes: Glutamate Clearance Is Locally Modulated by Presynaptic Neuronal Activity in the Cerebral Cortex

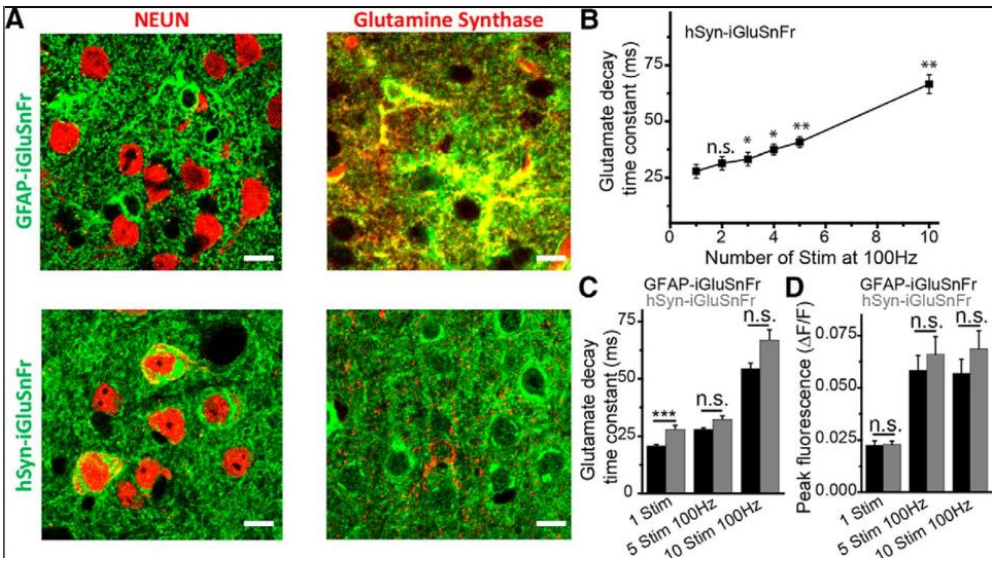
Article notes should be on separate sheets

Source Title	Glutamate Clearance Is Locally Modulated by Presynaptic Neuronal Activity in the Cerebral Cortex
Source citation (APA Format)	Armbruster, M., Hanson, E., & Dulla, C. G. (2016). Glutamate clearance is locally modulated by presynaptic neuronal activity in the cerebral cortex. *The Journal of Neuroscience*, *36*(40), 10404–10415. https://doi.org/10.1523/jneurosci.2066-16.2016
Original URL	https://doi.org/10.1523/jneurosci.2066-16.2016
Source type	Journal Article
Keywords	Activity, astrocyte, cortex, glutamate, glutamate uptake
#Tags	#glutamate #astrocytes
Summary of key points + notes (include methodology)	• EAATs - expressed by astrocytes, rapidly take away glutamate from extracellular environment, restrict temporal / spatial extent of glutamate signaling • capacity to remove glutamate = large, no saturation, even w/large glutamate challenges • neuronal activity rapidly + reversibly modulates EAAT-dependent glutamate transport • slowing of glutamate uptake depends on frequency + duration of

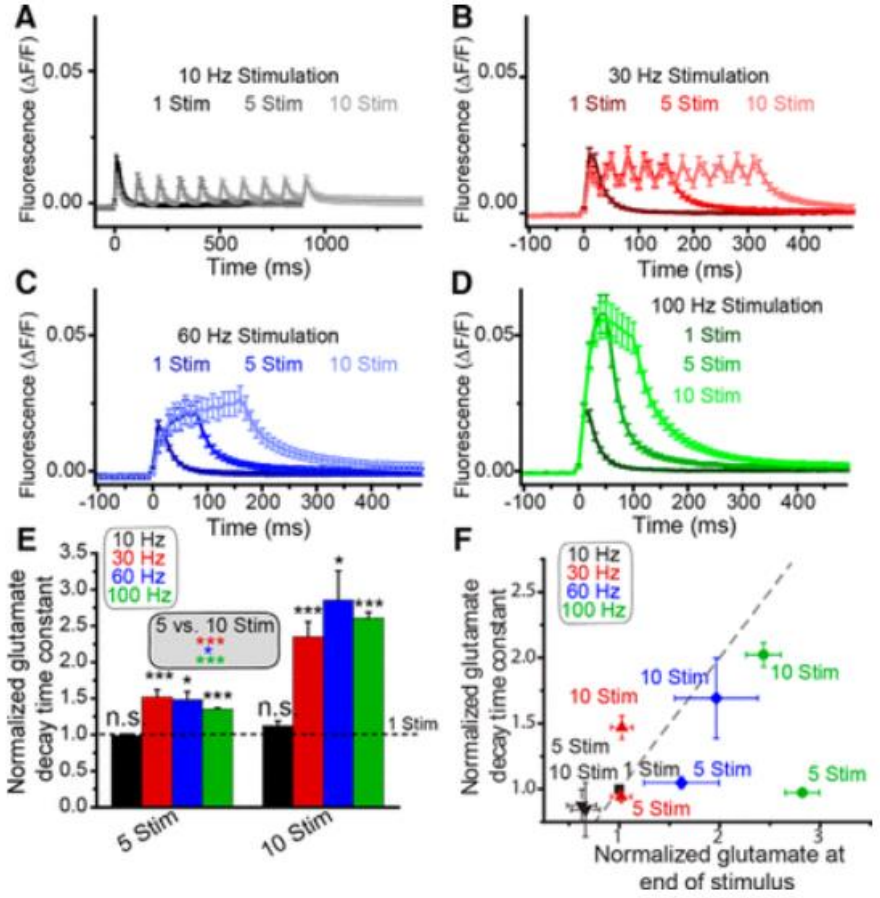
	presynaptic neuronal activity but independent of amount of glutamate released • astrocytic clearance of extracellular glutamate slowed in temporally and spatially specific manner after bursts of neuronal activity • these changes affect neuronal response to released glutamate • Maybe an unreported form of neuron-astrocyte interaction • Efficacious glutamate uptake after neuronal activity controls extracellular glutamate transients • It also constrains the activation of NMDA receptors + prevents seizures • majority of glutamate uptake occurs thru excitatory amino acid transporters (EAATs) --> expressed by astrocytes • brief & spatially restricted glutamate neurotransmission • kinetics of glutamate clearance independent of magnitude of glutamate challenge in vitro • astrocytic EAATs represent sink for extracellular glutamate (rarely saturated) • Some studies suggest that astrocytic glutamate uptake is more dynamic than previously known METHODS: • imaging-based approach used to assay glutamate dynamics in extracellular space • used established electrophysiological method to record glutamate transporter currents (GTCs) • With both methods, explored relationship between presynaptic activity + extracellular glutamate accumulation + glutamate clearance • C57BL/6 same amount of male and female mice were stereotaxtically injected postnatal with GFAP-iGluSnFr or hSyn-iGluSnFr in a single hemisphere, 3 injections sites • Mice anesthetized with isoflurane for surgery • Mice used for immunofluorescence/acute slice preparations 14–28 days post injection • Mice housed in 12/12 light/dark cycles post surgeries • Cortical brain slices (400 µm thickness), prepared from control (iGluSnFr-infected C57/B6 mice) • Mice anesthetized, decapitated, brains removed fast and put into cold slicing solution • Brain of mice glued to vibratome • Slices cut in coronal orientation • Slices placed in recovery chamber with aCSF • Slices for electrophysiology loaded with sulforhodamine before equibriliation • Slices returned to room temperature and used for live imaging/electrophysiology • Control (iGluSnFr) slices placed into a submersion chamber, held in place w/small gold wires, perfused w/aCSF, equilibrated, and circulated • tungsten concentric bipolar stimulating electrode (FHC) placed in deep

	cortical layers, upper cortical layers imaged with a 60× water-immersion objective on an Olympus BX51 microscope • two-stimulator experiments - second identical tungsten stimulating electrode placed in upper cortical layers • stimulus pulses generated every 30 s through stimulus isolators • Astrocytes identified by morphology • cells confirmed as astrocytes based on passive membrane properties, low membrane resistance, and hyperpolarized resting membrane potential • NMDA currents evoked identically to glutamate transporter currents process above • Brain sections blocked using blocking buffer • Cortical sections incubated w/diluted primary antibodies overnight at 4°C • Secondary antibodies diluted 1:500 in PBS w/ 5% blocking buffer, added to cortical sections for 2 h at room temperature • Slices imaged w/ Nikon A1R confocal microscope • Slices from 3 mice were stained for all experiments, 2-4 slides per mouse visualized • Analysis perfomed w/MATLAB and Origin • Normality tested w/Shapiro-Wilk test (alpha =0.05), and Grubb's test ($p<0.05$) • Error bars indicate SEM • all salts, glucose obtained from Sigma-Aldrich Results: • physiological activity can dynamically modulate glutamate clearance • iGluSnFr imaging can be used to measure glutamate clearance in adult cortex as reported in the striatum previously • stimulus-dependent slowing of iGSs we report is not artifact of glutamate accumulation during train stimulation but reflects slowed transport • presynaptic activity slows glutamate uptake by cortical astrocytes • signal is specific to synaptically released glutamate • glutamate clearance used to describe glutamate uptake by astrocytes • stimulus-dependent slowing of glutamate clearance not driven by amount of glutamate released • Hypothesis that iGS slowing occurs due to increased extracellular glutamate load --> refuted • Activity-dependent slowing of glutamate uptake affects glutamate dynamics at neuronal surfaces • steep transition in frequency dependence of activity-induced slowing of glutamate clearance and magnitude of slowing driven by # of stimuli delivered • rapid recovery of glutamate clearance following stimulus trains shown • activity-dependent slowing of iGSs occurs in input-specific manner • stimulus-dependent slowing of glutamate uptake occurs w/input specificity • slowed glutamate clearance modulates postsynaptic glutamate receptor activation

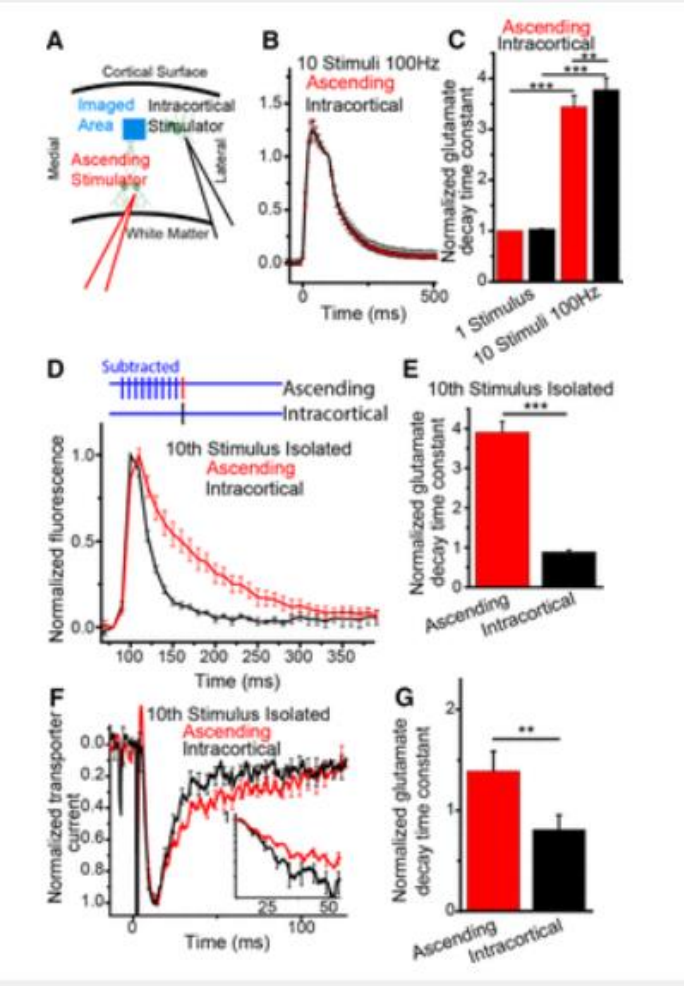
Research Question/Problem/ Need	What factors are involved with the modulation of glutamate uptake by presynaptic neuronal activity in the cerebral cortex?
Important Figures	 Stimulus-dependent slowing of glutamate clearance is shown in the graphs above.  These figures show how the slowing of glutamate clearance is independent of the amount of glutamate released.



These figures show how the stimulus-dependent slowing of glutamate uptake affects glutamate signals seen using neuronal expression of iGluSnFr.



These figures show the stimulus number and frequency dependence of glutamate clearance.



This figure shows how glutamate clearance slowing is locally modulated.

VOCAB: (w/definition)

- Activity: The work or action happening in the brain or body, like nerve cells sending signals or muscles moving
- Astrocyte: A star-shaped support cell in the brain and spinal cord that helps feed neurons, clean up chemicals, and keep the brain environment healthy
- Cortex: The outer layer of the brain that controls thinking, movement, speech, memory, and senses like seeing or hearing
- Glutamate: A powerful chemical messenger (neurotransmitter) in the brain that helps nerve cells talk to each other and is important for learning and memory
- Glutamate uptake: The process where special cells (like astrocytes) remove extra glutamate from around neurons to stop the brain from becoming overactive or damaged

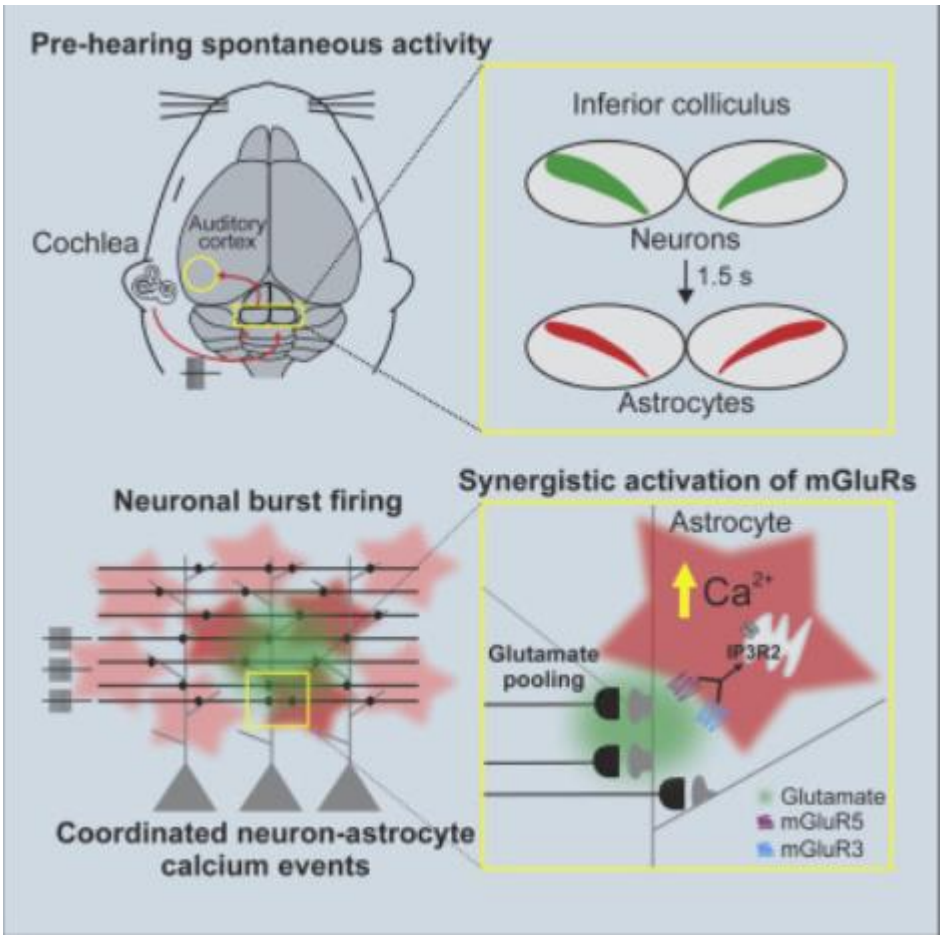
Cited references to follow up on	1. Bergles DE, Jahr CE (1997) Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* 19:1297–1308, doi:10.1016/S0896-6273(00)80420-1, pmid:9427252. 2. Bergles DE, Diamond JS, Jahr CE (1999) Clearance of glutamate inside the synapse and beyond. *Curr Opin Neurobiol* 9:293–298, doi:10.1016/S0959-4388(99)80043-9, pmid:10395570. 3. Chaudhry FS, Lehre KP, van Lookeren Campagne M, Ottersen OP, Danbolt NC, Storm-Mathisen J (1995) Glutamate transporters in glial plasma membranes: highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. *Neuron* 15:711–720, doi:10.1016/0896-6273(95)90158-2, pmid:7546749. 4. Clements JD, Lester RA, Tong G, Jahr CE, Westbrook GL (1992) The time course of glutamate in the synaptic cleft. *Science* 258:1498–1501, doi:10.1126/science.1359647, pmid:1359647.
Follow up Questions	1. What is the mechanism of the slowing of the glutamate clearance by presynaptic activity? 2. What are the regionally or developmentally specific synaptic or astrocytic properties that contribute to activity-dependent slowing of glutamate uptake? 3. How was the hypothesis that neuronal activity induces local depolarization of astrocyte microdemains, thereby reducing EAAT activity created? Is there significant background/evidence for why this is thought as a possible hypothesis and do other studies also support this hypothesis? How would this hypothesis be tested?

Article #11 Notes: Dual metabotropic glutamate receptor signaling enables coordination of astrocyte and neuron activity in developing sensory domains

Article notes should be on separate sheets

Source Title	Dual metabotropic glutamate receptor signaling enables coordination of astrocyte and neuron activity in developing sensory domains
Source citation (APA Format)	Ungless, M. A., Loriaux, A. L., & Bonci, A. (2003). Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. *Neuron*, *39*(3), 401–407. https://doi.org/10.1016/S0896-6273(03)00461-6
Original URL	https://doi.org/10.1016/S0896-6273(03)00461-6
Source type	Journal Article
Keywords	auditory system, astrocyte, spontaneous activity, mGluR, brain development
#Tags	#mGluR
Summary of key points + notes (include methodology)	• Before onset of sensory experience --> correlated groups of neurons in peripheral sensory organs assigned to discrete sensory domains fire periodic bursts of action potentials that propagate through sensory processing centers in CNS • This mapped to similar sensory space provides means to induce refinement of synapses through Hebbian mechanisms • This activity is conserved among species, resilient to perturbations, needed for precise maturation + refinement of neuronal circuitry • Mechanisms behind this poorly understood • astrocytes = critical elements of excitatory synapses • immature astrocytes appear in parenchyma (functional tissue of organ as distinguished from the connective and supporting tissue) shortly after neurons • They extend thin lamellar sheets that place glutamate transporters near sites of release and provide barrier to diffusion, limiting synaptic crosstalk • Initiated at time when immature synapses --> established + refined • Development coordinated, prevents runaway excitation, promote formation • Astrocytes secrete factors that promote formation, maturation, +

	refinement of excitatory synapses • Activity patterns of astrocytes not well studied • Neurons in developing auditory system experience high-frequency bursts of action potentials prior to onset of hearing • This ensures that they allow signal propagation from the cochlea to the auditory cortex (AC) • Burst firing = produce large glutamate transients that promote engagement of receptors outside the synaptic cleft, enhanced in developing CNS • In early developmental phase, astrocytes express metabotropic glutamate receptors that are optimized for detecting focal glutamate transients Methods: • performed time-lapse fluorescence imaging in mice that expressed the genetically encoded calcium indicator GCaMP3 under control of promoter/enhancer for GLAST • Cranial windows implanted over midbrain of young postnatal mice (P7–P11) to visualize superior colliculus (SC) + IC, where neurons exhibit prominent burst firing at this age • Imaging performed using widefield fluorescence microscope in awake (unanesthetized) pups, enabling macroscopic patterns of activity to be monitored simultaneously across brain hemispheres • performed two-photon imaging in mice that expressed GCaMP3 in astrocytes and jRGECO1a, a red-shifted calcium indicator, in neurons Results: • groups of astrocytes in these regions exhibited transient, spatially restricted increases in intracellular calcium --> distinct between auditory (IC) and visual (SC) regions • astrocyte activity in SC propagated as broad continuous waves, whereas activity in IC--> organized into discrete bands • Astrocytes in developing auditory center experience periodic, highly correlated activity aligned to future isofrequency domains • large amplitude spontaneous neuronal events are spatially and temporally correlated with astrocyte activity in central auditory center before to hearing onset • astrocytes in developing auditory midbrain and cortex experience periodic correlated activity within isofrequency lamina in concert with bursts of neuronal activity • large-scale coordinated activity in neurons and astrocytes restricted to narrow developmental time period before onset of sensory experience • neuronal burst firing in developing auditory pathway engages mGluR5 receptors in nearby astrocytes, eliciting correlated calcium transients in specific isofrequency lamina • synergistic activation of 2 distinct metabotropic glutamate receptors, mGluR5 and mGluR3, contribute to calcium rises in astrocytes in response to neuronal burst firing in auditory system before hearing onset • astrocytes in developing midbrain and cortex express functional mGluR5

	receptors that enable detection of spontaneous bursts of neuronal activity that course through emerging sensory pathways
Research Question/Problem/ Need	What is the effect of dual metabotropic glutamate receptors on the coordination of astrocyte and neuron activity in developing sensory domains?
Important Figures	 Graphical Abstract

