

**NJCBIR FINAL PROGRES REPORT
COVER PAGE**

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Name of Organization: University of Medicine and Dentistry of New Jersey, Newark

Grant Title: Tonic GABAergic Inhibition after Traumatic Brain Injury: Role in Epileptogenicity

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1. ORIGINAL AIMS OF THE PROJECT

The goal of the study was to determine if brain injury modifies extrasynaptic inhibition, a relatively novel non-synaptic form of GABAergic inhibition, in feed-forward and projection neurons of the dentate gyrus. We focused on the dentate gyrus because it is a region of the hippocampal structure that undergoes structural and functional alterations following concussive brain injury. Our general hypothesis was that brain injury induced alterations in extrasynaptic GABAergic inhibition are an essential mechanism underlying post-traumatic hyperec excitability in the dentate gyrus that can be targeted for therapeutic intervention. 3 Aims were defined.

Aim 1: Test the hypothesis that brain injury causes long-term alterations in tonic GABA currents in dentate granule cells and molecular layer interneurons (MLI). And determine using specific modulators of GABA subunits, whether subunit composition of GABA receptors contributing to tonic inhibition is altered after head injury. Strategy: patch clamp electrophysiological recordings from specific neurons in acute hippocampal slices from sham-injured control rats and those subject to moderate lateral fluid percussion injury

Aim 2: Determine how experimentally identified post-traumatic changes in tonic GABAergic inhibition in specific neuronal types alters dentate network activity. Strategy: Implement computational models of dentate networks and examine activity while manipulating tonic GABAergic currents in specific neuronal populations.

Aim 3: Determine whether modulation of tonic inhibition can decrease dentate excitability after experimental head trauma. Strategy: Assess epileptogenicity following use of GABA modulators *in vitro* and *in vivo*.

2. PROJECT SUCCESS

The studies proposed in Aim 1 were completed successfully and lead to novel findings that have been published (Gupta et al., 2012) explored further. To summarize, we identified post-traumatic increase in tonic GABA currents in dentate granule cells within one week after injury. Examination of neurons in the molecular layer revealed the presence of multiple subclasses including several inhibitory neuronal types and, unexpectedly, a **novel class of excitatory semilunar granule cells (SGCs)** with differential expression of tonic GABA currents in sham and head injured rats. Therefore, we **modified our experimental strategy to include morphological analysis** of the recorded cells to classify the different molecular layer neuronal types and determine the cell-type specific expression and post-injury changes in synaptic and extrasynaptic inhibition. One week after FPI, we identified diametrically opposite changes in tonic and synaptic GABA currents in granule cells and SGCs. We demonstrated that SGCs are unique among excitatory neurons in the in dentate gyrus in developing intrinsic hyperexcitability one week after brain trauma. Mechanistically, we identified that a **cell specific reduction in tonic GABAergic inhibition resulted in increased SGC excitability after brain injury**. Using pharmacological approaches, we showed that GABA receptors containing δ subunits contribute to tonic GABA currents in granule cells from control and post-TBI rats. These studies were published in the Journal of Neuroscience (Gupta et al., 2012-attached).

In parallel, we examined the time course of changes in tonic GABA currents in dentate projection neurons and identified that the early post injury increases in granule cell tonic inhibition do not persist 3 months after injury (Fig. 1). This important finding clarifies conflicting literature in the field. We have also identified that within one week after head trauma, synaptic and extrasynaptic GABA currents are significantly enhanced in a diverse class of MLI (Fig. 2&3). Given the diversity in molecular layer neuronal types (Fig. 2) we decided to restrict our studies to the early post traumatic time point. These studies have been **presented in abstracts at international meetings are part of manuscripts currently under preparation** (see Publications/Presentations).

In Aim 2, we had proposed to **expand on our earlier biophysically realistic dentate network model by incorporating mechanisms for tonic GABA currents and implement molecular layer neurons**. Implementation and expansion of the computational model was conducted in parallel with the physiological studies detailed above and lead to the successful expansion of the network model and implemented extrasynaptic GABA currents. The network model incorporating tonic GABA current mechanisms developed as part of the proposed studies was used to examine how seizure induced plasticity of tonic GABA currents in dentate basket cells alters network excitability. The **dentate network model with tonic GABA currents was published as part of our studies on pilocarpine induced seizures (Yu et al., J. Neurophys 2013-attached)**.

While the Yu et al (2013) study is not focused on experimental TBI, the dentate model used in our studies is widely used by the TBI and epilepsy research community. Thus, the **expansion of the dentate network model to include extrasynaptic inhibitory mechanisms is a significant contribution to theoretical studies in TBI and epilepsy.** Additionally, we have used the tonic GABA current mechanism developed during this study to explore the effect of basket cell tonic GABA currents on network oscillations. In a **computational study, currently under review**, we show that **changes in basket cell tonic GABA currents can increase the frequency of network oscillations and degrade the coherence of gamma frequency rhythms** critical for memory formation (**Proddatur et al-under review, attached**).

Since, as noted above (Fig. 2), MLI constitute a diverse group of neuronal types with different intrinsic physiology we decided to cancel our initial plans to develop biophysically realistic model MLI and chose to focus our attention on the novel excitatory SGCs. We conducted detailed morphological reconstructions of dentate granule cells and SGCs and have **developed detailed morphological models of granule cells and SGCs** (Fig. 4). Our **simulations including identical active and passive properties in the two cell types** has revealed that **cell morphology alone cannot account for the physiological differences between the two dentate projection neurons** (Fig. 4). The results of these simulations are part of a **manuscript currently under preparation describing the role of SGC morphology and location in mediating the unique synaptic physiology and post-traumatic plasticity of SGCs** (**Elgammal et al., abstract to American Epilepsy Society 2013 is attached**).

In experiments relevant to Aim 3, we found that THIP, a selective agonist of tonic GABA currents mediated by delta subunits, **failed to show consistent changes in dentate network excitability and paired pulse inhibition in hippocampal slices from sham and post-FPI rats** (Fig. 5). We reasoned that the differential post-injury changes in tonic GABA currents between granule cells and SGCs and diverse classes of MLI may have contributed to the lack of modulation by THIP. Given the lack of robust response to THIP we decided to abandon our proposed studies examining if modulators of tonic GABA currents modify post-traumatic dentate excitability.