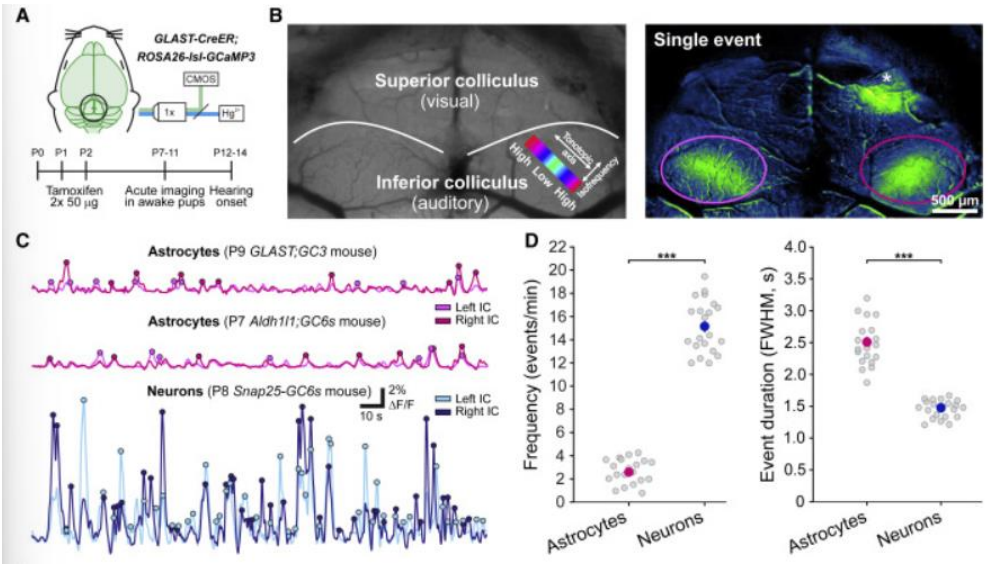


Figure A: Configuration and timeline for widefield imaging of the midbrain of neonatal mice

Figure B: Imaging field of view with superior colliculus (SC) and inferior colliculus (IC). Tonotopic and isofrequency axes are overlayed. Right: single astrocyte calcium event in left and right IC (regions of interest [ROI] circles) and calcium wave in SC (asterisk) in a P9 GLAST

Figure C: (Top) Astrocyte calcium transients (filled circles) from ROIs in (B). (Middle) astrocyte calcium transients from a P7 Aldh1l1-CreER;ROSA26-lsl-GCaMP6s (Aldh1l1;GC6s) mouse. (Bottom) neuronal calcium transients in a P8 Snap25-T2A-GCaMP6s mouse

Figure D: Average frequency (left) and duration of events (right; full width at half-maximum) for each mouse (gray circles). Colored circles denote the mean, error bars represent SEM (contained within the mean when not visible).

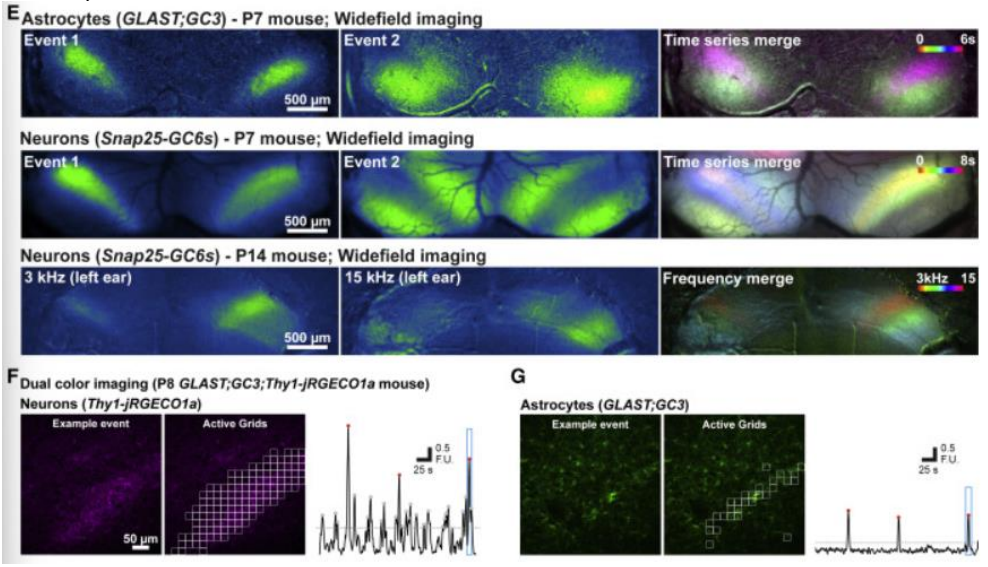


Figure E: Spatial localization of spontaneous astrocyte (top) and neuronal (middle) calcium events in IC of P7 mice. Bottom: sound-evoked neuronal calcium responses to 3- and 15-kHz 100-dB sound pressure level [SPL]

tones presented to the left ear in a P13 mouse after hearing onset. Right: color-coded representation of events over time (top and middle) and frequencies
Figure F: One calcium event (left) and active ROIs (middle, see STAR Methods). Right: fluorescence changes for one ROI in the field (red circles, astrocyte-associated events; gray circles, non-astrocyte associated events; dashed line, threshold) for neurons
Figure G: One calcium event (left) and active ROIs (middle, see STAR Methods). Right: fluorescence changes for one ROI in the field (red circles, astrocyte-associated events; gray circles, non-astrocyte associated events; dashed line, threshold) for astrocytes

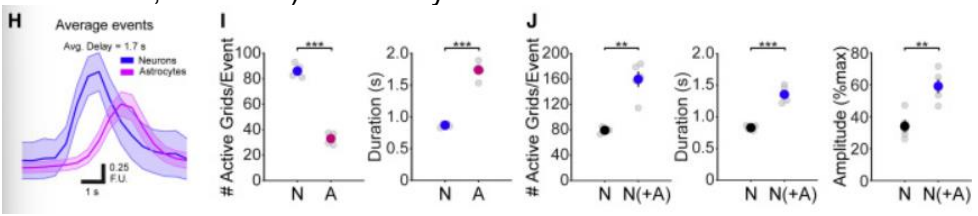


Figure H: Average calcium transients of astrocyte and preceding neuronal events
Figure I: Average number of active grids (left) and duration of events between all neuronal events (N) and astrocyte events (A)
Figure J: Average number of active grids (left), duration of events (middle), and amplitude of events (right), between neuronal events not associated with astrocyte activation (N, black) and neuronal events associated with astrocyte activation

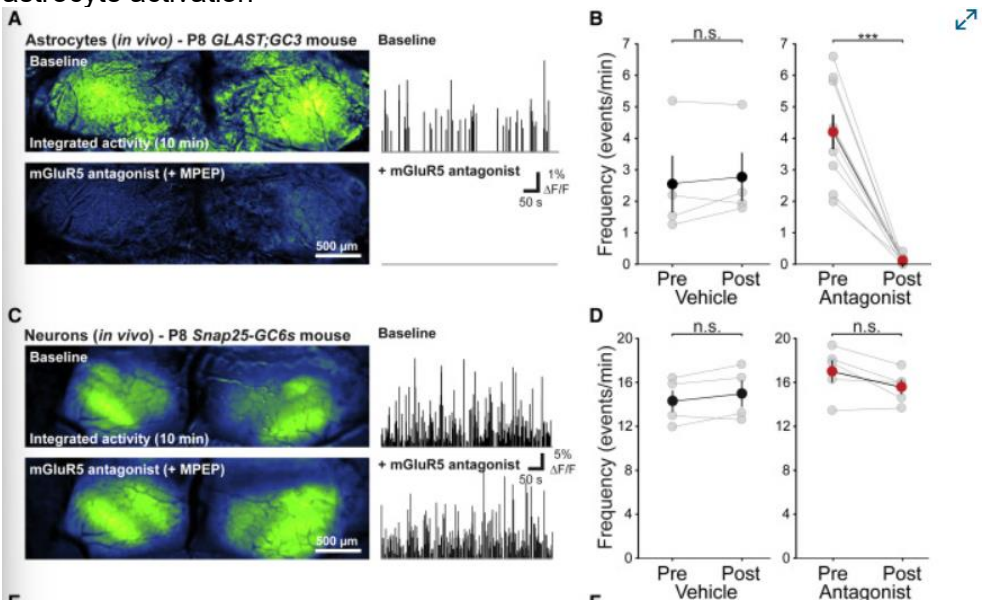


Figure A: Astrocytes (in vivo) activity in the Baseline vs. With mGluR5 antagonist (+MREP) for 10 mins
Figure B: Average Frequency of Astrocytes (events/min) pre and post of the vehicle vs. Antagonist
Figure C: Neurons in vivo activity in the baseline vs. With mGluR5 antagonist (+MREP) for 10 mins
Figure D: Average Frequency of Neurons (events/min) pre and post of the

vehicle vs. Antagonist

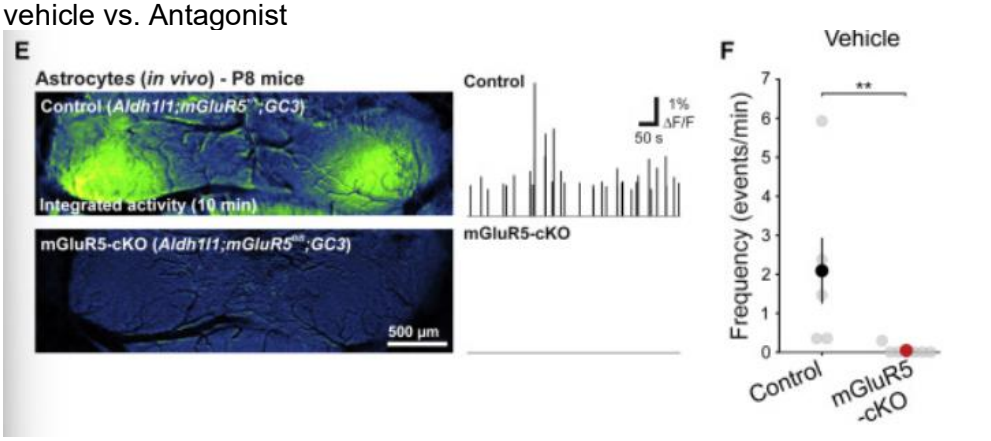


Figure E: Astrocytes in vivo activity in the control vs. MGluR5-cKO
Figure F: Average Frequency (events/min) of astrocytes in vivo with the control vs. MGluR5-cKO

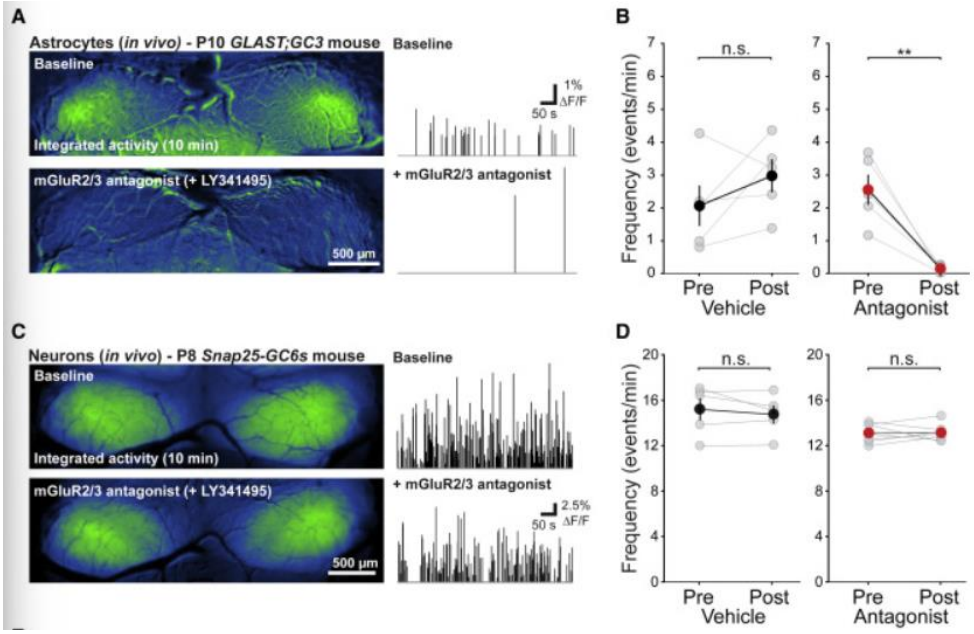
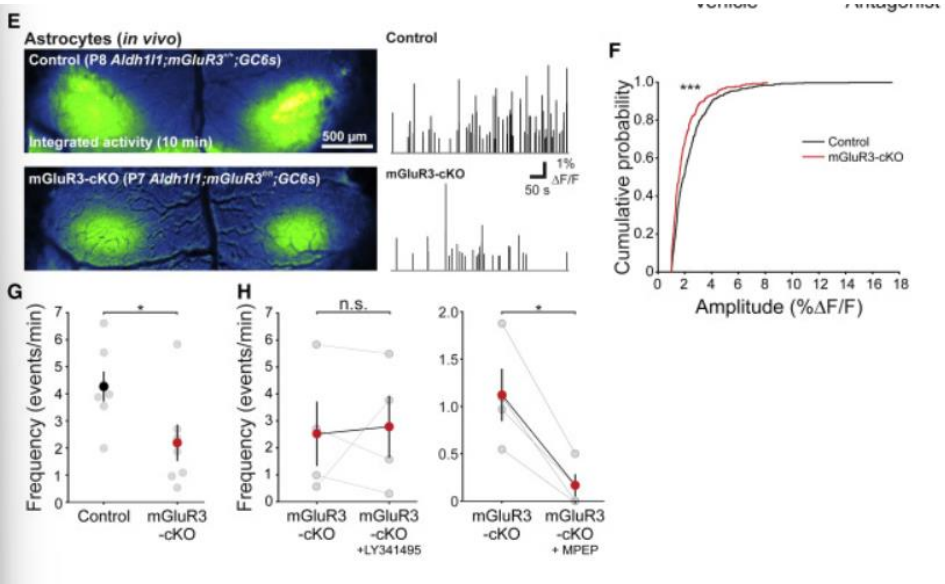


Figure A: Astrocytes in vivo activity in the baseline vs. With mGluR2/3 antagonist
Figure B : Average frequency of astrocytes pre and post administration of the vehicle vs. Antagonist
Figure C: Neurons in vivo activity in the baseline vs. With mGluR2/3 antagonist
Figure D: Average frequency of neurons pre and post administration of the vehicle vs. Antagonist

	 Figure E: Astrocytes in vivo activity in the control vs with mGluR3-cKO Figure F: Cumulative probability over amplitude in the control vs. With mGluR3-cKO Figure G: Frequency (events/min) in the control, mGluR3-cKO, mGluR3-cKO +MREP, and mGluR3-cKO + a blocker (LY341495)
VOCAB: (w/definition)	auditory system - The sensory system for hearing, converting sound waves into neural signals processed by the ear and brain astrocyte - Star-shaped glial cell that provides support, maintains the blood-brain barrier, and manages the chemical environment of neurons spontaneous activity - Neural firing or oscillations that occur without an external stimulus, crucial for wiring the developing brain mGluR - Metabotropic Glutamate Receptor, A G-protein coupled receptor that uses second messengers to indirectly modulate neuronal excitability and synaptic strength brain development - The growth and formation of the brain from gestation through adulthood, involving neurogenesis, migration, synaptogenesis, and pruning
Cited references to follow up on	Allen, N. J., Bennett, M. L., Foo, L. C., Wang, G. X., Chakraborty, C., Smith, S. J., & Barres, B. A. (2012). Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature*, *486*(7403), 410–414. **https://doi.org/10.1038/nature11059** Bergles, D. E., & Jahr, C. E. (1997). Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron*, *19*(6), 1297–1308. https://doi.org/10.1016/s0896-6273(00)80420-1

	Cai, Z., Schools, G. P., & Kimelberg, H. K. (2000). Metabotropic glutamate receptors in acutely isolated hippocampal astrocytes: developmental changes of mGluR5 mRNA and functional expression. *Glia*, *29*(1), 70–80. https://doi.org/10.1002/(sici)1098-1136(20000101)29:1<70::aid-glia7>3.0.co;2-v
Follow up Questions	1. Why is there not more research done on the mechanisms by which the early patterned activity initiates diverse cellular and synaptic changes in the central nervous system? 2. Why are the activity patterns of astrocytes not well studied if other areas are? Is it difficult to study this? 3. Is there proof that coordinated astrocyte calcium activity, once triggered by glutamate, feeds back and further refines the neuronal circuits?

Article #12 Notes: Neuromuscular NMDA Receptors Modulate Developmental Synapse Elimination

Article notes should be on separate sheets

Source Title	Neuromuscular NMDA Receptors Modulate Developmental Synapse Elimination
Source citation (APA Format)	Personius, K. E., Slusher, B. S., & Udin, S. B. (2016). Neuromuscular NMDA receptors modulate developmental synapse elimination. *Journal of Neuroscience*, *36*(34), 8783–8789. https://doi.org/10.1523/JNEUROSCI.1181-16.2016
Original URL	https://doi.org/10.1523/JNEUROSCI.1181-16.2016
Source type	Journal Article
Keywords	Competition, glutamate, neuromuscular junction, polyneuronal, synapse elimination
#Tags	#glutamate #neuromuscularjunction #synapse
Summary of key points + notes (include methodology)	• All mammalian skeletal muscle fibers --> innervated by multiple motor neurons, but after few weeks only one remains, others retract • Differential activity between inputs controls this • Acetylcholine is assumed to mediate this activity-dependent process • glutamatergic transmission also occurs at the neuromuscular junction • normal adult muscle=each muscle fiber innervated by single axon, at birth, fibers are multiply innervated • Glutamate at NMJ --> derived from enzymatic breakdown of N-acetylaspartylglutamate (NAAG) = released from the motor nerve terminal • NAAG hydrolyzed within synaptic cleft into glutamate and N-acetylaspartate by glutamatecarboxypeptidase II (GCPII)-->which is expressed extracellularly by terminal Schwann cells • Glutamate then available to bind postsynaptic NMDA and AMPA receptors at end plate • AMPA and NMDA receptors -->documented in rodent myotubes Materials + Methods: • postnatal day 4 (P4)–P28 pups or adult CD-1 mice of either sex • euthanized by intraperitoneal injection of ketamine/xylazine • Elvax 40W beads washed in 3–4 changes of 95% ethanol for 1 week with continuous stirring, dried on filter paper • 100 mg of beads dissolved in 900 µl of methylene chloride in glass culture tube • Elvax mixture vortexed, medium speed for 3 min, poured onto glass slide with Parafilm cut to form spacer • slide placed on powdered dry ice • second slide clamped on top of first slide

	• Drug-impregnated Elvax placed in 1 leg and control Elvax placed in opposite leg • Muscles were immersion fixed for 15 min in 4% paraformaldehyde, rinsed 3× in PBS, stained with 10% rhodamine-conjugated α-bungarotoxin, bathed overnight in primary antibodies SV2, visualized by secondary antibody Alexa Fluor 488 donkey anti-mouse • Slides coverslipped w/ Vectashield mounting medium • Primary antibodies visualized • Immunostained NMJs analyzed by conventional epifluorescence microscopy • constructed 25 base vivo-morpholino to knock down expression of GluN1 subunit of NMDA receptor • scrambled-sequence vivo-morpholino injected in contralateral limb • Innervation of NMJ assessed by immunohistochemistry at P11 • magnesium-free Ringer's medium used for all experiments • Experiments performed at room temperature • attached nerve not stimulated during these experiments except via depolarization during final high potassium exposures • data collected from responsive fibers • 2 tailed T-Test used to analyze data Results: • ionotropic glutamate receptors normally promote reduction of polyneuronal innervation, even in presence of normal cholinergic transmission • NMDA receptor = key ionotropic glutamate receptor involved in pruning excess inputs at growing NMJ • locally derived glutamate activates NMDA receptors at NMJ and contributes to synapse elimination • reducing NMDA-receptor-mediated transmission slow reduction of polyneuronal innervation of NMJ • chronic activation of NMDA receptors with exogenous NMDA, speeds reduction of polyneuronal innervation • NMDA --> significant impact on juvenile muscles during same period when axon pruning occurs, but not on older muscles
Research Question/Problem/ Need	How do Neuromuscular NMDA receptors modulate developmental synapse elimination in the neuromuscular junction?