Instead we pursued our **exciting novel finding that excitatory SGCs have unique intrinsic and post-traumatic changes in inhibition.** In addition to intrinsic differences in synaptic inhibition, we have identified that **SGCs have significantly greater excitatory synaptic inputs than granule cells.** In contrast to the differential post-traumatic plasticity of synaptic inhibition, both **granule cells and SGCs show an increase in excitatory synaptic inputs a week after FPI** (Fig. 6). We explored whether differences in cellular morphology may underlie the divergent intrinsic pattern and post-traumatic plasticity of synaptic inputs between SGCs and granule cells. Morphometric analysis of granule cells and SGCs filled during physiological recordings and reconstructed using NeuroLucida revealed a greater dendritic contraction angle in SGCs. Although, the **total dendritic length was not different between the two cell types, SGCs had more numerous first and second order branches and greater dendritic length in lower order branches than granule cells** (Fig. 7). Proximal to the soma, the length of SGC dendrites was greater than that of granule cells. However, granule cells had greater dendritic length than SGCs at locations distal to the soma. These findings have lead us to a testable hypothesis concerning the role of dendritic morphology in distinctive post-traumatic plasticity of granule cells and SGCs. Together with the morphometric simulations, the results of these studies are part of a **manuscript currently under preparation** (**Elgammal et al., abstract to American Epilepsy Society 2013 is attached**).

Unexpectedly, our analysis of SGCs in sham control rats also revealed **distinctive developmental changes in tonic GABA currents in granule cells and SGCs** which we are examining in an independent study. **Our demonstration that granule cells and SGCs express prox1 and arise from the same niche for adult neurogenesis provides unique insights into dentate development** (Gupta et al., 2012-attached).

In addition to the above studies that were part of the funded proposal, establishment of the FPI model system paved the way in our exploration of the **involvement of neuroimmune interactions in post-traumatic dentate hyperexcitability** (**Li et al., manuscript in preparation –attached**) and in examination of how the **kinematics of injury influence dentate pathology** (**Neuberger et al., submitted – attached**).

3. PROJECT CHALLENGES

The first year of the grant period was marked by several issues that lead to considerable delays in establishing the model system. We faced **major delays in purchase and delivery of the rodent fluid**

percussion injury device due to delays at the sole source manufacturing company. Additionally, **device installation and calibration was delayed** due to a defective the pressure calibration system modified shape of the transducer nozzle which required repairs by the manufacture. Finally, the shape of the transducer tip was not a tight fit with the luer-loc cap resulting in loss of pressure requiring modification of surgical procedures. We also faced an early **delay in recruitment of research staff in year 1**.

On commencement of the study, **unanticipated research finding including identification of distinctive neuronal population in MLI necessitated setup of new experimental protocols**. We had to established cell filling and immunohistochemical protocols to identify filled cells using the morphology. While establishing the combined physiological and immunohistological analysis to characterize of the neuronal types did delay the experimental analysis of post-traumatic inhibitory changes in molecular layer neurons, **the additional analysis yielded valuable insights into the cell specific changes in post-traumatic plasticity and were instrumental in our novel findings on SGCs**.

The diversity of MLI morphology and physiology and non-uniform changes in inhibition among different MLI proved to be complex to examine and simulate. This resulted in cancellation of our initial plans to develop biophysically realistic model MLI and lead to a shift in focus on the novel excitatory SGCs.

Since, modulators of dentate neuronal tonic GABA currents **failed to show consistent changes in dentate network excitability in vitro** (Fig 5), we abandoned planned in vivo experiments. Instead, we focused on the novel findings relating SGC morphology to physiology.

While logistical challenges lead to an early delay, we were able to ramp up our experiments and demonstrate considerable productivity. The **scientific challenges, served to move the study in new and exciting directions** that will greatly benefit TBI and hippocampal studies. Our publication on post-traumatic plasticity of SGCs (Gupta et al., 2012) is the first demonstration of the role of this novel cell type in neurological disease.

4. Implications for future research and/or clinical treatment

Our studies have characterized the role for a novel neuronal type in pathology following TBI which is relevant to epilepsy and related neurological disorders. **Gupta et al., 2012 has been cited by 10 publications and highlighted on Psychologyprogress.com as a key paper relevant to neuropsychiatric disorders**. Our demonstration SGCs show unique developmental changes tonic inhibition with a peak expression in adolescence is exciting, since tonic GABA currents are modulated by alcohol and neuroactive drugs. This finding has profound implication for hippocampal function in adolescence. Our expansion of the original dentate network model (cited in over 100 studies) performed during this study is likely to serve as an important tool for future computational analysis of the role of dentate inhibition in TBI and neurological disease. With respect to clinical treatment, there has been an increasing push to develop drugs targeting tonic GABA currents to reduce acquired epilepsy. Our findings demonstrate that there are cell-specific and temporal changes in tonic inhibition. Thus use of modulators of tonic GABA currents is likely to lead to complex outcome depending on age and pathology.

5. Plans to continue the research, including applications submitted to other sources for ongoing support

Our studies have shown that there is alteration in extrasynaptic inhibition in the dentate gyrus, and have identified the hitherto unknown diversity in the interneuronal population in the molecular layer. We plan to focus on the diversity in the molecular layer interneurons and differential inhibition of SGCs and their response to injury. Since this region which is crucial for feed-forward inhibition and SGCs have been proposed to play a critical role in working memory formation, the data from this project provides the invaluable preliminary data for federal funding to examine the circuit changes following brain injury that could underlie memory and cognitive impairments. An NIH R01 application examining interneuronal and SGC plasticity after concussive brain injury is currently under preparation. We are also in the process of submitting a revised 3 year individual research grant examining mechanisms of neuroimmune plasticity of dentate physiology to NJCBIR.

An **R01 application to NIH (R01 NS069861), examining dentate perisomatic interneuronal inhibition in acquired epilepsy has been funded for a period of 5 years**. Additionally, CURE Foundation, Kirby Foundation and NJCBIR have funded ongoing studies on post-traumatic epilepsy as detailed below.

6. Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations.

Extramurally funded TBI projects in obtained as a result of successful leverage of NJCBIR funding:

- A. **Cure Foundation** 2011 “Prevention of Acquired Epilepsy” Award (Santhakumar PI:259051): Modulation of toll-like receptors to decrease post-traumatic epileptogenicity. 3 year award of 250,000, June 2011 to May 2014
- B. **NJCBIR** 2011 Multi Investigator Grant (Santhakumar Co-PI: CBIR11PJT003): Effect of mild, high rate and repetitive brain injury on hippocampal circuits. 3 year award of 450,000, June 2011 to May 2014
- C. **Kirby Foundation** Award for examining synaptic plasticity in acquired epilepsy. 1 year award of 37,500, June 2013 to May 2014
- D. **NJCBIR** 2011 Postdoctoral research grant (Santhakumar Mentor: 11-3223-BIR-E-O). Role of Semilunar Granule Cells in Post-traumatic Hyperexcitability. 3 year award of 70,000, June 2011 to May 2014

7. List and include a copy of all publications emerging from this research, including those used in preparation.

1. Elgammal FS, Gupta A, Proddutur A, Chika-Nwosuh O, Swietek B, Santhakumar V (*in preparation-AES 2013 abstract attached*) Structural differences between granule cells and semilunar granule cells: role in differential post-traumatic plasticity of synaptic inputs.
2. Gupta A, Elgammal F, Proddutur A, Santhakumar V. (*in preparation- SFN 2012 abstract attached*) Early changes in synaptic inputs to dentate molecular layer neurons following concussive brain injury.
3. Li Y, Korgaonkar A, Wang J, Townes-Anderson E, Elkabes S, Santhakumar V (*in preparation-attached*) Toll-like receptor 4 signaling contributes to early increase in dentate excitability after concussive brain injury by NMDA receptor independent mechanisms
4. Proddutur A, Yu J, Elgammal FS, Santhakumar V. (*submitted-attached*) Seizure-induced plasticity of fast-spiking basket cell GABA currents modulates frequency and coherence of gamma oscillation in network simulations.
5. Neuberger EJ, Abdul-Wahab R, Jayakumar A, Elgammal FS, Pfister BJ, Santhakumar V. (*submitted-attached*) Distinct effect of impact rate on immediate and early neuropathology after brain injury.
6. Yu J, Proddutur A, Elgammal FS, Ito T, Santhakumar V. (2013) Depolarizing shift in GABA reversal limits network effects of enhanced basket cell tonic GABA currents after status epilepticus. Journal of Neurophysiology. 109(7):1746-63
7. Gupta A, Elgammal FS, Proddutur A, Shah S, Santhakumar V (2012) Decrease in Tonic Inhibition Contributes to Increase in Dentate Semilunar Granule Cell Excitability after Brain Injury. The Journal of Neuroscience 32(7): 2523-2537.
8. Abdul-Wahab R, Swietek B, Mina S, Sampath S, Santhakumar V, Pfister BJ (2011) Precisely controllable traumatic brain injury devices for rodent models. Bioengineering Conference (NEBEC), 2011 IEEE 37th Annual Northeast, 1-2

Awards and Conference Presentations:

1. 2012 **Outstanding achievement award** for Dr. Gupta’s poster at 2012UMDNJ Research Symposium on Advances in ChildHealth
2. 2012 Gupta et al poster **voted the best presentation** at the 2012 Annual Post-doc Appreciation Day Symposium
3. 2013 Invited presentation at the Spring Hippocampal Research Conference, Taormina, Sicily
4. 2013 Digital Reconstruction of Neuronal Morphology: Recognizing the Breakthroughs. George Mason University, Krasnow Institute, Fairfax, VA

Meeting Abstracts

1. Elgammal FS, Gupta A, Proddutur A, Chika-Nwosuh O, Swietek B, Santhakumar V. Structural differences between granule cells and semilunar granule cells: role in differential post-traumatic plasticity of synaptic inputs. (American Epilepsy Society), 2013
2. Li Y, Korgaonkar A, Wang J, Townes-Anderson E, Elkabes, S, Santhakumar V, Enhanced Tonic GABA currents in Dentate Fast-Spiking Basket Cells after Status Epilepticus. (American Epilepsy Society), 2012
3. Neuberger EJ, Abdul-Wahab R, Jayakumar A, Elgammal FS, Pfister BJ, Santhakumar V. Similar enhancement in dentate network excitability despite marked differences in early cellular injury following fast- and standard rate concussive brain trauma. (Society for Neuroscience), 2012
4. Proddutur A, Yu J, Santhakumar V, Network Effects of Seizure-Induced Changes in Dentate Fast-Spiking Basket Cell Inhibition: A computational study. (Society for Neuroscience), 2012
5. Gupta A, Elgammal F, Proddutur A, Santhakumar V. Early Changes in Synaptic Inputs to Dentate Molecular Layer Neurons Following Concussive Brain Injury. (Society for Neuroscience), 2012
6. Korgaonkar A, Li Y, Wang J, Townes-Anderson E, Elkabes, S, Santhakumar V, Early enhancement of toll-like receptor expression in the hippocampus following concussive brain injury: Innate immune response to neurotrauma. (Society for Neuroscience), 2012
7. Santhakumar V, Gupta A, Proddutur A, Elgammal F. POST-TRAUMATIC INHIBITORY PLASTICITY AUGMENTS EXCITABILITY OF ATYPICAL DENTATE GUTAMATERGIC PROJECTION NEURONS. (National Neurotrauma Society), 2012
8. Gupta A, Proddutur A, Elgammal F, Ito T, Santhakumar V. Decreased Tonic GABAergic Inhibition and Enhanced Excitability of Dentate Semilunar Granule Cells after Traumatic Brain Injury (American Epilepsy Society), 2011
9. Gupta A, Proddutur A, Elgammal F, Ito T, Santhakumar V. Tonic GABA currents in dentate fast-spiking basket cells are enhanced following status epilepticus. (Society for Neuroscience), 2011

8. Financial summary.

The allocated funds have been fully spent over the last 4 years. A detailed financial report will be directly communicated by the grants office.