Important Figures

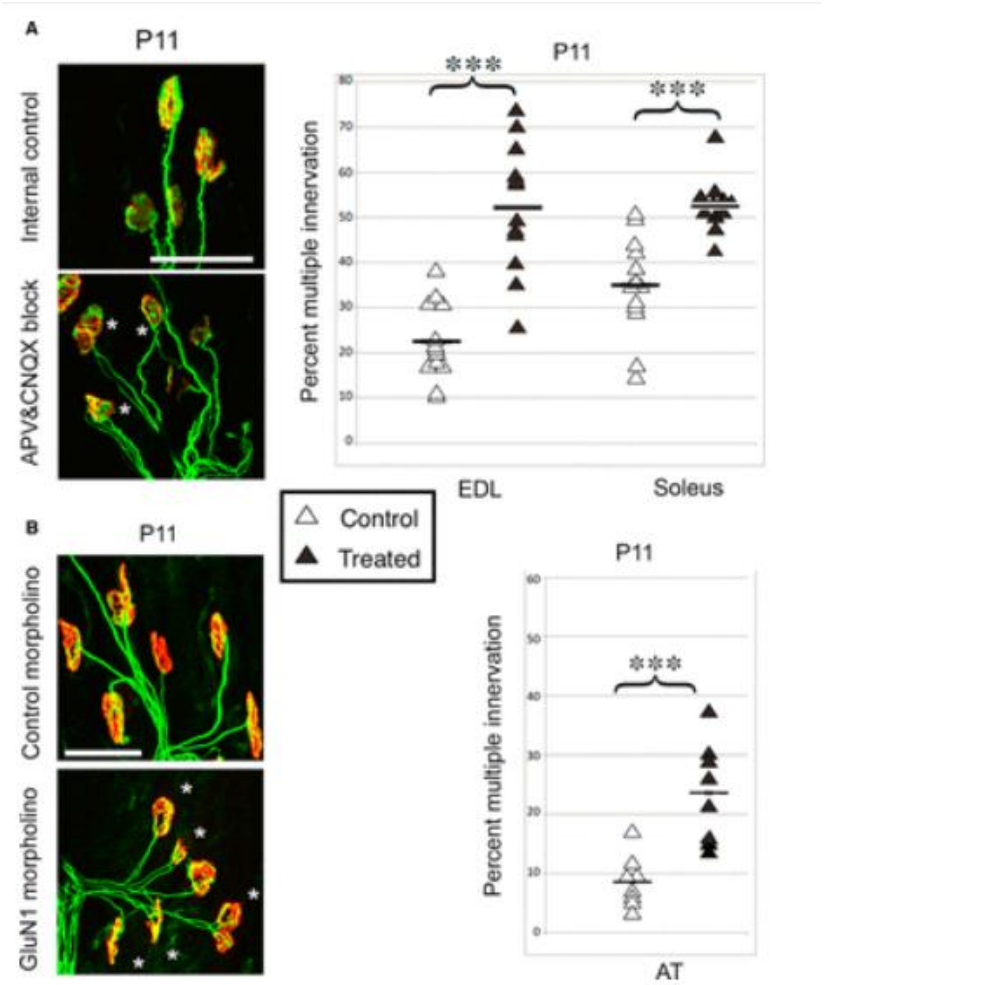


Figure A: Pharmacological reduction of NMDA and AMPA receptor activity with AP5 and CNQX between P4 and P11
Figure B: Vivo-morpholino downregulation of GluN1 expression between P4 and P11

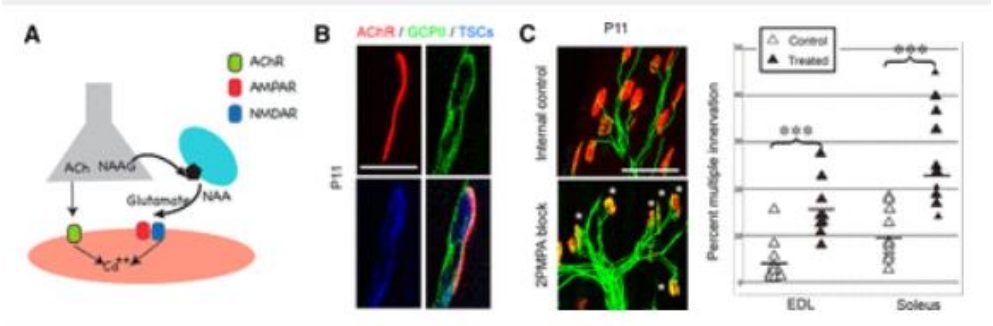
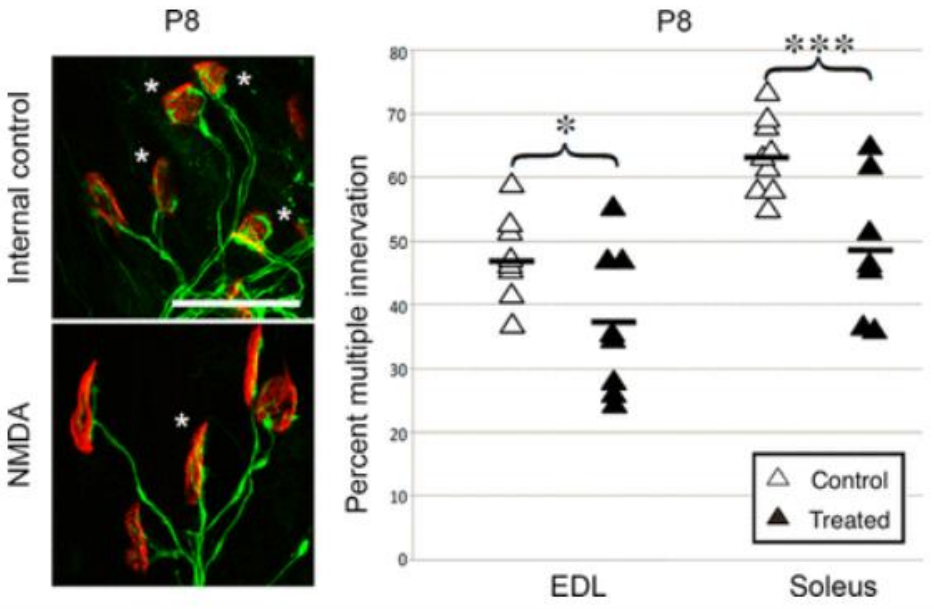


Figure A: Model of glutamate production and induction of calcium level influx at the NMJ
Figure B: Presynaptic expression of the enzyme GCPII during the period of synapse elimination
Figure C: GCPII antagonist slows synapse elimination by reducing glutamate at the NMJ



This shows how exogenous NMDA accelerates removal of excess innervation

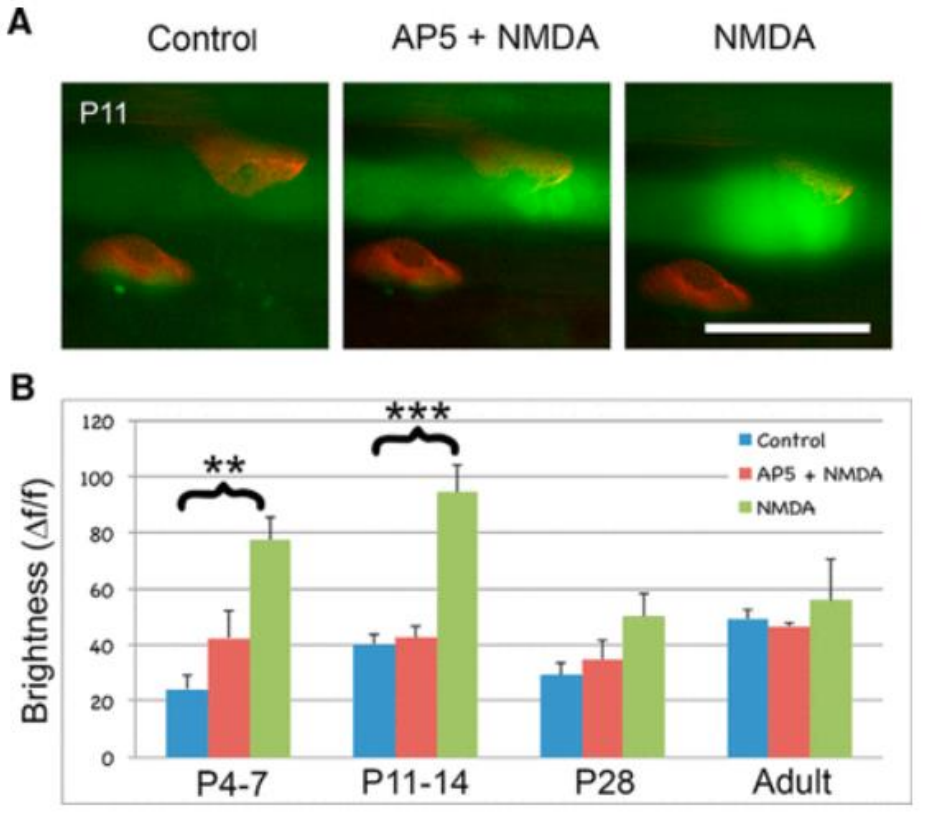


Figure A: Calcium response to bath-applied NMDA (200 μm) in an EDL of a P11 CD-1 pup. AP5 (200 μm) blocked most of the response to NMDA
Figure B: NMDA significantly evoked Calcium responses in P4–P14 pups compared with control

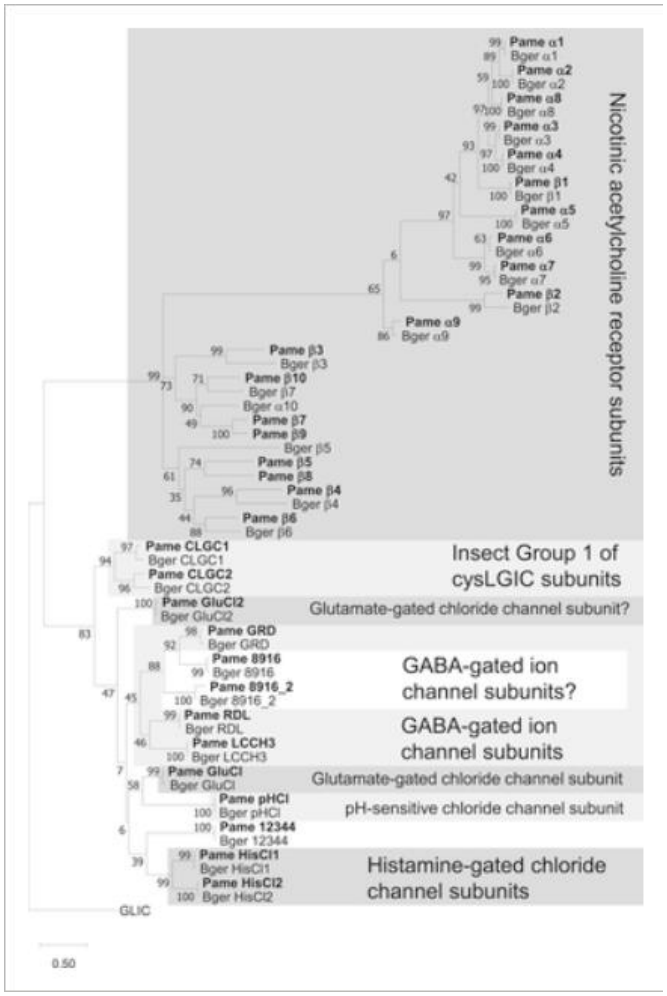
VOCAB: (w/definition)	• Competition: A process where multiple neural inputs vie for survival and territory on a single target cell, often leading to the strengthening of one and the elimination of others. • Glutamate: The primary excitatory neurotransmitter in the central nervous system (CNS) and a critical signaling molecule for synapse formation and plasticity. • Neuromuscular Junction: The specialized chemical synapse where a motor neuron's axon terminal meets a skeletal muscle fiber, causing muscle contraction. • Polyneuronal: The state of a target cell, such as a muscle fiber or neuron, being temporarily innervated by multiple axons/neurons. • Synapse Elimination: The developmental process of refining neural circuits by removing excess or redundant synaptic connections, leading to the one-to-one adult state.
Cited references to follow up on	Berger UV, Carter RE, Coyle JT (1995) The immunocytochemical localization of N-acetylaspartyl glutamate, its hydrolysing enzyme NAALADase, and the NMDAR-1 receptor at a vertebrate neuromuscular junction. *Neuroscience* 64:847–850, doi:10.1016/0306-4522(95)92578-8, pmid:7753384 Herzog E, Gilchrist J, Gras C, Muzerelle A, Ravassard P, Giros B, Gaspar P, El Mestikawy S (2004) Localization of VGLUT3, the vesicular glutamate transporter type 3, in the rat brain. *Neuroscience* 123:983–1002, doi:10.1016/j.neuroscience.2003.10.039, pmid:14751290 Malomouzh AI, Nurullin LF, Arkhipova SS, Nikolsky EE (2011) NMDA receptors at the end plate of rat skeletal muscles: precise postsynaptic localization. *Muscle Nerve* 44:987–989, doi:10.1002/mus.22250, pmid:22102472.
Follow up Questions	1. How do NMDA receptors play a role in the maturation of neuromuscular innervation? 2. Does reducing NMDA receptor activation lead to permanent polyinnervation? 3. Why is there overlap between the influence of NMDA receptors on axon elimination and the influence of the MHC1 members of the major histocompatibility complex at the NMJ?

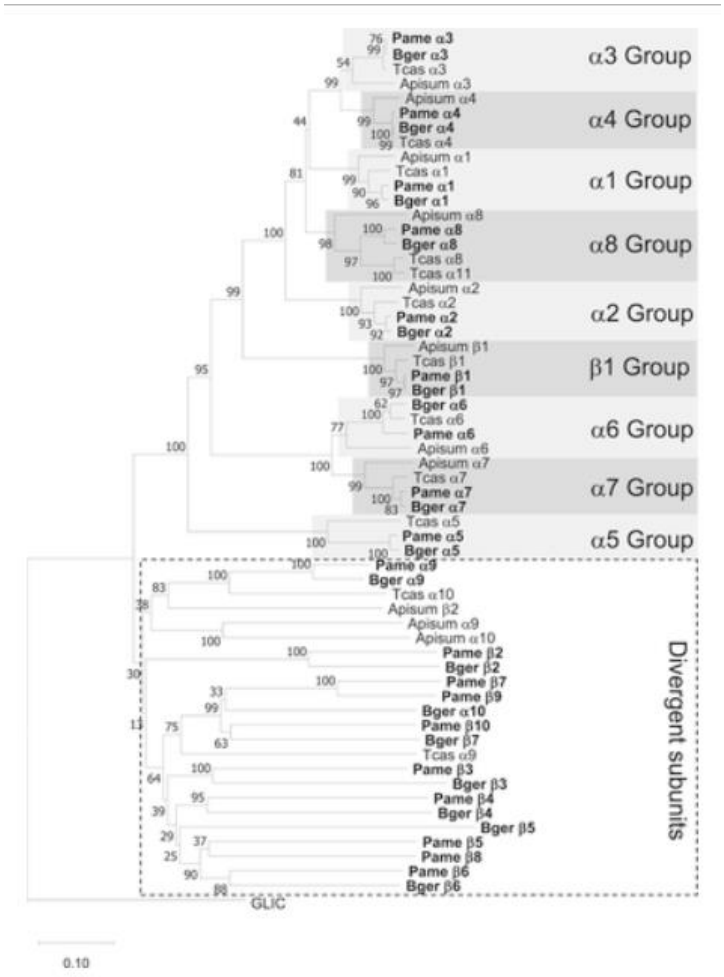
Article #13 Notes: The cys-loop ligand-gated ion channel gene superfamilies of the cockroaches Blattella germanica and Periplaneta americana

Article notes should be on separate sheets

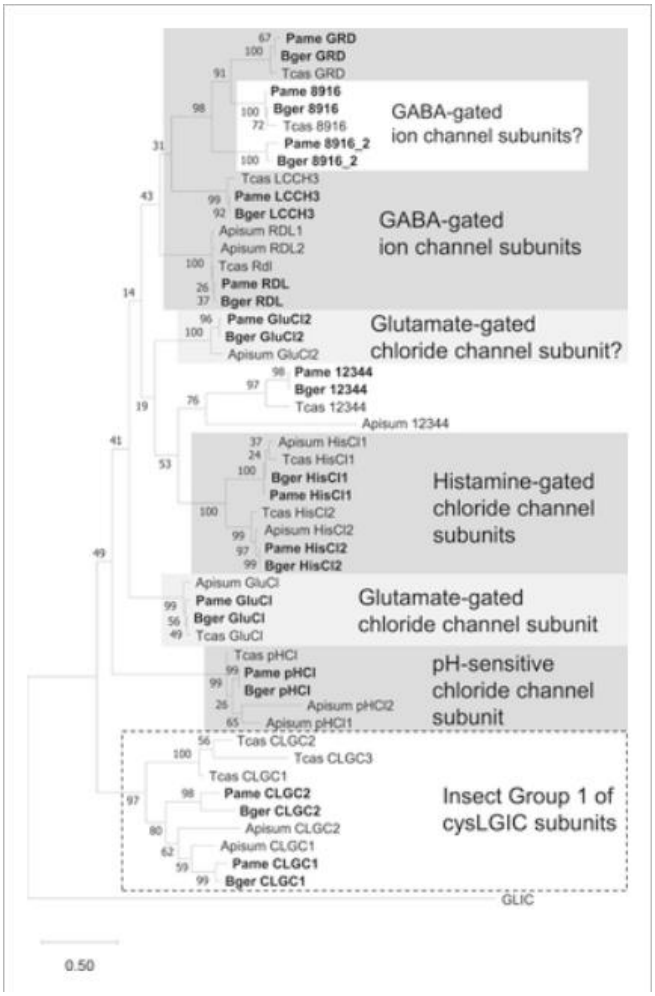
Source Title	The cys-loop ligand-gated ion channel gene superfamilies of the cockroaches Blattella germanica and Periplaneta americana
Source citation (APA Format)	Jones, A. K., Goven, D., Froger, J.-A., Bantz, A., & Raymond, V. (2021). The cys-loop ligand-gated ion channel gene superfamilies of the cockroaches *Blattella germanica* and *Periplaneta americana*. *Pest Management Science*, *77*(8), 3787–3799. https://doi.org/10.1002/ps.6245
Original URL	https://doi.org/10.1002/ps.6245
Source type	Journal Article
Keywords	Cys-loop Ligand-Gated Ion Channels (cysLGIC), Glutamate, Nicotinic acetylcholine receptor subunit, Insecticide resistance, Blattella germanica / Periplaneta americana, Subunit-encoding genes
#Tags	#glutamate #acetycholine
Summary of key points + notes (include methodology)	• Insects: members of cys-loop ligand-gated ion channel (cysLGIC) superfamily control fast excitatory and inhibitory synaptic transmission in nervous system and may be performing non-neuronal roles • Cockroaches = serious urban pests, can transfer disease-causing microorganisms + trigger allergic reactions + asthma • cockroach neurons resulted in many instructive studies investigating actions of insecticides on cysLGICs in insect nervous system • reports of cockroaches showing resistance to insecticidal bait containing fipronil, abamectin and imidacloprid found • Emphasizes need for improved knowledge of resistance mechanisms and detection + development of novel control agents Methods + Materials: • Candidate cockroach cysLGIC subunits identified based on considerable sequence homology w/previously characterized subunits particularly in N-terminal ligand-binding domain and 4 transmembrane regions • tBLASTn28 used to screen B. germanica genome assembly or

	transcript sequences • For some of them cockroach cysLGIC sequences corrected based on protein sequence alignments or RT-PCR sequence data • Adult male cockroach P. americana used for RT-PCR experiments were maintained under standard laboratory conditions • Cockroaches came from lab-maintained strain susceptible to insecticides • To study cysLGIC subunits expressed in P. americana central nervous system + confirm coding sequences identified in cockroach genome, RT-PCR carried out on terminal abdominal ganglion (TAG), (removed from nerve cord of cockroaches) Results: • several of the cockroach divergent nAChRs are expressed • GluCl2 is transcribed • additional cysLGIC subunit gene was found in the genomes of *B. germanica* and *P. Americana* • Cockroaches have unusually large cysLGIC gene superfamilies • Cockroach and aphid cysLGIC gene superfamilies share unusual features
Research Question/Problem/ Need	How do the cys-loop ligand-gated ion channel gene superfamilies function in the cockroaches *Blattella germanica* and the *Periplaneta americana*?