Briefly, during the 4 year period, granted funds of \$450,000 have been spent in accordance with the original budget on the following major categories as needed for the research progress: ~ \$218,000 has been spent on research personnel salary including fringes for a post-doctoral fellow and research assistants and PI salary (~\$25,000). \$17,000 was spent on purchase of major equipment needed for TBI research and the equipment are currently located and in use in the PI's lab. Funds amounting to ~ \$42,000 were used to purchase and house animals needed for the studies and \$124,000 was used to obtain experimental supplies. A total of \$6000 was used to travel to scientific meetings and present the research findings at the Society for Neuroscience and Neurotrauma meetings by the PI and research personnel. \$2000 was spent on miscellaneous expenses including poster printing, journal submission and publication costs and transportation and repair of major equipment. In addition to the above direct costs, the indirect cost was \$39,000.

Figures

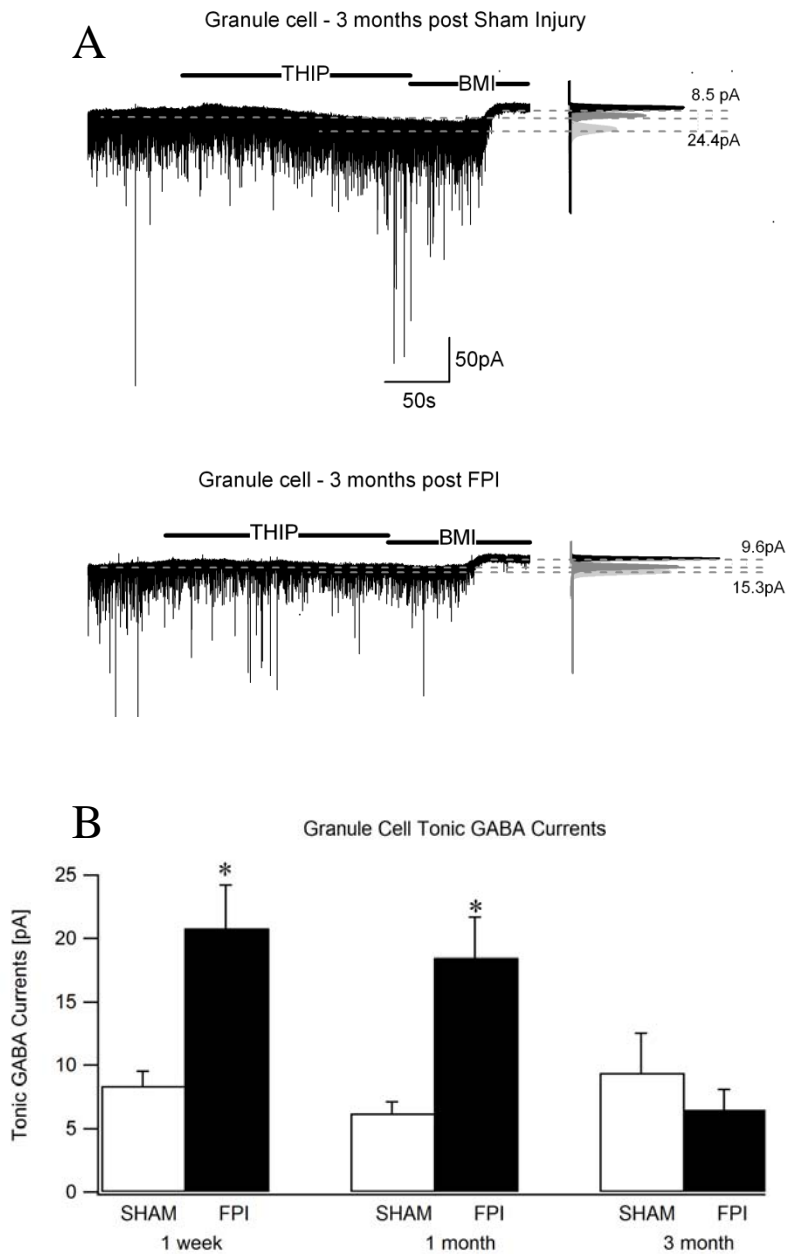


Figure 1: Recovery of early post-injury increase in granule cell tonic GABA currents within 3 months after FPI.

A. Representative traces from a dentate granule cell 3 months after sham injury (above) and fluid percussion injury (below) shows the tonic GABA currents in control solutions and after addition of THIP (1 μ M). Tonic GABA currents were measured as the baseline currents blocked by a saturating concentration of BMI (100 μ M). Panels to the right show all-points histograms derived from 30s recording periods. The dashed lines indicate Gaussian means and difference currents are noted. **B.** Summary histogram of tonic GABA currents in dentate granule cells (measured in the presence of 3 mM kynurenic acid) from sham-injured and FPI rats in control conditions 1 week, 1 month and 3 months after FPI.