Important Figures	 This is a tree showing relationships of the complete complement of Blattella germanica and Periplaneta americana cysLGIC subunit protein sequences



This is a tree showing relationships of Blattella germanica, Periplaneta americana, Acyrthosiphon pisum and Tribolium castaneum nAChR subunit protein sequences



This is a tree showing relationships of Blattella germanica, Periplaneta americana, Acyrthosiphon pisum and Tribolium castaneum non-AChR subunit protein sequence

VOCAB: (w/definition)

- Cys-loop Ligand-Gated Ion Channels (cysLGIC): A superfamily of proteins in the nervous system that function as molecular targets for many pesticides. They open an ion channel when a neurotransmitter (ligand) binds.
- Glutamate: A common neurotransmitter that, when binding to a cysLGIC (like the glutamate-gated chloride channel mentioned), typically causes an inhibitory effect in insects.
- Nicotinic acetylcholine receptor subunits: Components of an excitatory cysLGIC that are the primary molecular targets of many neonicotinoid and other modern insecticides.

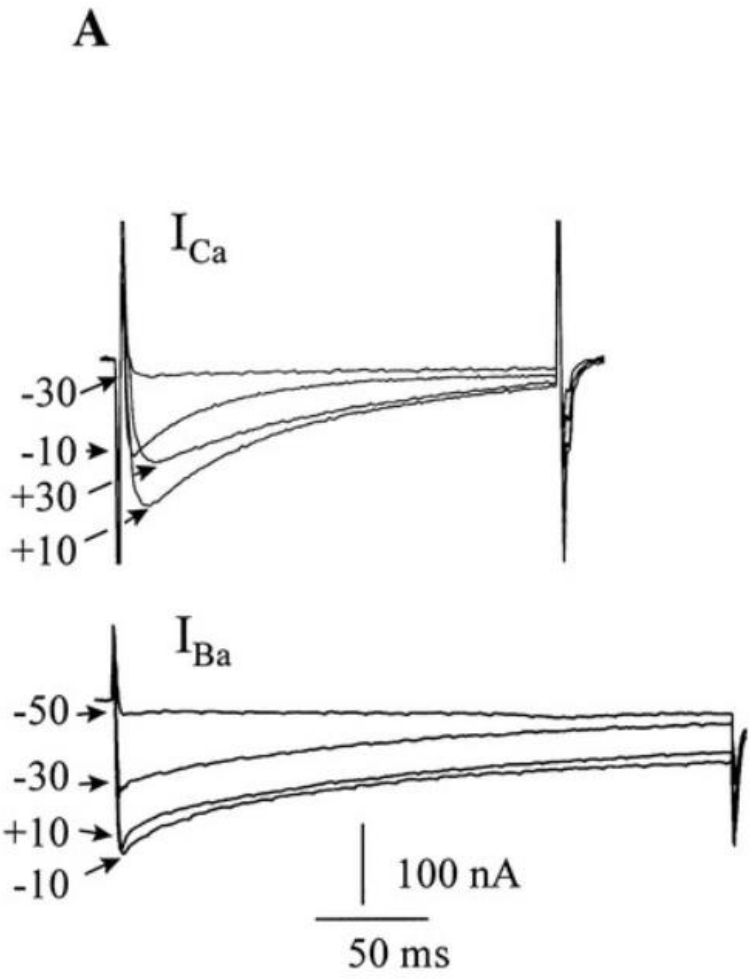
	• Insecticide resistance: The evolutionary ability of a pest population (like cockroaches) to withstand the effects of pesticides, often due to genetic changes in the cysLGIC targets. • *Blattella germanica* / *Periplaneta americana*: The scientific names for the German and American cockroaches, two major urban pests studied for insecticide target development. • Subunit-encoding genes: Genes that contain the instructions for building the individual protein pieces that assemble to form a functional cysLGIC.
Cited references to follow up on	Wolstenholme, A. J. (2012). Glutamate-gated chloride channels. *The Journal of Biological Chemistry*, *287*(48), 40232–40238. https://doi.org/10.1074/jbc.R112.406280 Ihara, M., Buckingham, S. D., Matsuda, K., & Sattelle, D. B. (2017). Modes of action, resistance and toxicity of insecticides targeting nicotinic acetylcholine receptors. *Current Medicinal Chemistry*, *24*(27), 2925–2934. https://doi.org/10.2174/0929867324666170206142019 Buckingham, S. D., Ihara, M., Sattelle, D. B., & Matsuda, K. (2017). Mechanisms of action, resistance and toxicity of insecticides targeting GABA receptors. *Current Medicinal Chemistry*, *24*(27), 2935–2945. https://doi.org/10.2174/0929867324666170613075736
Follow up Questions	1. Does the expression of other alpha7 splice variants result in more robust expression in *P. americana*? 2. Why is it unusual that the cockroach genome shares the feature of having a second putative GluCL subunit with *Acyrthosiphon pisum*? 3. Why do cockroaches and Acyrthosiphon pisum GluCl2 not clearly cluster with other subunits?

Article #14 Notes: Electrical Properties of a Cockroach Motor Neuron Soma Depend on Different Characteristics of Individual Ca Components

Article notes should be on separate sheets

Source Title	Electrical Properties of a Cockroach Motor Neuron Soma Depend on Different Characteristics of Individual Ca Components
Source citation (APA Format)	Mills, J. D., & Pitman, R. M. (1997). Electrical properties of a cockroach motor neuron soma depend on different characteristics of individual Ca components. *Journal of Neurophysiology*, *78*(5), 2455–2466. https://doi.org/10.1152/jn.1997.78.5.2455
Original URL	https://doi.org/10.1152/jn.1997.78.5.2455
Source type	Journal Article
Keywords	Motor Neuron Soma (Df), Active Electrical Properties, Inward Currents (ICa), Plateau Potentials, Action Potentials (Spikes), Calcium Components, Calcium-Dependent Inactivation
#Tags	#motorneuron #actionpotentials
Summary of key points + notes (include methodology)	• calcium currents have properties that don't correspond to vertebrate channels in terms of pharmacology, activation threshold, or kinetics • soma of cockroach fast coxal depressor motor neuron, Df, generate either action potentials or plateau potentials on depolarization, each calcium dependent • soma of this neuron not normally possess tetrodotoxin (TTX)-sensitive sodium channels • Soma does exhibit calcium currents • inactivation characteristics of calcium currents may be important in determining electrical activity in Df Methods: • metathoracic "fast" coxal depressor motor neuron, Df used of adult male cockroaches • Animals - decapitated, mesothoracic and metathoracic ganglia + first three abdominal ganglia dissected out • metathoracic ganglion desheathed for electrophysiological recording • Experiments performed in circulating oxygenated saline containing (in mM) 214 NaCl, 3.1 KCl, 9 CaCl2, + 10 N-tris(hydroxymethyl)methyl-2-

	aminoethanesulfonic acid (TES) buffer • Appropriate concentrations of ethanol applied to preparation as controls • Twenty- or 200-µl aliquots of nifedipine + verapamil (Sigma) added to side compartment of chamber • oxygenation system mixed + diluted agents before they reached preparation • Concentrations expressed as final values attained after agents mixed in experimental chamber • electrodes filled w/solution containing 100 mM BAPTA + 100 mM KCl. • Experiments carried out at room temp (20–23°C) • Df somata penetrated by 2 thin-walled, fiber-filled borosilicate glass microelectrodes • Microelectrodes for current-clamp recording had 2 M potassium acetate and resistances of 12–20 MΩ • Outward potassium currents blocked by adding 50 mM tetraethylammonium chloride • leakage of cesium ions facilitated by adding train of positive pulses • Current monitored using laboratory-built “virtual earth” amplifier circuit connected to reference electrode in experimental chamber • Data from current-clamp experiments recorded on tape with DTR 1204 digital tape recorder + displayed on Gould 1604 oscilloscope Results: • Inward current has a low-threshold component • low-threshold Ni-sensitive and Ni-insensitive calcium currents identified in cockroaches • higher concentrations of Cd2+-->selectivity was lost, currents activated at lower potentials suppressed • Components of inward current show differential sensitivities to nifedipine and micromolar cadmium • block by nifedipine decreases, whereas block by Cd2+ increases as membrane stepped from holding potential of −80 mV to progressively more positive potentials • nifedipine-sensitive current underlie plateau potential, but both nifedipine- and Cd2+-sensitive currents underlie action potentials • some steady-state inactivation of Ca currents occurs at potentials close to resting potential of neurons • faster component of decay of I Ca due to calcium-dependent inactivation process • reason for the U-shaped inactivation curve is reduction in extent of inactivation at positive membrane potentials from influx of calcium or other cations or some undescribed inactivation component • recovery from inactivation is faster for I Ba than for I Ca • longer interpulse intervals--> little difference in extent of inactivation between I Ca and I Ba

Research Question/Problem/ Need	How do individual Calcium components affect the electrical properties of a Cockroach Motor Neuron Soma?
Important Figures	 Figure A: I_{Ca} (Calcium currents) and I_{Ba} (Barium currents) elicited by stepping from a holding potential of −70 mV to command potentials indicated. Note faster decay of I_{Ca} compared with I_{Ba}.

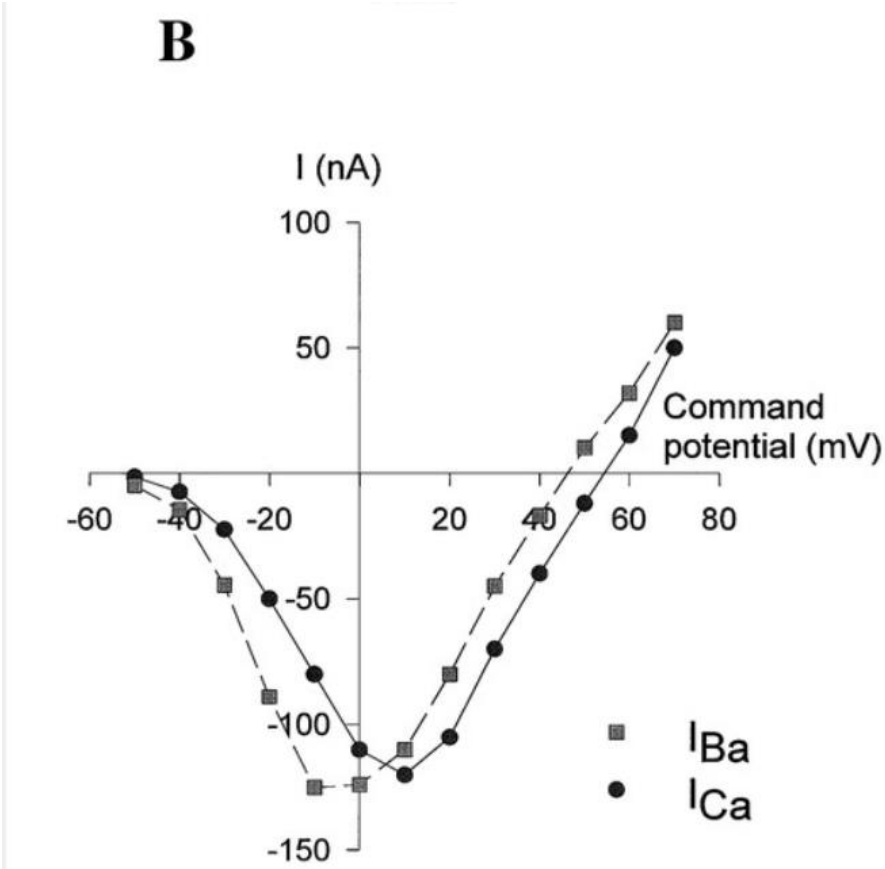
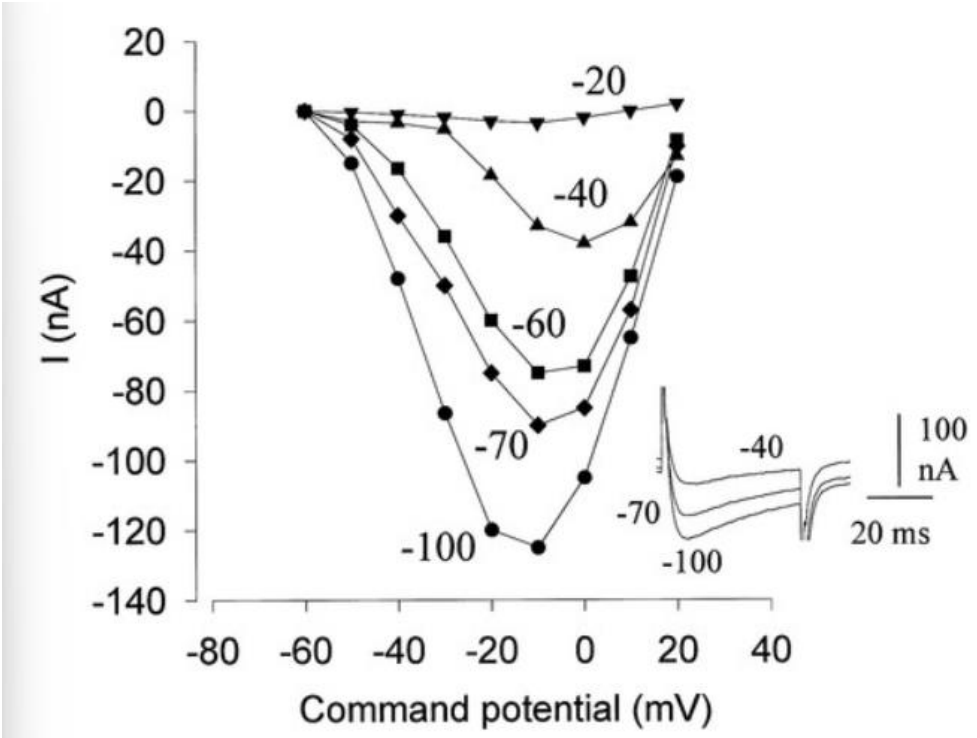
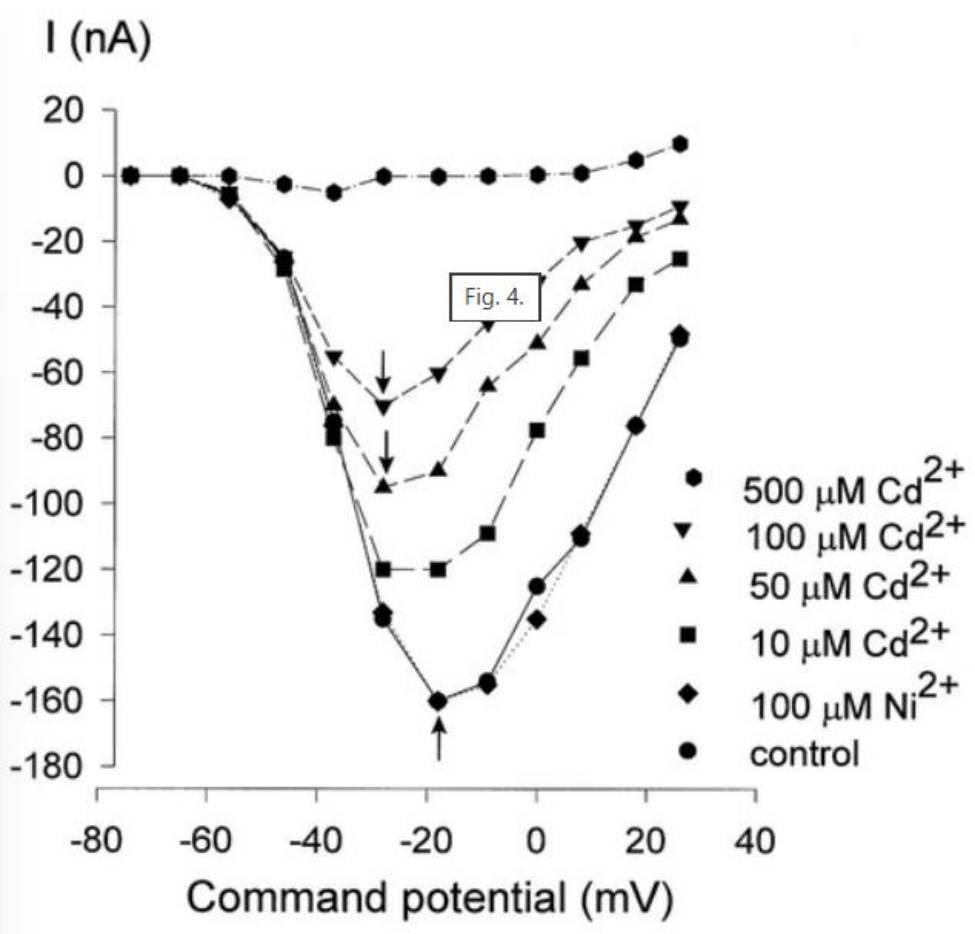


Figure B: Current-voltage (*I*-*V*) relationship for peak inward I_{Ca} and I_{Ba} measured in same cell first in calcium saline, then after changing to a barium saline.



Effect of holding potential on the *I-V* relationship of I_{Ba}.



Effect of Ni^{2+} and Cd^{2+} ions on the *I-V* relationship for I_{Ba}.

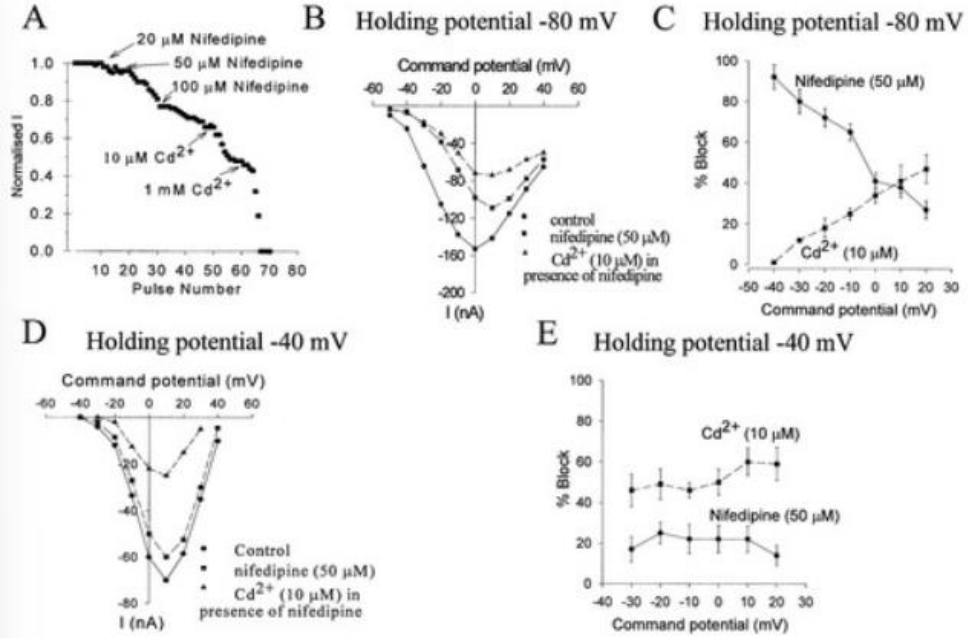


Figure A: time course of inhibition of I_{Ca}; the cell was held at −70 mV and stepped to 0 mV once every 15 s.