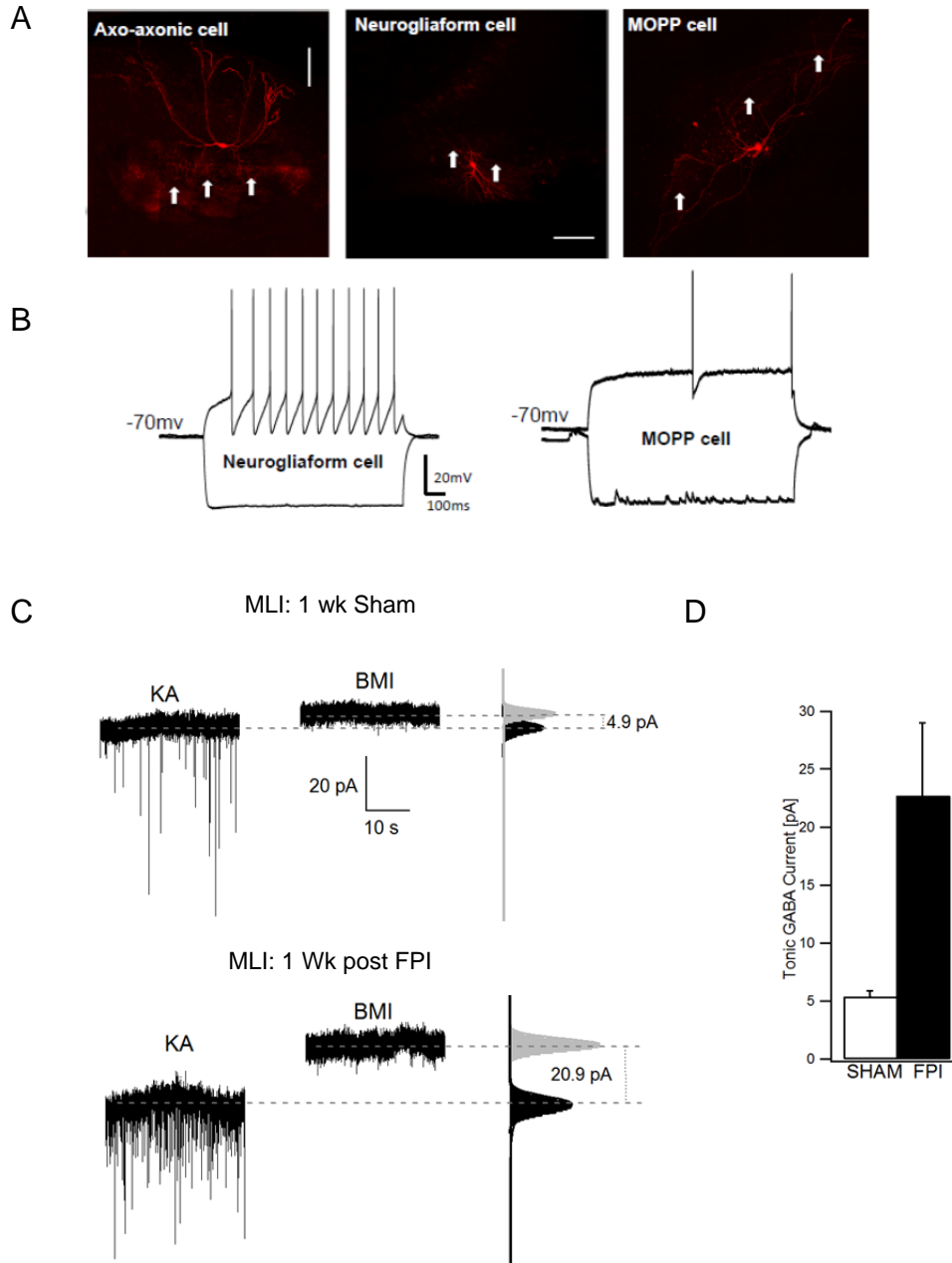


Figure 2: Extrasynaptic GABA currents in molecular layer interneurons (MLI).

A. Photomicrographs of distinctive types of MLI filled with biocytin during physiological recordings. Examples of an Axo-axonic cell (left), Neurogliaform cell (middle panel) and MOPP cell (right) are shown. Arrows indicate location of axon collaterals. **B.** Sample voltage traces show the distinctive firing pattern of a morphologically identified neurogliaform cell (right) and MOPP cell (left). **C.** Representative current traces from MLI one week after sham injury (above) and fluid percussion injury (below) shows the tonic GABA currents in 3mM kynurenic acid (KA) blocked by a saturating concentration of BMI (100 μ M). Panels to the right show all-points histograms derived from 30s recording periods. The dashed lines indicate Gaussian means and difference currents are noted. **D.** Summary histogram of tonic GABA currents in MLI cells.

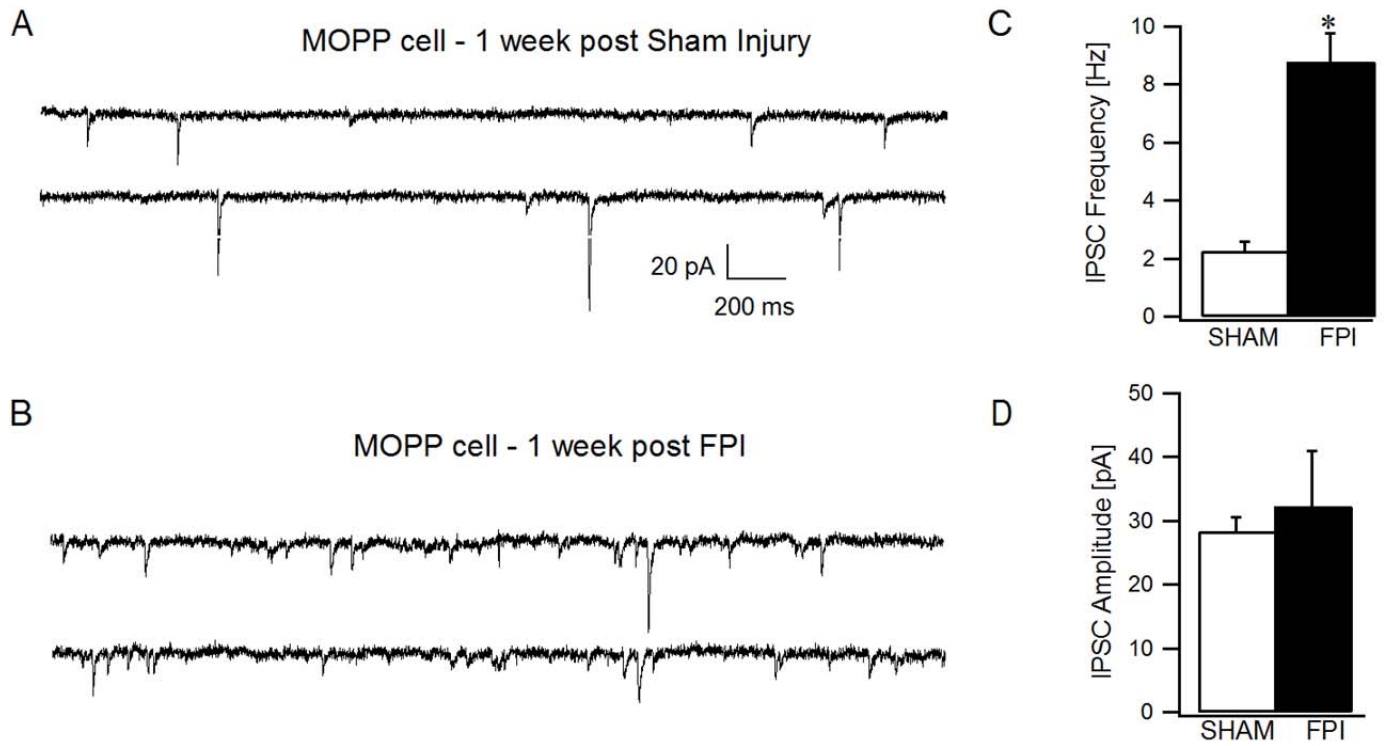


Figure 3: Post-injury increase in synaptic inhibition among Molecular layer interneurons