Figure B: effect of 50 μM nifedipine and 10 μM Cd^{2+} on the *I-V* relationship of I_{Ca} (holding potential −80 mV)
Figure C: mean percentage block of I_{Ca} obtained for peak inward currents elicited by stepping the membrane potential from a holding potential of −80 mV to command potentials between −40 and +20 mV by either nifedipine
Figure D: effects of 50 μM nifedipine and 10 μM Cd^{2+} on the *I-V* relationship of I_{Ca} at a holding potential of −40 mV
Figure E: mean percentage block of I_{Ca} obtained for peak inward currents elicited from a holding potential of −40 mV to command potentials between −30 and +20 mV by either nifedipine or Cd^{2+}

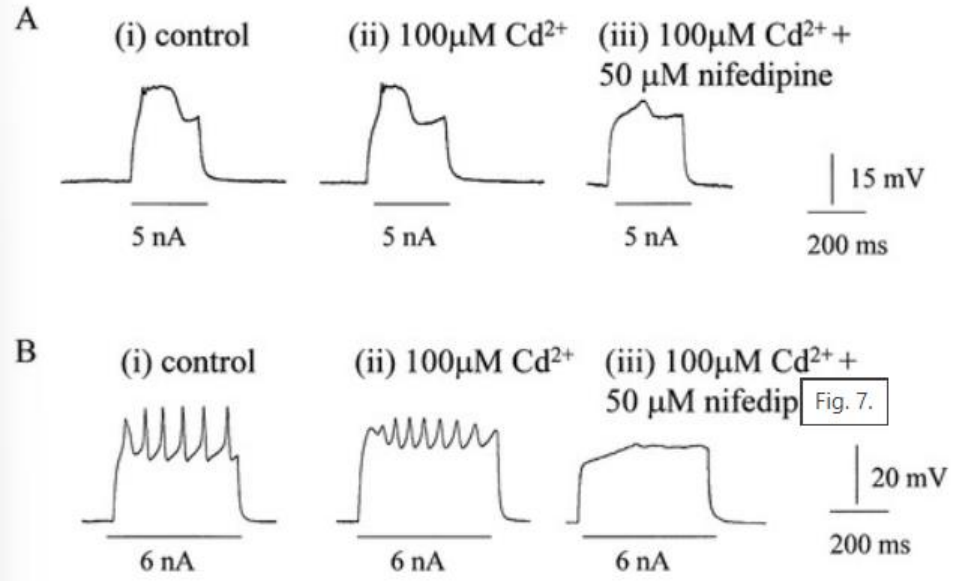
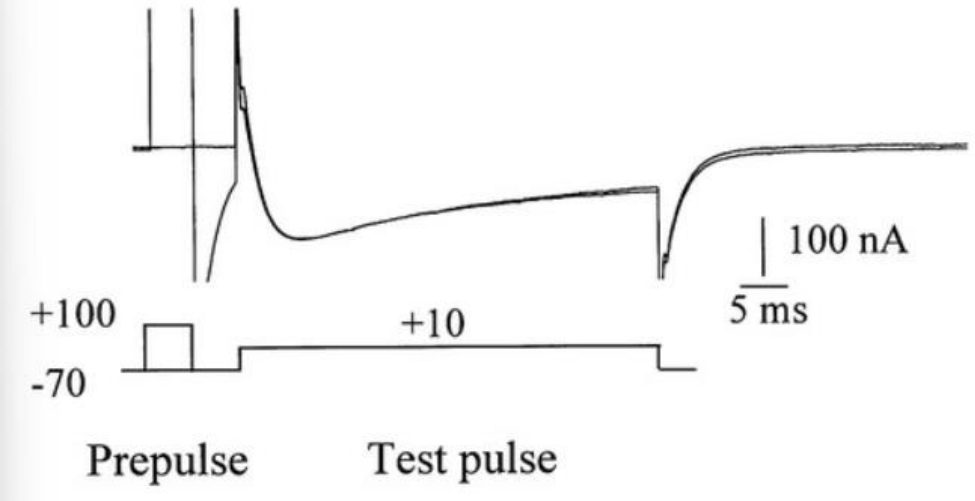


Figure A: a plateau potential elicited by depolarization (*Ai*) is not blocked by 100 μM Cd^{2+} (*Aii*) but is blocked by 50 μM nifedipine
Figure B: time-dependent spikes require a combination of micromolar Cd^{2+} and nifedipine for complete suppression



I_{Ca} does not display facilitation at positive membrane potentials

VOCAB: (w/definition)	• Motor Neuron Soma (Df): The cell body of the cockroach motor neuron studied; it can exhibit active electrical properties (generating its own signals). • Active Electrical Properties: The ability of the cell body to generate its own electrical events, specifically action potentials or plateau potentials. • Inward Currents (ICa): Electrical currents, primarily carried by calcium ions (Ca2+), that flow into the neuron and are responsible for initiating active signals. • Plateau Potentials: Prolonged, sustained membrane depolarizations that rely only on the nifedipine-sensitive calcium component. • Action Potentials (Spikes): Brief, rapid electrical signals that rely on the combination of both the nifedipine-sensitive and the Cd^{2+}-sensitive calcium components. • Calcium Components: Two distinct types of calcium channels identified by their drug sensitivity (nifedipine vs. Cd2+), each contributing differently to the cell's electrical output. • Calcium-Dependent Inactivation: A regulatory mechanism where the buildup of internal calcium (Ca2+) causes the calcium channels to close (inactivate), reducing the neuron's excitability.
Cited references to follow up on	Akaike, N., Tsuda, Y., & Oyama, Y. (1988). Separation of current- and voltage-dependent inactivation of calcium current in frog sensory neuron. *Neuroscience Letters*, *84*(1), 46–50. https://doi.org/10.1016/0304-3940(88)90335-7 Bean, B. P. (1989). Classes of calcium channels in vertebrate cells. *Annual Review of Physiology*, *51*, 367–384. https://doi.org/10.1146/annurev.ph.51.030189.002055 Christensen, B. N., Larmet, Y., Shimahara, T., Beadle, D., & Pichon, Y. (1988). Ionic currents in neurones cultured from embryonic cockroach (*Periplaneta americana*) brains. *Journal of Experimental Biology*, *135*(1), 193–214. https://doi.org/10.1242/jeb.135.1.193
Follow up Questions	1. If a chemical like glutamate were applied to this neuron, would it make the cell more or less likely to fire an action potential? 2. Could glutamate change the balance between the plateau potential and the short spike in this neuron?

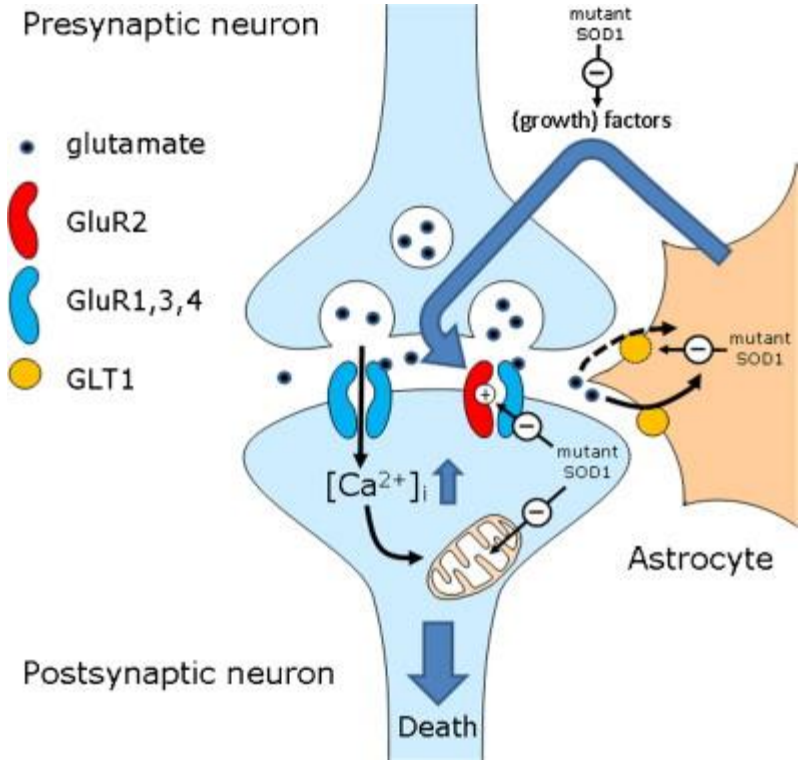
	3. How could an inhibitory glutamate receptor affect the special calcium currents that control this motor neuron's firing?

Article #15 Notes : Calcium dysregulation in amyotrophic lateral sclerosis

Article notes should be on separate sheets

Source Title	Calcium dysregulation in amyotrophic lateral sclerosis
Source citation (APA Format)	Grosskreutz, J., Van Den Bosch, L., & Keller, B. U. (2010). Calcium dysregulation in amyotrophic lateral sclerosis. *Cell Calcium, 47*(2), 165–174. https://doi.org/10.1016/j.ceca.2009.12.002
Original URL	https://doi.org/10.1016/j.ceca.2009.12.002
Source type	Journal Article
Keywords	Amyotrophic lateral sclerosis, Calcium deregulation, Endoplasmic reticulum, Mitochondria, AMPA receptor, GluR2, Neurodegeneration, Calcium buffe, rSelective vulnerability, Motor neuron
#Tags	#ALS #motorneuron #glutamate #neurodegeneration
Summary of key points + notes (include methodology)	Introduction: • In ALS motor neurons degenerate with signs: organelles breaking, Ca2+ overflow in mitochondria, broken transport from axons, and build up of proteins in intracellular cells • Riluzole, only known effective treatment that seems to reduce glutamatergic input • Ca2+ dysregulation - central role in amyotrophic lateral sclerosis (ALS) • Leads to muscle atrophy, paralysis and death of the patient on average within 3–4 years • Most striking characteristic of ALS: selectivity of the disease process for motor neurons • Spinal motor neurons receive very strong glutamatergic input • ALS-vulnerable spinal and brain stem motor neurons in mice have been shown to display low endogenous Ca2+ buffering capacities • Consumption of excitotoxins can give rise to selective motor neuron death

	• Data indicate that overconsumption of excitotoxins is neurotoxic and that motor neurons seem to be most vulnerable to this type of toxicity • selective loss of the astroglial glutamate transporter, GLT1 • motor neurons have intrinsic properties that make them extremely vulnerable to AMPA receptor-mediated excitotoxicity • GLT1 transporter present in the astrocytes removes glutamate from the synaptic cleft and factors secreted Methods: • Calcium imaging using fluorescent Ca^{2+} indicators to measure intracellular calcium dynamics • Electric recordings to see calcium and glutamate receptor activity • Use of ALS disease models (SOD1 mutant mice, cultured motor neurons) • Manipulation of calcium signaling (glutamate, AMPA) • Scientists analyzed proteins that handle calcium • Observation of calcium cycling in mitochondria and mitochondrial dysfunction • Compared vulnerable vs. resistant motor neuron populations based on calcium buffering capacity Results: • Motor neurons in ALS - elevated intracellular calcium levels compared to normal neurons • Less expression of calcium reducing proteins increases motor neuron vulnerability • Dysregulated mitochondria and endoplasmic reticulum calcium cycling leads to mitochondrial calcium overload • Mitochondrial calcium overload is related to oxidative stress and problematic energy metabolism • Calcium elevation activates calpains promoting neuronal damage • ALS models (SOD1 mutants) have eventual worsening of calcium homeostasis over ALS progression • Disruption of calcium signaling causes motor neuron degeneration, indicating a role in ALS
Research Question/Problem/ Need	How does dysregulation of intracellular calcium contribute to motor neuron degeneration in amyotrophic lateral sclerosis?

Important Figures	 Figure 1. Infographic of excitotoxicity and motor neuron death and the role of the mutant SOD1

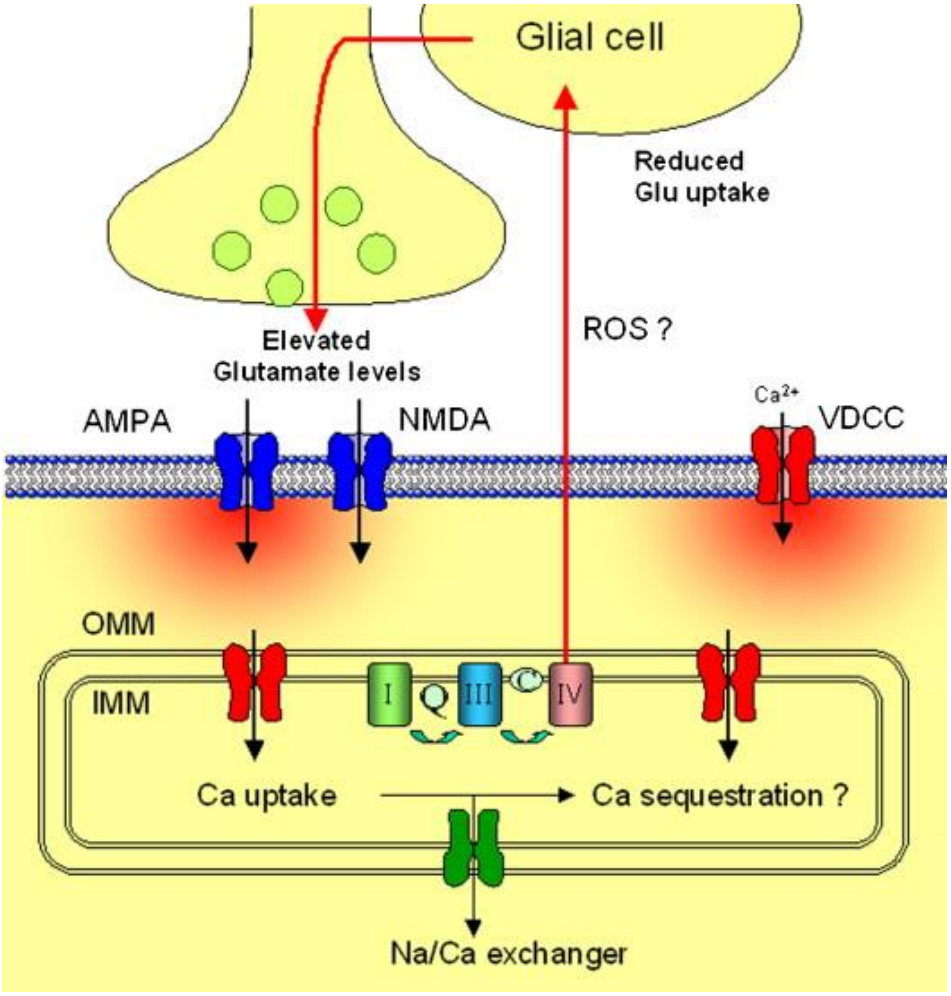
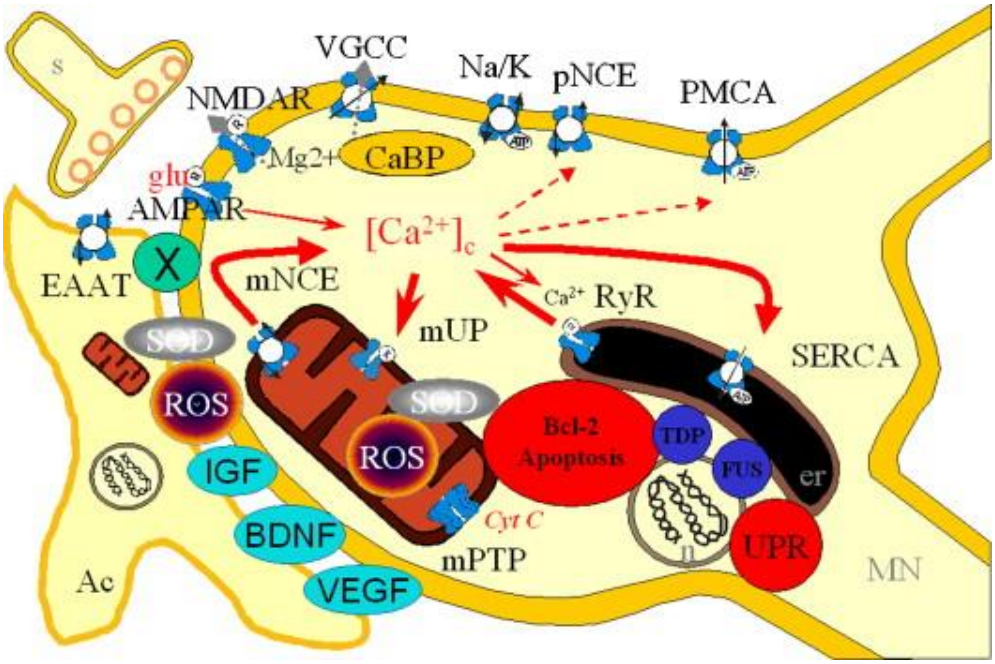


Figure 2. Interaction of Calcium and mitochondria in ALS as a local feedback mechanism.

	 Figure 3. Model of the Endoplasmic reticulum & mitochondria Calcium cycle (ERMCC) as the central regulatory system in motor neuron (MN) Calcium homeostasis.
VOCAB: (w/definition)	ALS: Neurodegenerative disease affecting motor neurons. Calcium deregulation: Abnormal intracellular calcium levels. Endoplasmic reticulum (ER): Organelle storing and regulating calcium, folding proteins. Mitochondria: Energy-producing organelle; regulates calcium and apoptosis. AMPA receptor: Glutamate receptor; some forms let calcium in. GluR2: AMPA subunit controlling calcium permeability. Neurodegeneration: Progressive loss of neuron function or death. Calcium buffer: Protein that binds calcium to maintain safe levels. Selective vulnerability: Some neurons are more prone to damage than others. Motor neuron: Nerve cell that controls muscles.
Cited references to follow up on	Van Den Bosch, L., Van Damme, P., Bogaert, E., & Robberecht, W. (2006). The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1762*(11-12), 1068-1082. https://doi.org/10.1016/j.bbadis.2006.05.002 Fray, A. E., Ince, P. G., Banner, S. J., Milton, I. D., Usher, P. A., Cookson, M. R., & Shaw, P. J. (1998). The expression of the glial glutamate transporter protein EAAT2 in motor neuron disease: An immunohistochemical study. *European Journal of Neuroscience*, *10*(8), 2481-2489. https://doi.org/10.1046/j.1460-

	9568.1998.00273.x Kawahara, Y., Kwak, S., Sun, H., Ito, K., Hashida, H., Aizawa, H., Jeong, Y., & Kanazawa, I. (2003). Human spinal motoneurons express low relative abundance of GluR2 mRNA: An implication for excitotoxicity in ALS. *Journal of Neurochemistry*, *85*(3), 680-689. https://doi.org/10.1046/j.1471-4159.2003.01703.x
Follow up Questions	1. How does glutamate receptor activity differ between vulnerable motor neurons and calcium-resistant neurons? 2. What role do calcium-buffering proteins play in protecting resistant neurons from calcium-mediated damage? 3. At what point does elevated intracellular calcium trigger activation of calcium-dependent proteases in ALS models?

Article #16 Notes : The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis

Article notes should be on separate sheets

Source Title	The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis
Source citation (APA Format)	Van Den Bosch, L., Van Damme, P., Bogaert, E., & Robberecht, W. (2006). The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1762*(11-12), 1068-1082. https://doi.org/10.1016/j.bbadis.2006.05.002
Original URL	https://doi.org/10.1016/j.bbadis.2006.05.002
Source type	Journal Article
Keywords	ALS, Neurodegeneration, Motor neuron, Ca2+ metabolism,, Glutamate, AMPA receptors, GluR2
#Tags	#Glutamate #Calciumsignaling #AMPA #MotorNeuron

Summary of key points + notes (include methodology)	Introduction: • Glutamate released from the presynaptic neuron activates ionotropic glutamate receptors present on the postsynaptic neuron • Disintegration of neuronal cells causes a further increase of extracellular glutamate and amplifies the excitotoxic damage • The neuron no longer meet the demands of its many ATP dependent processes, becomes damaged and ultimately dies • AMPA receptors are the most important glutamate receptors to mediate fast excitatory transmission • GluR2 have a very low calcium permeability compared to GluR2-lacking receptors • Co-administration of GABA enhanced the Calcium influx during AMPA receptor stimulation and resulted in an increased Calcium influx and enhanced cell death Methods: • Lit review of studies on glutamate excitotoxicity in ALS • Calcium imaging to measure glutamate-induced Calcium increase in motor neurons • Electrophysiology to see AMPA/NMDA receptor activity and Calcium • Pharmacological glutamate stimulation to cause excitotoxic stress • ALS animal models (SOD1 mutant mice) to study motor neuron degeneration • Biochemical assays to measure calcium-dependent protease activation and neuronal damage • Comparative analysis of vulnerable vs. resistant motor neuron populations Results: • Not everything in the pathogenesis of ALS can be reduced to the involvement of excitotoxicity • Sensitize motor neurons is inflammation and concomitant microglial activation • Excitotoxicity could also be responsible for the selective vulnerability of motor neurons during the course of the disease • To increase the resistance of motor neurons to high intercellular Calcium concentrations by inducing defense mechanisms • To inhibit the downstream pathways activated by an increased intracellular Ca^{2+} concentration
Research Question/Problem/ Need	How does glutamate-induced excitotoxicity contribute to motor neuron degeneration in amyotrophic lateral sclerosis?