A. Representative current traces showing IPSCs in a MOPP cell from a sham injured animal. **B.** Representative trace shows IPSC in a MOPP cell from a head-injured animal. **C.** Summary plot shows the post-injury increase in IPSC frequency 1 week after brain injury. **D.** Summary histogram shows that the IPSC amplitude is not altered after injury. Recordings were obtained in the glutamate receptor antagonist kynurenic acid (3mM).

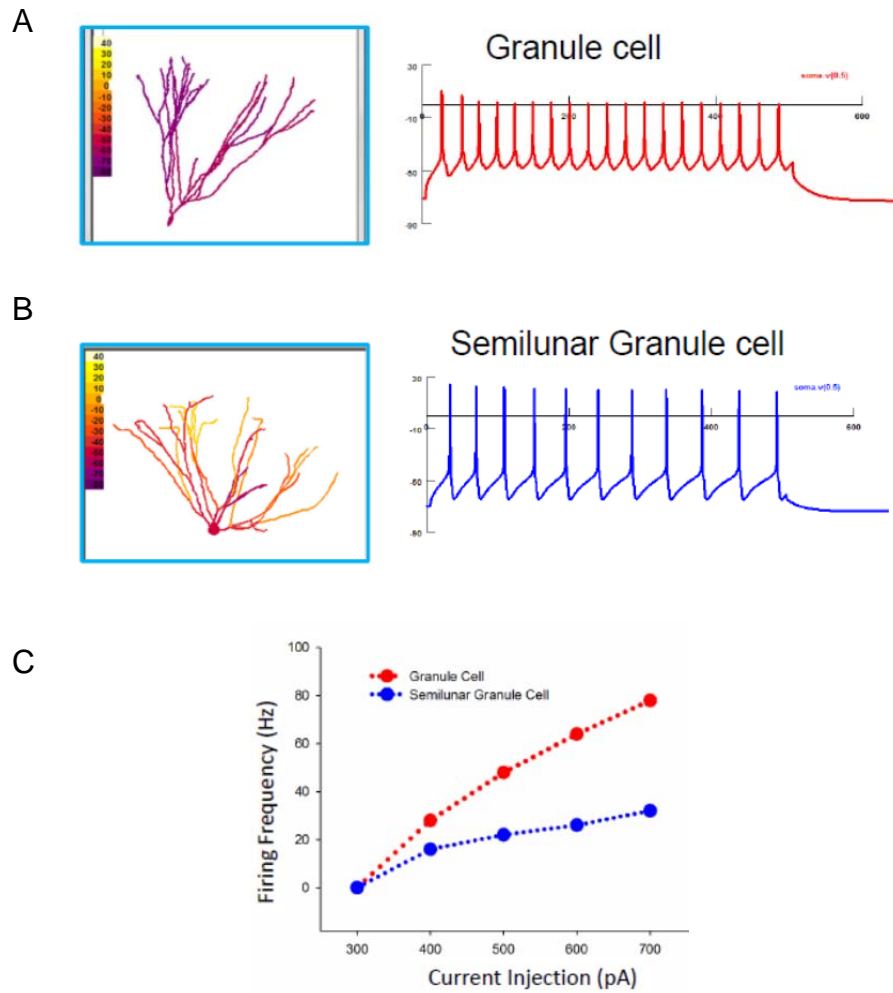


Figure 4: Structural differences between granule cells and SGCs do not fully explain the differences in intrinsic physiology

A. NEURON simulation of a morphologically realistic granule cell. Pseudo-color voltage plot is shown on the left and the membrane voltage in response to current injection on the right. **B.** NEURON simulation of a morphologically realistic SGC. Pseudo-color voltage plot is shown on the left and the membrane voltage in response to current injection on the right. **C.** Summary current/frequency plot shows that the simulated SGC has lower firing in response to the same current injection than the model granule cell even when all channel conductances are maintained identical. This contrasts with the higher firing in SGCs observed in biological recordings.

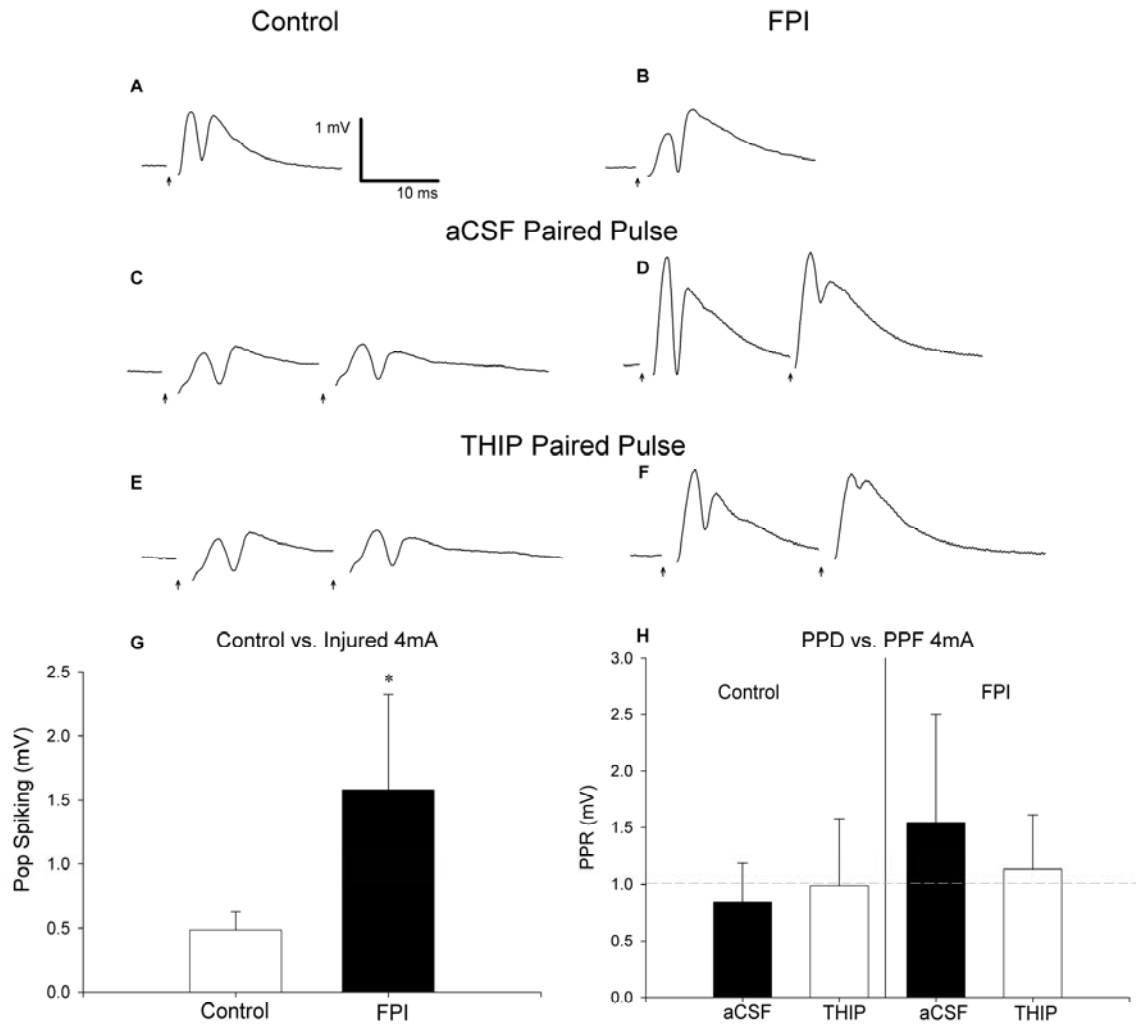


Figure 5: Effect of enhancing tonic GABA currents on in vitro excitability of the dentate gyrus.

Averages of representative granule cell field responses to single afferent activation from sham operated control (A) and FPI animals (B) 1 week after injury illustrate the enhanced excitability in aCSF. Average responses to paired pulse afferent activation (20 ms inter-stimulus interval) show paired pulse depression, indicating a functional feedback inhibitory circuit function in sham-injured (C) and FPI (D) animals. Average response to paired pulse activation of the afferent pathway in the presence of the GABA_AR δ subunit agonist THIP (1 μ M) in sham-injured (E) and FPI (F) animals. The arrow indicates location of stimulus artifact which was removed for clarity. Stimulation intensity was 4mA. G. Summary plot shows the post injury increase in evoked population spike amplitude. H. Summary histogram shows that THIP decreases paired pulse depression in controls and decreases the trend towards paired pulse facilitation after brain injury. PPR: paired pulse ratio = ratio of amplitude of second pop spike to first. Stimuli for paired pulse were delivered at 20 ms inter-stimulus interval.