Important Figures

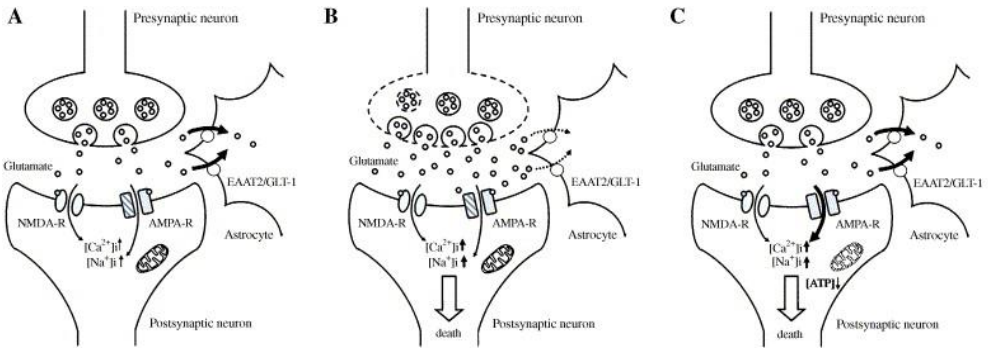


Figure 1. Glutamate neurotransmission and excitotoxicity. Under normal conditions, glutamate released from the presynaptic neuron activates the NMDA and AMPA receptors. These figure depicts the process. Figure A shows the glutamate coming into the neuron. Figure B shows more glutamate in the motor neurons. Figure C shows the glutamate leaving the motor neuron.

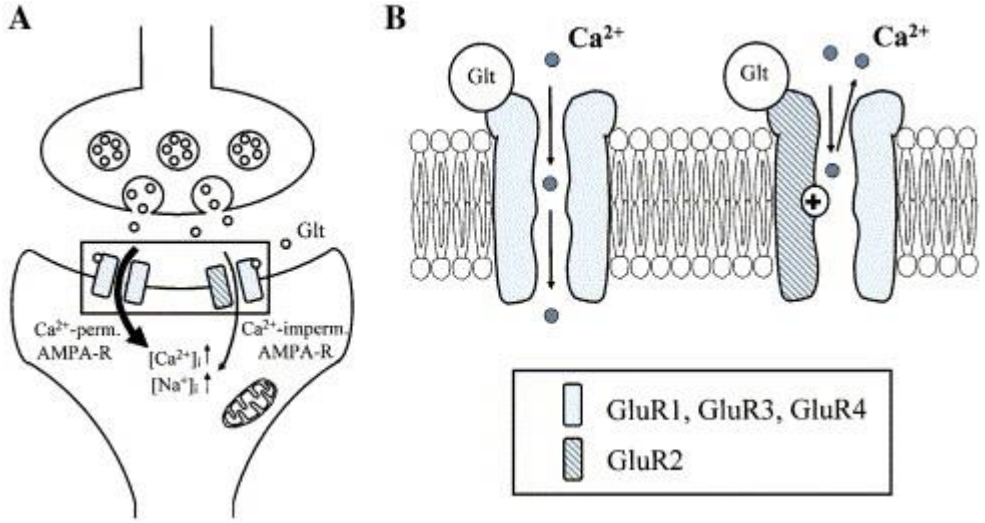


Figure 2. The Calcium permeability of AMPA glutamate receptors is determined by the presence/absence of GluR2 in the receptor area. This figure depicts the process. Figure A shows the motor neuron. Figure B shows the internal processes.

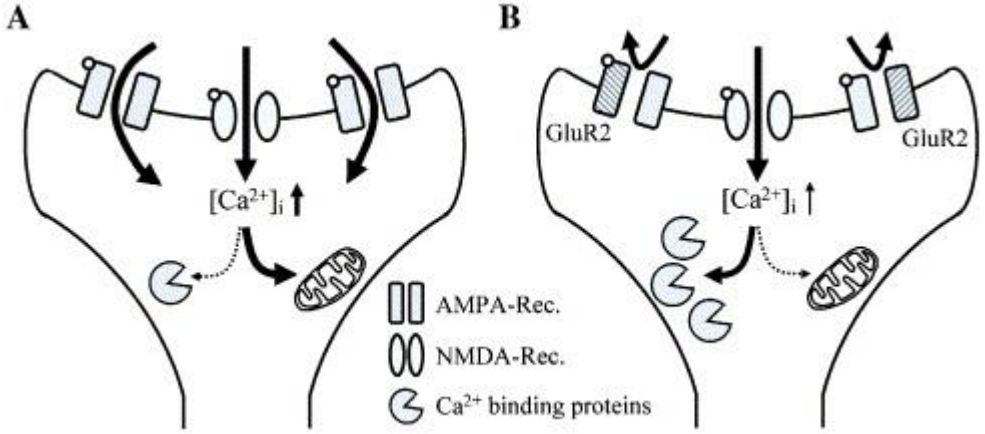
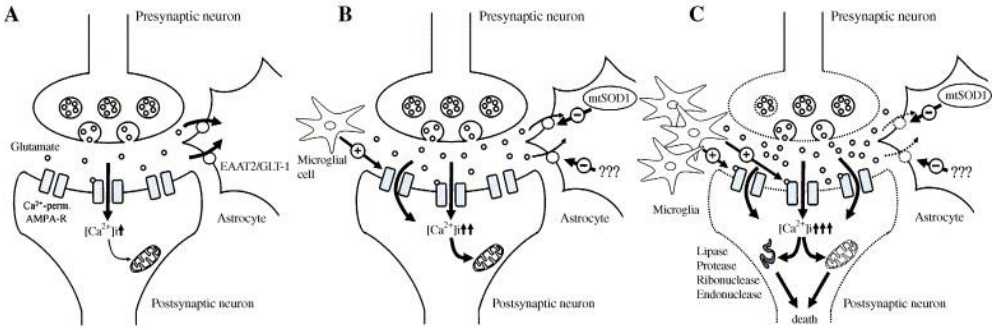
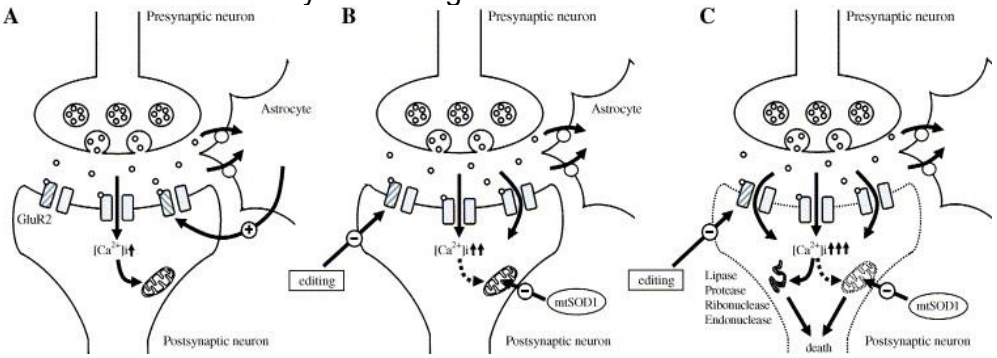


	Figure 3. Difference in calcium metabolism between different neurons depicted above. The entry pathway of calcium is different in motor neurons than compared to other neurons.Figure A shows the calcium going into the motor neuron, Figure B shows the calcium leaving.  Figure 4. Mechanisms of excitotoxicity in ALS. Figure A shows the normal motor neuron under these conditions. Figure B shows a motor neuron with the glutamate transporter damaged. Figure C shows a further increase in both factors released by the microglial cells.  Figure 6. Mechanisms of slow excitotoxicity in ALS. Figure A shows mitochondrial processes in a astrocyte. Figure B shows editing and calcium going into the mitchondria of an astrocyte. Figure C shows editing, more calcium entering the mitochondria of the astrocyte and death.
VOCAB: (w/definition)	• ALS (Amyotrophic lateral sclerosis): A progressive disease that causes degeneration of motor neurons. • Neurodegeneration: Gradual loss of neuron structure and function. • Motor neuron: Neuron that sends signals from the brain/spinal cord to muscles. • Ca^{2+} metabolism: Cellular processes that regulate calcium levels and signaling. • Glutamate: The main excitatory neurotransmitter in the nervous system. • AMPA receptors: Glutamate receptors that mediate fast synaptic transmission and can allow Ca^{2+} entry. • GluR2: AMPA receptor subunit that limits calcium permeability.

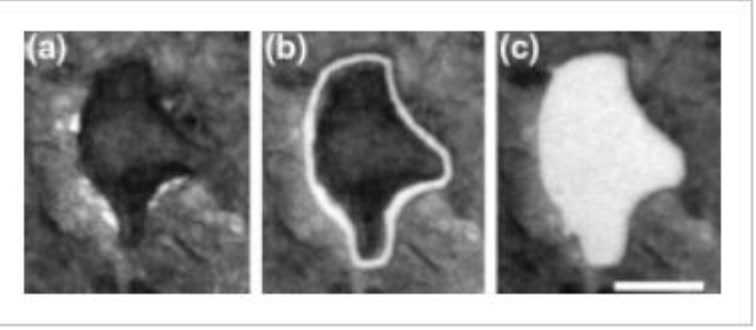
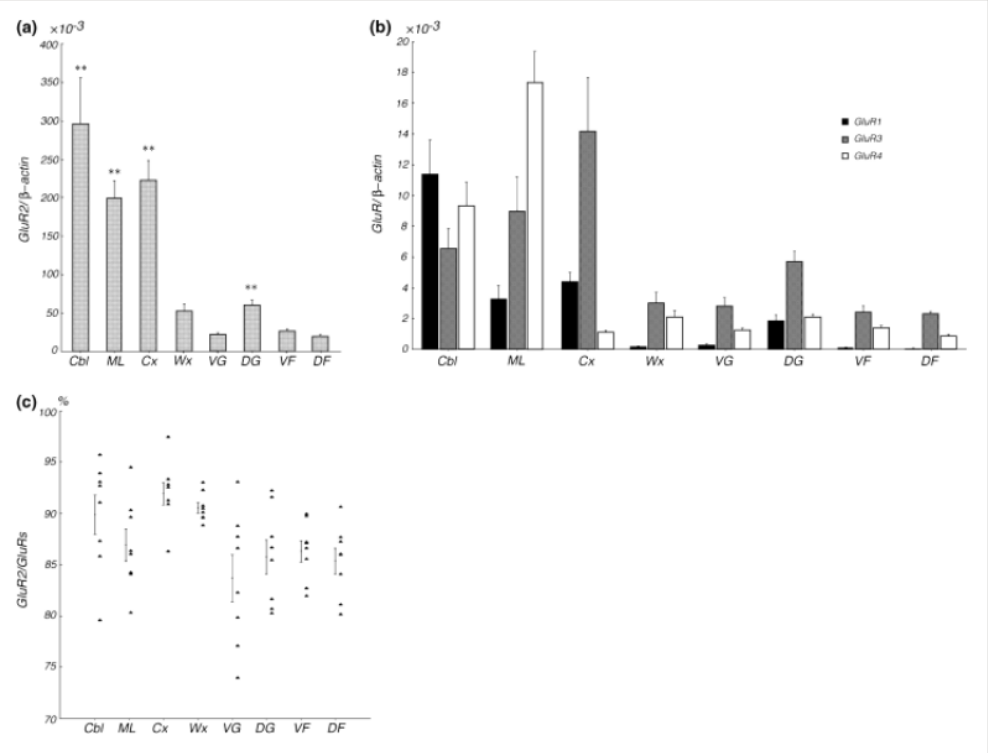
Cited references to follow up on	Coyle, J. T., & Puttfarcken, P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, *262*(5134), 689–695. https://doi.org/10.1126/science.7901908 Doble, A. (1999). The Role of Excitotoxicity in Neurodegenerative Disease: Implications for Therapy. *Pharmacology & Therapeutics*, *81*(3), 163-221. https://doi.org/10.1016/S0163-7258(98)00042-4 Collingridge, G. L., & Lester, R. A. (1989). Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacological Reviews*, *41*(2), 143-210. https://doi.org/10.1016/S0031-6997(25)00025-0
Follow up Questions	1. Which types of glutamate receptors (AMPA, NMDA, etc.) are most responsible for calcium-mediated excitotoxicity in ALS motor neurons? 2. How does excessive calcium influx trigger downstream pathways that lead to motor neuron death? 3. Why are some motor neurons more resistant to excitotoxicity than others in ALS?

Article #17 Notes : **Human spinal motoneurons express low relative abundance of GluR2 mRNA: an implication for excitotoxicity in ALS**

Article notes should be on separate sheets

Source Title	Human spinal motoneurons express low relative abundance of GluR2 mRNA: an implication for excitotoxicity in ALS

Source citation (APA Format)	Kawahara, Y., Kwak, S., Sun, H., Ito, K., Hashida, H., Aizawa, H., Jeong, Y., & Kanazawa, I. (2003). Human spinal motoneurons express low relative abundance of GluR2 mRNA: An implication for excitotoxicity in ALS. *Journal of Neurochemistry*, *85*(3), 680-689. https://doi.org/10.1046/j.1471-4159.2003.01703.x
Original URL	https://doi.org/10.1046/j.1471-4159.2003.01703.x
Source type	Journal Article
Keywords	ALS, amyotrophic lateral sclerosis, motoneuron, excitotoxicity, glutamate, AMPA receptor, GluR2, RNA editing, Q/R site, Ca^{2+} permeability, spinal cord, neurodegeneration, single-cell RT-PCR, laser microdissection
#Tags	#ALS #motoneuron #excitotoxicity #glutamate #AMPA
Summary of key points + notes (include methodology)	• Investigates GluR2 (AMPA receptor subunit) mRNA expression in human spinal motoneurons • Goal – to understand if low GluR2 expression or editing deficiency contributes to motoneuron vulnerability in ALS • Finds motoneurons naturally have lower GluR2 mRNA than other neurons • Suggests ALS-related motoneuron death is more linked to deficient GluR2 RNA editing (Q/R site) than to overall GluR2 reduction Methodology: • Samples: Spinal cord, brain tissue from 8 ALS patients and 8 controls • Single cell isolation: Laser microdissection of motoneurons, Purkinje cells, pyramidal neurons • RNA extraction + cDNA synthesis: TRIZOL reagent, DNase I treatment, reverse transcription • Quantitative RT-PCR: Measured mRNA levels of GluR1-GluR4 and β-actin as control • Regional expression in spinal cord areas compared to expression in motoneurons and other neuron types • ALS vs. control cases Results: • Normal neurons: • GluR2 mRNA dominant AMPA subunit in all neurons • Motoneurons had lowest GluR2 mRNA among neuron types (77.8%), but still majority • GluR1, GluR3, GluR4 mRNA levels variable across neuron types • GluR1 very low in motoneurons. • ALS vs. control: • No significant difference in GluR2 mRNA copy number in spinal motoneurons or spinal cord regions between ALS and controls

	• Single-cell analysis - no selective GluR2 reduction in ALS motoneurons • Suggests selective motoneuron death in ALS is not due to reduced GluR2 expression • Possibly due to deficient RNA editing at GluR2 Q/R site + increasing Ca^{2+} permeability + excitotoxicity
Research Question/Problem/ Need	Does reduced GluR2 mRNA expression or dysfunctional RNA editing at the Q/R site in human spinal motoneurons contribute to selective neuronal death in ALS?
Important Figures	 Figure 1. Single motoneuron isolation from spinal cord.  Figure 2. mRNA expression in GluR subunits in normal patients

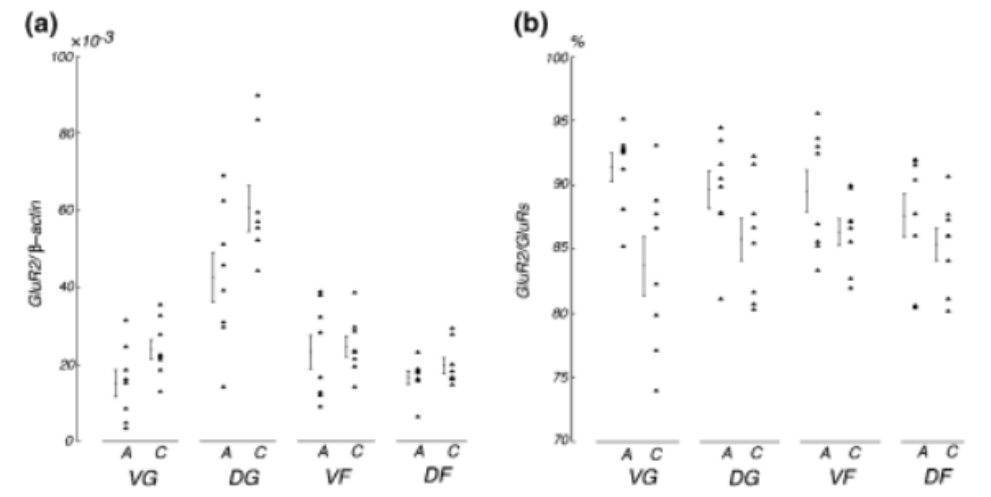
<table>
<tr><td></td><td> 

Figure 3. Level of expression of GluR2 mRNA in spinla cord regions of ALS patients and control patients</td></tr>
<tr><td>VOCAB: (w/definition)</td><td>

ALS (Amyotrophic Lateral Sclerosis): A progressive neurodegenerative disease causing loss of upper and lower motoneurons and muscle wasting
Motoneuron: Neurons that send signals from the spinal cord to muscles to control movement
Excitotoxicity: Neuronal damage or death caused by excessive stimulation by neurotransmitters such as glutamate
Glutamate: A major excitatory neurotransmitter in the central nervous system
AMPA Receptor: A type of ionotropic glutamate receptor involved in fast synaptic transmission and calcium influx
GluR2: Subunit of AMPA receptor; presence reduces Ca^{2+} permeability, providing neuroprotection
RNA Editing (Q/R site): Post-transcriptional modification where a glutamine (Q) codon is converted to arginine (R) in GluR2 mRNA, affecting calcium permeability
Single-cell RT-PCR: Technique to measure gene expression in individual neurons
Laser Microdissection: Method to isolate single neurons from tissue sections for molecular analysis

</td></tr>
<tr><td>Cited references to follow up on</td><td>Bar-Peled, O., O’Brien, R. J., Morrison, J. H., & Rothstein, J. D. (1999).

Cultured motor neurons possess calcium-permeable AMPA/kainate</td></tr>
</table>

	receptors. *Neuroreport*, *10*(4), 855–859. https://doi.org/10.1097/00001756-199903170-00034 Carriedo, S. G., Yin, H. Z., & Weiss, J. H. (1996). Motor neurons are selectively vulnerable to AMPA/Kainate Receptor-Mediated InjuryIn vitro. *Journal of Neuroscience*, *16*(13), 4069–4079. https://doi.org/10.1523/jneurosci.16-13-04069.1996 Geiger, J., Melcher, T., Koh, D., Sakmann, B., Seeburg, P., Jonas, P., & Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron*, *15*(1), 193–204. https://doi.org/10.1016/0896-6273(95)90076-4
Follow up Questions	1. How does dysfunctional GluR2 Q/R site editing affect AMPA receptor function in motoneurons? 2. Could modulating GluR2 expression or editing be a treatment for ALS? 3. How might species differences (human vs. rat) affect the relevance of AMPA receptor studies in animal models of ALS?

Article #18 Notes : **Motor Neurons Are Selectively Vulnerable to AMPA/Kainate Receptor-Mediated Injury *In Vitro***

Article notes should be on separate sheets

Source Title	Motor Neurons Are Selectively Vulnerable to AMPA/Kainate Receptor-Mediated Injury In Vitro
Source citation (APA Format)	Carriedo, S. G., Yin, H. Z., & Weiss, J. H. (1996). Motor neurons are selectively vulnerable to AMPA/Kainate Receptor-Mediated InjuryIn vitro. *Journal of Neuroscience*, *16*(13), 4069–4079. https://doi.org/10.1523/jneurosci.16-13-04069.1996
Original URL	https://doi.org/10.1523/jneurosci.16-13-04069.1996
Source type	Journal Article
Keywords	motor neuron, AMPA, kainate, excitotoxicity, Ca^{2+} influx, glutamate receptor, vulnerability, neurotoxicity, spinal cord neurons, in vitro culture, glutamate reuptake blocker, reactive oxygen species
#Tags	#motorneuron #AMPA #glutamate
Summary of key points + notes (include methodology)	• Motor neurons - critical for controlling muscle movement but are selectively vulnerable in neurodegenerative diseases like ALS • Previous research suggested that excitotoxicity (cell damage caused by excessive activation of glutamate receptors) could explain why motor neurons are more likely to die • Study used in vitro spinal cord cultures to test whether motor neurons are more vulnerable than other neurons to excitotoxic injury mediated by AMPA and kainate receptors and whether Ca^{2+} influx is a key factor • Investigated how blocking glutamate reuptake (which increases extracellular glutamate) affects neuron survival Methodology: • Cell culture: Spinal cord neurons from rodents were cultured in vitro • Motor neurons were identified using morphological characteristics + motor neuron markers • Treatment: Cultures were exposed to AMPA or kainate - to activate

	receptors • Glutamate reuptake blocker (PDC) to increase extracellular glutamate • Manipulations of extracellular Ca^{2+} to measure role of calcium influx • Injury: Loss of motor neuron markers + cell morphology changes + cell survival recorded • Intracellular Ca^{2+} levels measured during treatment • Motor neurons compared to other spinal neurons in same cultures to see if selectively vulnerable Results: • Motor neurons significantly more sensitive to AMPA- and kainate-induced injury than other spinal neurons • Most motor neurons showed cell death after 12-24 hours of receptor activation, but other neurons survived under the same conditions • Intracellular Ca^{2+} increased more in motor neurons than in other neurons during AMPA/kainate exposure • Removing extracellular Ca^{2+} reduced motor neuron death, indicating that Ca^{2+} influx through AMPA/kainate receptors is primary cause of excitotoxicity • Increasing extracellular glutamate (thru PDC) enhanced motor neuron injury • Supports idea that motor neurons highly vulnerable to glutamate accumulation • Higher concentrations of AMPA/kainate caused more rapid motor neuron death • Low doses caused slower injury but still selectively affected motor neurons • Motor neurons might express a higher proportion of Ca^{2+}-permeable AMPA/kainate receptors, which is why they have selective vulnerability compared to other spinal neurons
Research Question/Problem/ Need	Are motor neurons more vulnerable to AMPA/kainate receptor-mediated excitotoxic injury compared to other spinal neurons, and is this vulnerability connected to Ca^{2+} increase?

Important Figures

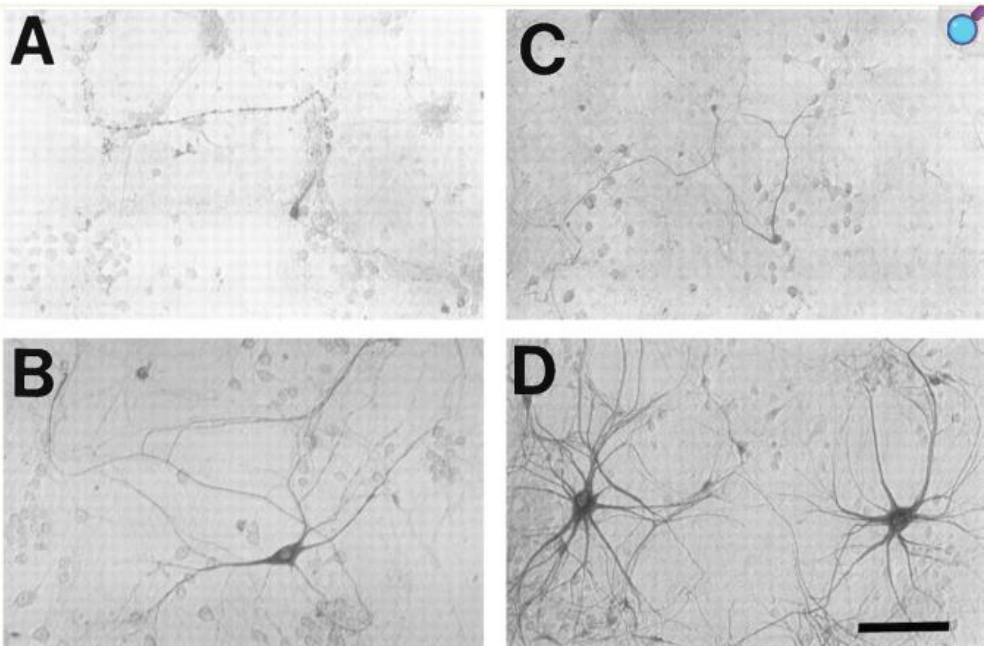


Figure 1. Spinal cord motor nerve cultures exposed to kainate with calcium or without

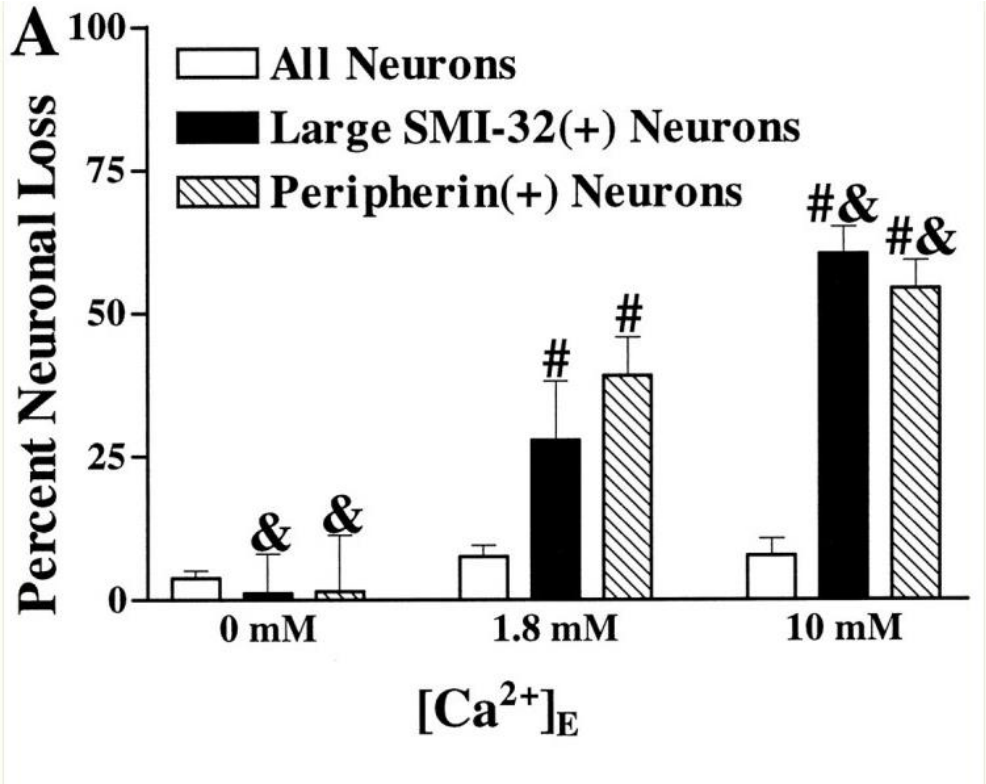
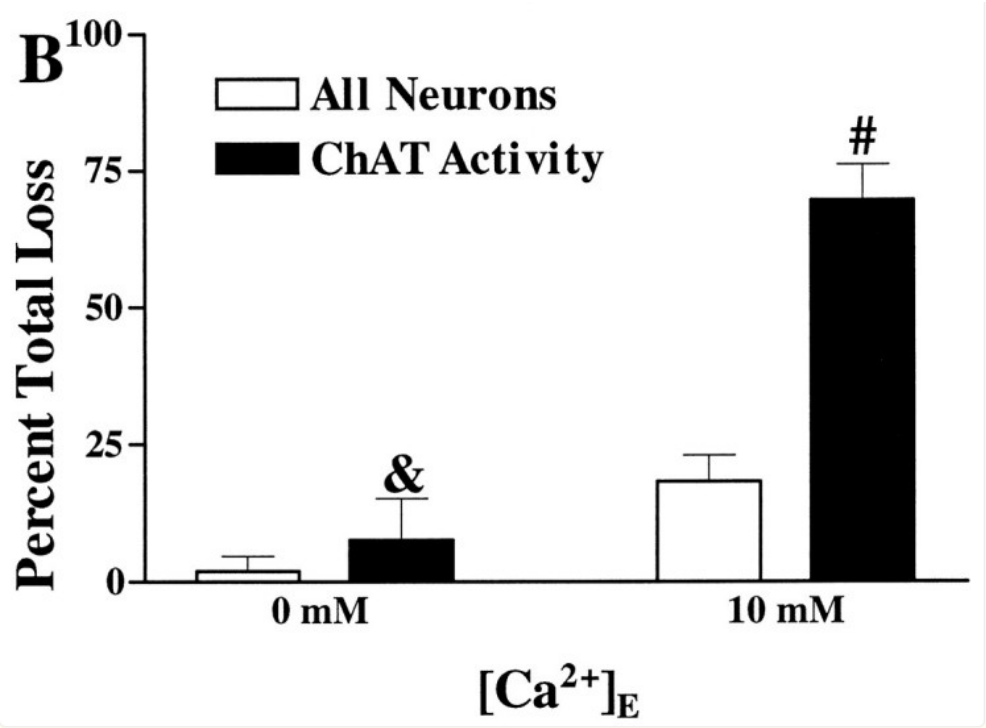


Figure 2. Percent Neuronal Loss for each type of neuron with calcium

	 Figure 3. Percent Total Loss for all neurons and ChAT activity with calcium
VOCAB: (w/definition)	• Motor neuron: A neuron that sends signals from the spinal cord to muscles • AMPA receptor: A glutamate-activated ion channel that mediates excitatory synaptic transmission; some forms allow Ca^{2+} entry • Kainate receptor: Another subtype of non-NMDA glutamate receptor that contributes to excitatory signaling and can be excitotoxic • Excitotoxicity: Neuronal injury or death caused by excessive activation of glutamate receptors and Ca^{2+} overload • Ca^{2+} influx: Entry of calcium ions into neurons, often triggering damaging downstream effects when excessive • Glutamate: The main excitatory neurotransmitter that activates AMPA and kainate receptors • Glutamate reuptake blocker: A chemical that prevents reuptake of glutamate, increasing extracellular glutamate and excitotoxic stress • In vitro culture: Laboratory culture of cells outside the organism, often used to study cellular mechanisms
Cited references to follow up on	Bochet, P., Audinat, E., Lambolez, B., Crépel, F., Rossier, J., Iino, M., Tsuzuki, K., & Ozawa, S. (1994). Subunit composition at the single-cell level explains functional properties of a glutamate-gated channel.

	Neuron, *12*(2), 383–388. https://doi.org/10.1016/0896-6273(94)90279-8 Brorson, J. R., Zhang, Z., & Vandenberghe, W. (1999). CA2+Permeation of AMPA receptors in cerebellar neurons expressing GLU receptor 2. *Journal of Neuroscience*, *19*(21), 9149–9159. https://doi.org/10.1523/jneurosci.19-21-09149.1999 Choi, D. W. (1992). Excitotoxic cell death. *Journal of Neurobiology*, *23*(9), 1261–1276. https://doi.org/10.1002/neu.480230915
Follow up Questions	1. Does blocking Ca^{2+} entry through AMPA/kainate receptors shield motor neurons from excitotoxicity injury? 2. Are motor neurons more vulnerable than other spinal neurons because they have more Ca^{2+}-permeable glutamate receptors? 3. Can controlling glutamate uptake reduce motor neuron vulnerability to excitotoxicity damage?

Article #19 Notes : Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS

Article notes should be on separate sheets

Source Title	Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS
Source citation (APA Format)	Geiger, J., Melcher, T., Koh, D., Sakmann, B., Seeburg, P., Jonas, P., & Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron*, *15*(1), 193–204. https://doi.org/10.1016/0896-6273(95)90076-4
Original URL	https://doi.org/10.1016/0896-6273(95)90076-4
Source type	Journal Article
Keywords	AMPA receptor, GluR2, RNA expression, Ca^{2+} permeability, glutamate receptor, interneuron, principal neuron, synaptic transmission, receptor subunits, electrophysiology, patch-clamp, subunit composition, excitotoxicity
#Tags	#glutamate #excitotoxicity #AMPA
Summary of key points + notes (include methodology)	• AMPA receptors - composed of combinations of four subunits (GluR1-GluR4) • Presence of GluR2 in AMPA receptor reduces Ca^{2+} permeability, because when GluR2 is present and properly edited at Q/R site, blocks Ca^{2+} flux • Neurons with high GluR2 expression have AMPA receptors with low Ca^{2+} permeability, but neurons with low GluR2 have more Ca^{2+}-permeable AMPA receptors • Work showed that different classes of neurons express different

	ratios of GluR2 and other subunits, leading to functionally different AMPA receptors • Differences help explain why some neurons are more vulnerable to Ca^{2+}-dependent excitotoxicity than others Methodology: • Measured currents through AMPA receptors in cultured neurons under controlled conditions • Determined the relative abundance of individual AMPA receptor subunit mRNAs (GluR2 vs GluR1) in the same neurons that were electrophysiologically recorded • Matched functional receptor properties (Ca^{2+} permeability) with subunit expression profiles to identify how composition influenced physiology • Principal neurons and interneurons were studied to see how subunit composition varied across cell classes and affected receptor function Results: • Neurons with highGluR2 expression had AMPA receptors that were more impermeable to Ca^{2+}, but neurons with lower GluR2 allowed significant Ca^{2+} entry • Interneurons often had lower GluR2 expression and thus exhibited higher Ca^{2+} permeability compared to principal neurons • Within a single neuron class, variation in subunit expression resulted in measurable differences in receptor gating and Ca^{2+} conductance • Mechanistic link suggests cells with low GluR2/low editing are more vulnerable to Ca^{2+}-mediated excitotoxicity-relevant factor in neurodegenerative diseases like ALS
Research Question/Problem/ Need	How does expression of AMPA receptor subunits, like GluR2, affect the Ca^{2+} permeability and gating properties of AMPA receptors in different types of CNS neurons?

Important Figures

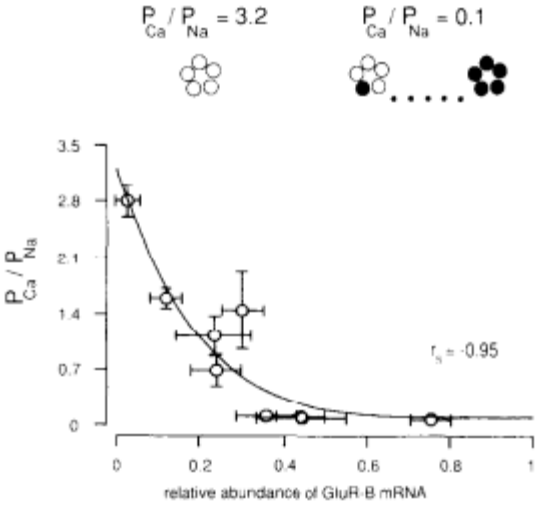


Figure 1. The abundance of GluR mRNA shows how permeable the calcium is of Native AMPARs.

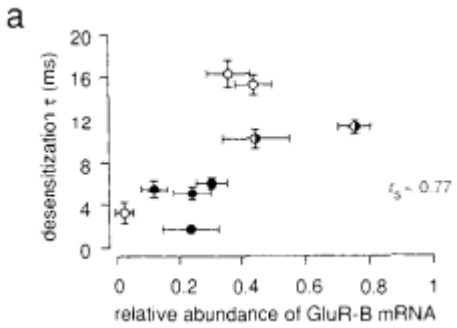


Figure 2. Desensitization of the time constant against the relative abundance of GluR-B mRNA

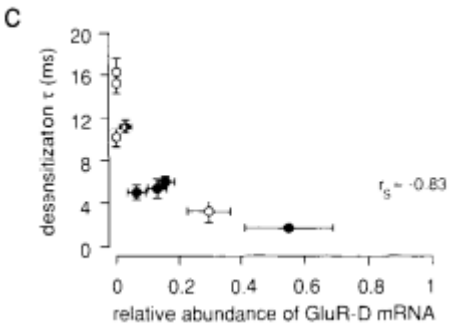


Figure 3. Desensitization of the time constant against the relative abundance of GluR-D mRNA

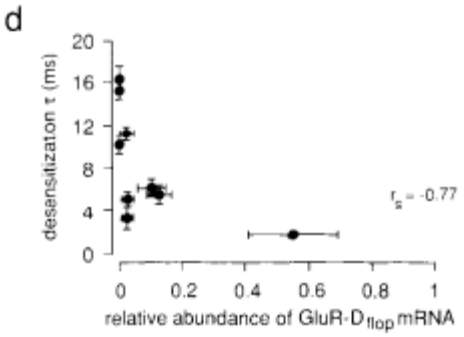


Figure 4. Desensitization of the time constant against the relative abundance of GluR-D top mRNA

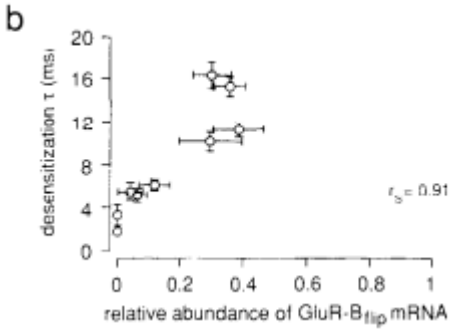


Figure 5. Desensitization of the time constant against the relative abundance of GluR-B top mRNA

VOCAB: (w/definition)

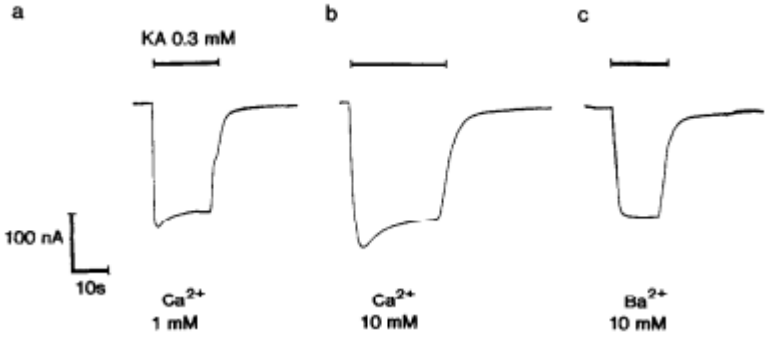
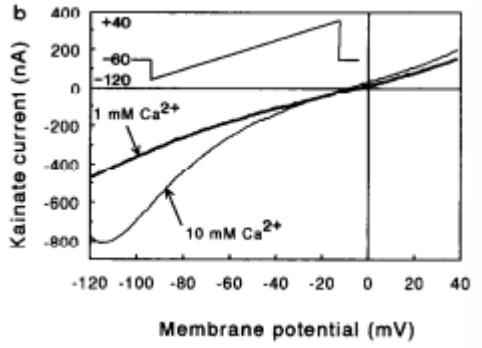
- AMPA receptor: A type of ionotropic glutamate receptor that mediates most fast excitatory synaptic transmission in the brain
- GluR2: A subunit of the AMPA receptor; its presence critically determines whether the receptor is permeable to Ca^{2+}
- Ca^{2+} permeability: The ability of an ion channel to allow calcium ions to pass through, central to signaling but also to excitotoxic damage if excessive
- Glutamate receptor: A receptor activated by glutamate, the principal excitatory neurotransmitter in the CNS
- Interneuron: A local circuit neuron, often inhibitory, that connects other neurons within a brain region
- Principal neuron: A major output neuron (e.g., pyramidal cell) that typically has projection functions
- Subunit composition: The particular combination of protein subunits that assembles into a receptor, determining its functional properties
- Patch-clamp electrophysiology: A laboratory technique to measure ionic currents through individual neurons' receptors or channels
- Excitotoxicity: Neuronal damage/death due to excessive stimulation, often involving Ca^{2+} influx through glutamate receptors

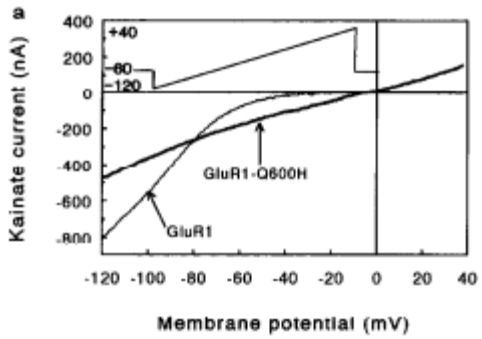
Cited references to follow up on	Burnashev, N. (1993). Recombinant ionotropic Glutamate Receptors: Functional Distinctions Imparted by Different Subunits. *Cellular Physiology and Biochemistry*, *3*(5–6), 318–331. https://doi.org/10.1159/000154696 Hestrin, S. (1993). Different glutamate receptor channels mediate fast excitatory synaptic currents in inhibitory and excitatory cortical neurons. *Neuron*, *11*(6), 1083–1091. https://doi.org/10.1016/0896-6273(93)90221-c Hume, R. I., Dingledine, R., & Heinemann, S. F. (1991). Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science*, *253*(5023), 1028–1031. https://doi.org/10.1126/science.1653450
Follow up Questions	1. How does changing the ratio of GluR2 to other AMPA receptor subunits change Ca^{2+} permeability in neurons? 2. Do neurons with low GluR2 expression show more vulnerability to excitotoxicity damage? 3. Can changing GluR2 expression levels in a neuron shield against excitotoxicity in neurodegenerative disease models?