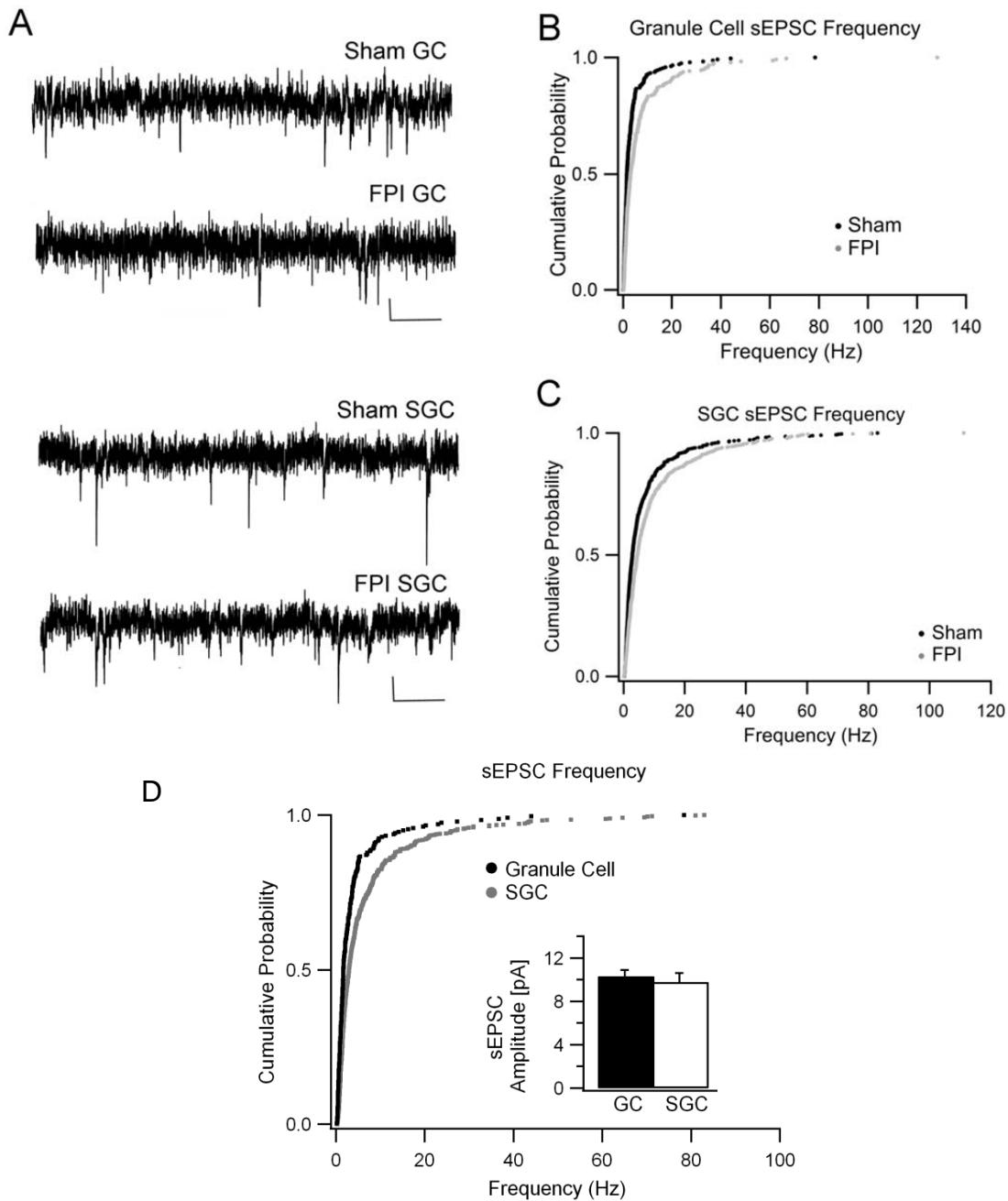


Figure 6: Post-injury increase in synaptic excitation in granule cells and SGCs

A. Representative current traces showing sEPSCs in granule cells (GC) and SGCs from sham and head-injured rats one week after FPI. Scale bar 5 pA and 200 ms. **B-C.** Cumulative probability plots show the post-injury increase in sEPSC frequency in both granule cells (B) and SGCs (C) 1 week after brain injury. **D.** Cumulative probability plot of sEPSC frequency in granule cells and SGCs from sham rats shows the greater sEPSC frequency in SGCs. Inset: summary histogram of sEPSC amplitude in granule cells and SGCs from sham rats.

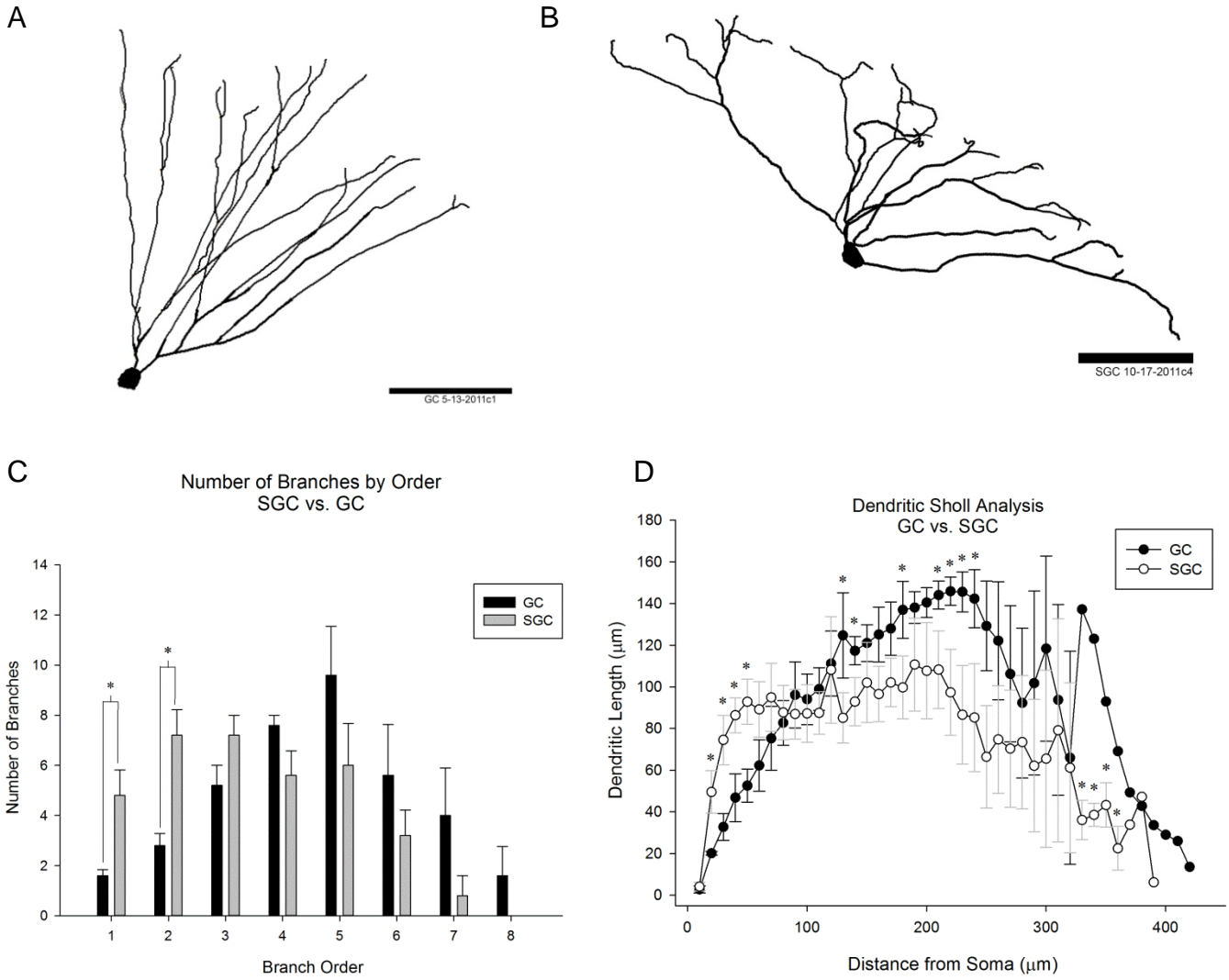


Figure 7: Quantitative morphometrics of granule cells and SGCs

A-B. Representative neurolucida reconstruction of a granule cell (A) and SGC (B) from a 4 week old sham-injured rat. Scale bar 5 pA and 200 ms. **C.** Summary histogram illustrates the difference in dendritic branch order. **D.** Plot compares the dendritic Sholl analysis between granule cells and SGCs. * indicates $p < 0.05$, $n = 5$ granule cells and 5 SGCs.