Article #20 Notes : In the GluRl glutamate receptor subunit a glutamine to histidine point mutation suppresses inward rectification but not calcium permeability

Article notes should be on separate sheets

Source Title	In the GluRl glutamate receptor subunit a glutamine to histidine point mutation suppresses inward rectification but not calcium permeability
Source citation (APA Format)	Curutchet, P., Bochet, P., De Carvalho, L. P., Lambolez, B., Stinnakre, J., & Rossier, J. (1992). In the GluR1 glutamate receptor subunit a glutamine to histidine point mutation suppresses inward rectification but not calcium permeability. *Biochemical and Biophysical Research Communications*, *182*(3), 1089–1093. https://doi.org/10.1016/0006-291x(92)91843-f
Original URL	https://doi.org/10.1016/0006-291X(92)91843-F
Source type	Journal Article
Keywords	GluR1, glutamate receptor, point mutation, inward rectification, calcium permeability, AMPA receptor, ion channel, subunit mutation, electrophysiology, synaptic transmission, patch-clamp
#Tags	#AMPA #glutamate #calcium #electrophysiology
Summary of key points + notes (include methodology)	• Investigated effect of single amino acid point mutation (glutamine to histidine) in GluR1 subunit of AMPA receptors • Determine if inward rectification and calcium permeability are functionally linked • Found that the point mutation reduced inward rectification, allowing more symmetric current flow • Calcium permeability - unchanged, means rectification and Ca^{2+} conductance can be independently modulated • Concluded subunit structure directly controls channel properties, and these properties can be genetically separated • Helps explain how neurons may regulate Ca^{2+} entry independently of rectification • Relevant for excitotoxicity in neurodegenerative diseases Methodology: • Used molecular biology techniques to introduce point mutation into GluR1 subunit cDNA • Expressed mutant + wild-type GluR1 receptors in Xenopus oocytes for electrophysiological recordings • Performed two-electrode voltage clamp to measure ionic currents through AMPA receptors

	• Tested rectification properties by recording current at different holding potentials • Measured calcium permeability w/Ca^{2+}-sensitive solutions and electrophysiological measurements • Compared results from mutant Q→H receptors + wild-type receptors to determine functional effects of mutation Results: • Point mutation in GluR1 subunit got rid of inward rectification of AMPA receptor currents • Despite this change, mutant receptor remained permeable to Ca^{2+} at similar levels as wild-type receptor • Results indicate that inward rectification + Ca^{2+} permeability not obligatorily linked in AMPA receptors, can be modulated separately by subunit structure • Suggests that Ca^{2+} influx through AMPA receptors not solely depend on rectification properties but on specific molecular determinants within channel pore
Research Question/Problem/ Need	Does a specific mutation in the GluR1 subunit of AMPA receptors affect the calcium permeability of the receptor channel?
Important Figures	 Figure 1. Kainate caused currents displayed in mv, and different calcium levels  Figure 2. Membrane potentail versus Kainate Current with different amounts of calcium (1mM-10mM)

	 Figure 3. Membrane potentail versus Kainate Current with different subunits of GluR (GluR1 or GluR1 – Q600H)
VOCAB: (w/definition)	• GluR1: One of the four subunits (GluR1–GluR4) that assemble into AMPA-type glutamate receptors • Glutamate receptor: An ion channel activated by glutamate, the primary excitatory neurotransmitter in the nervous system • Point mutation: A change in a single amino acid in a protein sequence due to a DNA mutation • Inward rectification: A property of ion channels where current flows more easily into the cell than out; associated with channel block by intracellular polyamines • Calcium permeability: The ability of an ion channel to allow Ca^{2+} ions to pass through, which can influence excitotoxicity and intracellular signaling • AMPA receptor: A type of ionotropic glutamate receptor responsible for fast synaptic transmission; its properties depend on subunit composition and editing • Subunit mutation: A change in one component (subunit) of a multimeric receptor that alters its functional properties • Electrophysiology: Techniques like patch-clamp used to record electrical currents through receptor channels
Cited references to follow up on	Miller, R. (1991). The revenge of the kainate receptor. *Trends in Neurosciences*, *14*(11), 477–479. https://doi.org/10.1016/0166-2236(91)90054-x Barish, M. E. (1983). A transient calcium-dependent chloride current in the immature Xenopus oocyte. *The Journal of Physiology*, *342*(1), 309–325. https://doi.org/10.1113/jphysiol.1983.sp014852

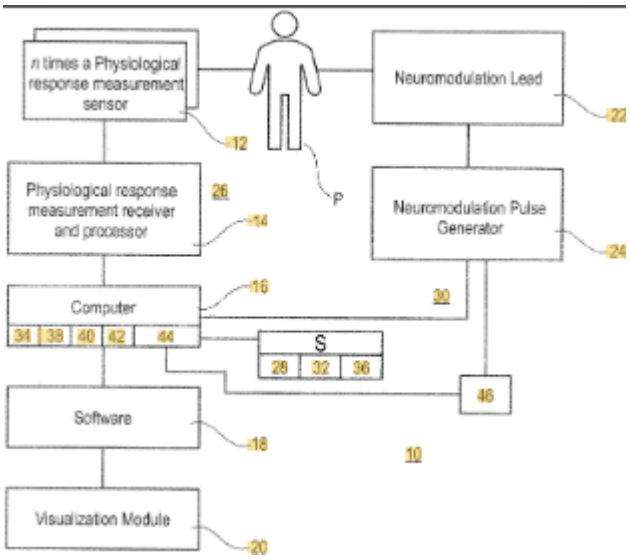
	Hollmann, M., Hartley, M., & Heinemann, S. (1991). CA2 + permeability of KA-AMPA—Gated glutamate receptor channels depends on subunit composition. *Science*, *252*(5007), 851–853. https://doi.org/10.1126/science.1709304
Follow up Questions	1. How does the GluR1 point mutation change interactions with intracellular blockers like polyamines that normally cause inward rectification? 2. Do other subunit mutations, in GluR2 or GluR3, similarly divide rectification from calcium permeability? 3. In disease models of excitotoxicity (like ALS), do changes in subunit or editing show the effects seen in the point mutation and what are the consequences for motor neuron survival?

Patent #1 Notes: System for planning and/or providing neuromodulation

Article notes should be on separate sheets

Source Title	System for planning and/or providing neuromodulation
Source citation (APA Format)	Wagner, F., Minassian, K., Capogrosso, M., Courtine, G., Brouns, R., Bakker, J., Kleibeuker, A., Bakker, B., Delattre, V., De Lausanne Epfl, E. P. F., & Nv, O. M. (2017, December 5). *US11511116B2 - System for planning and/or providing neuromodulation - Google Patents*. https://patents.google.com/patent/US11511116B2/en

Original URL	https://patents.google.com/patent/US11511116B2/en
Source type	Patent
Keywords	Neuromodulation, neurostimulation, electrical stimulation, stimulation parameters, neural response, physiological data, response mapping, electrode, pulse generator, data analysis, functional mapping, stimulation planning
#Tags	#neuromodulation #neurostimulation
Summary of key points + notes (include methodology)	• A system that connects electrical stimulation parameters to neural responses • Goal - to optimize neuromodulation by observing how different stimulation settings affect activity • Emphasizes connections made with evidence of electrical stimulation inputs, motor outputs • System designed to compare expected vs actual response and change stimulation parameters based on that • Developed for neuromodulation in professional settings, doesn't depend on a specific species and applies to all experimental models, including non-mammals systems • Supports idea that quantifying stimulation and response relationships is needed for understanding neuromodulation effects Methodology: • Data Input: • Apply electrical pulses through electrodes • Record neuron or body responses with sensors • Response Mapping: • Create a map linking stimulation to responses • Include timing of responses • Include size/magnitude of responses • Analysis & Comparison: • Compare actual responses to expected ones • Identify differences • Determine how well stimulation worked • Optimization: • Adjust stimulation settings based on results • Refine pulses, frequency, or timing • Repeat process to improve effectiveness • Output & Planning: • Suggest best settings for future experiments

	• Save and visualize data • Reuse data for further refinement Results: • Does not include experimental outcome data such as physiological measurements, clinical trials, or neural recordings • Describes neuromodulation system design and workflow without reporting quantified testing results • Outlines modules and data structures for linking stimulation parameters to response data, implying how the system could be evaluated, but no specific performance numbers provided • Claims that its system can plan stimulation based on stored stimulation and response data, but it does not show real-world validation results showing this
Research Question/Problem/ Need	How can electrical stimulation parameters be optimized to achieve desired neural or physiological responses?
Important Figures	 Figure 1. Image depicting framework of patented device.

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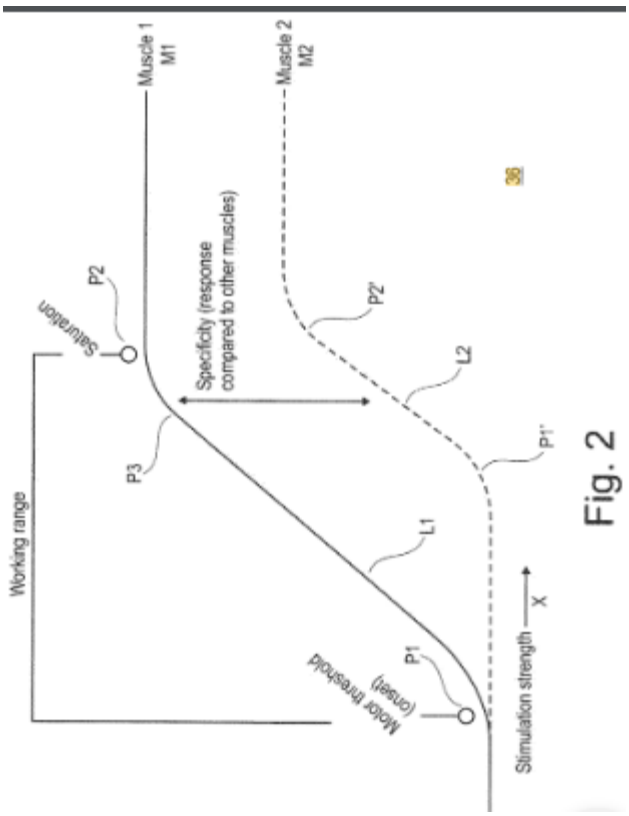
<td> 

Figure 2. Image depicting the stimulation strength and muscle responses.</td>
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<td>VOCAB: (w/definition)</td>
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Neuromodulation – The process of altering nerve activity by delivering targeted stimuli, such as electrical signals.
Neurostimulation – Applying electrical or other stimuli to neurons to elicit or modify their activity.
Electrical stimulation – Use of controlled electrical pulses to activate or inhibit neural tissue.
Stimulation parameters – Adjustable settings of electrical pulses, including amplitude, frequency, duration, and pulse width.
Neural response – The measurable activity of neurons in reaction to stimulation, such as firing rate or calcium changes.
Physiological data – Quantitative measurements of biological functions resulting from stimulation, calcium signaling, voltage changes.
Response mapping – Linking specific stimulation parameters to observed neural or physiological outcomes.
Electrode – Conductive device that delivers electrical pulses to neural tissue.
Pulse generator – Device that produces controlled electrical pulses for neuromodulation.
Data analysis – Computational or statistical evaluation of stimulation-response data to identify patterns or optimize parameters.
Functional mapping – Visualization or representation of how neural

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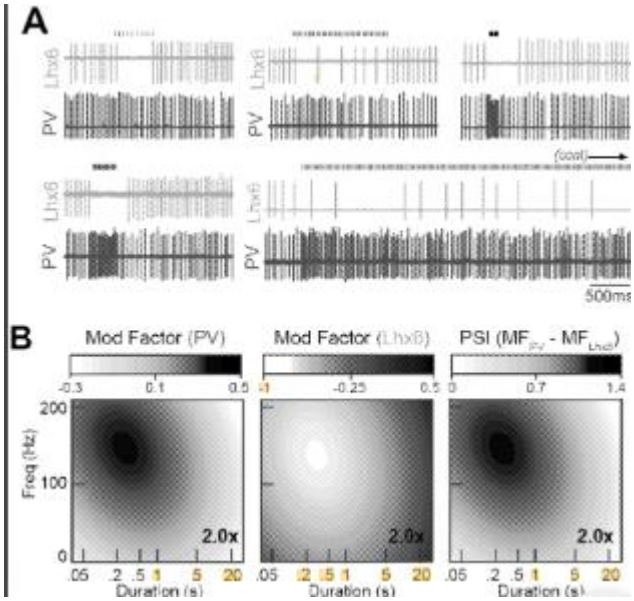
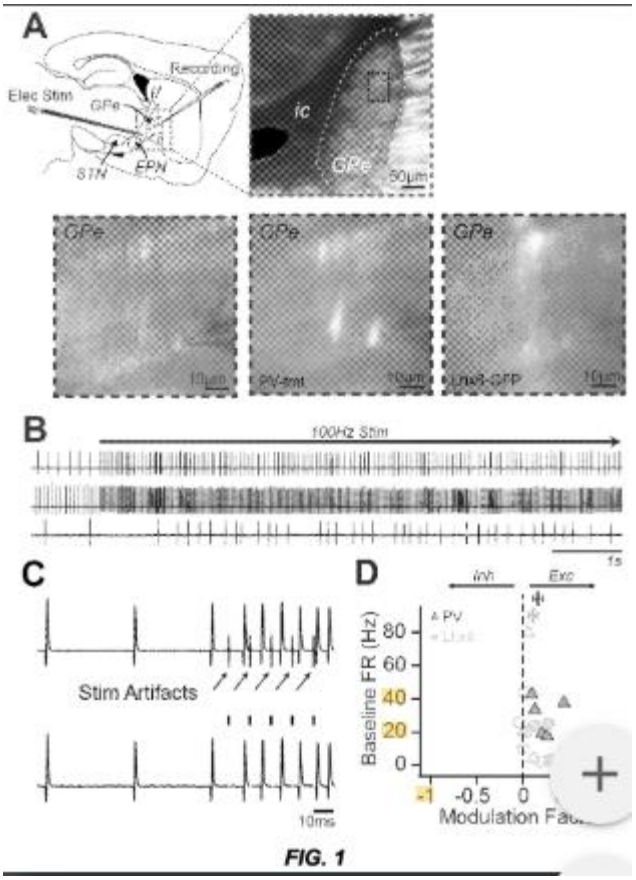
	function varies with different stimulation settings. • Stimulation planning – Designing and selecting stimulation protocols to achieve desired neural responses.
Cited references to follow up on	Gharib, J. E., Kaula, N., Blewett, J., & Inc, N. (2001, July 11). *WO2003005887A2 - System and methods for determining nerve proximity, direction, and pathology during surgery - Google Patents*. https://patents.google.com/patent/WO2003005887A2/en Bokil, H., Carcieri, S., Carlton, K. R., Moffitt, M. A., Yoo, P. J., & Corp, B. S. N. (2014, July 30). *US20160030750A1 - Systems and methods for stimulation-related volume analysis, creation, and sharing with integrated surgical planning and stimulation programming - Google Patents*. https://patents.google.com/patent/US20160030750A1/en Blum, D. A., Schulte, G. T., Kokones, S., Carlton, K., & Corp, B. S. N. (2010, June 14). *US20150066111A1 - Programming interface for spinal cord neuromodulation - Google Patents*. https://patents.google.com/patent/US20150066111A1/en
Follow up Questions	1. How does changing stimulation parameters (like frequency or pulse width) affect the neural response? 2. Can the system predict the optimal stimulation settings to achieve a desired physiological outcome? 3. How is the mapping between stimulation input and neural/physiological output stored and analyzed?

Patent #2 Notes: Neuromodulation Using Electrical Stimulation

Article notes should be on separate sheets

Source Title	Neuromodulation Using Electrical Stimulation
Source citation (APA Format)	Gittis, A., & Spix, T. A. (2020, January 15). *US20230064864A1 - Neuromodulation using electrical stimulation - Google Patents*. https://patents.google.com/patent/US20230064864A1/en
Original URL	https://patents.google.com/patent/US20230064864A1/en
Source type	Patent
Keywords	Stimulation pattern, electrical pulses, alternating bursts, neuromodulation, neurons, frequency, electrode lead, pulse generator, movement disorder therapy, basal ganglia, therapeutic effect
#Tags	#neuromodulation
Summary of key points + notes (include methodology)	• A method + system for neuromodulation using patterned electrical stimulation of neurons • Focuses on delivering bursts of electrical pulses and then pauses to influence neural activity • Is meant to improve treatment of movement disorders (Parkinson's disease) by modulating neural circuits • Uses specific timing and frequencies for pulses + pauses to increase therapeutic effects while continually stimulating Methodology: • Apply electrical stimulation in bursts (first periods with pulses) • Use pauses with no stimulation between bursts • Choose burst durations of 100–400 ms and pause durations of 500–1900 ms • Deliver pulses at 100–250 Hz frequency during bursts • Implant an electrode in the target region of the nervous system • Use pulse generator connected to electrode to control stimulation • Repeat pattern as long as therapeutic effects are expected • The stimulation can improve motor symptoms like tremor or rigidity Results:

	• Burst-pause electrical stimulation modulates neuronal activity more selectively than continuous stimulation • Excitatory postsynaptic currents (EPSCs) show increased response during bursts • Inhibitory postsynaptic currents (IPSCs) are recruited differently depending on stimulation intensity • Neurons respond differently based on cell type, showing that some neurons have capability, others don't • Statistical analysis indicates significant differences ($p < 0.05$) between burst-pause and continuous stimulation in synaptic responses • The results support the claim that alternating burst-pause stimulation can achieve controlled and targeted neuromodulation
Research Question/Problem/ Need	What stimulation pattern of alternating bursts and pauses of electrical pulses can effectively control neural activity for treating movement disorders and other neurological problems?
Important Figures	 Figure 1. Image comparing the frequency (hz), duration in seconds, and mod factor (PV)

	 Figure 2. Comparison of the modulation factor versus the baseline frequency (hz).
VOCAB: (w/definition)	• Stimulation pattern – A specific sequence of electrical pulses and pauses used to modulate neural activity • Electrical pulses – Brief bursts of electrical current used to activate or inhibit neurons • Alternating bursts – Intervals when pulses are delivered followed by intervals with no pulses • Neuromodulation – Changing neural activity through targeted electrical stimulation • Neurons – Nerve cells whose activity is influenced by the stimulation • Frequency – Rate at which electrical pulses are delivered (100–250 Hz) • Electrode lead – A conductive implant or lead that delivers electrical pulses to target tissue • Pulse generator – Device that creates and controls the pulse stimulation. • Movement disorder therapy – Clinical use of stimulation to improve symptoms like tremor or rigidity • Basal ganglia – Brain region targeted in therapies for movement disorders (Parkinson's) • Therapeutic effect – The improvement in disease or symptom after

	stimulation
Cited references to follow up on	Moffitt, M. A., Peterson, D. K., Meadows, P. M., & Corp, A. B. (2007, November 2). *US20090118787A1 - Closed-loop feedback for steering stimulation energy within tissue - Google Patents*. https://patents.google.com/patent/US20090118787A1/en Wu, J., Nelson, D. E., & Inc, M. (2011, April 20). *US20120271375A1 - Electrical brain therapy parameter determination based on a bioelectrical resonance response - Google Patents*. https://patents.google.com/patent/US20120271375A1/en De Ridder, D., & Individual. (2014, September 11). *US20190015666A1 - System and Method for Nested Neurostimulation - Google Patents*. https://patents.google.com/patent/US20190015666A1/en
Follow up Questions	1. What range of stimulation frequencies and burst/pause durations produces the best neural modulation? 2. Does using a single implanted electrode lead work as well as multiple leads? 3. How long do therapeutic benefits persist after stimulation is stopped?