NJCBIR FINAL PROGRES REPORT
COVER PAGE

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

Grant Title: Role of Semilunar Granule Cells in Post-traumatic hyperexcitability

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1. ORIGINAL AIMS OF THE PROJECT

The goal of the study was to determine whether if head trauma modifies the intrinsic and synaptic properties of dentate semilunar granule cells (SGCs) and increases connectivity between SGCs and granule cells. The hypothesis is that injury-induced changes in the structure and function of SGCs are a critical mechanism underlying dentate dysfunction after brain trauma. 3 Aims were defined. Specific Aim 1: Test the hypothesis that the intrinsic and synaptic physiology of SGCs is altered after brain injury. Specific Aim 2: Test the hypothesis that brain injury results in morphological changes in SGCs. Specific Aim 3: Determine the contribution of SGCs to the early and long-term network dysfunction after brain injury.

2. PROJECT SUCCESS

The **studies proposed in Aim 1 were completed successfully and the results have been published (Gupta et al., 2012).** To summarize, we identified post-traumatic increase in excitability of semilular granule cells in dentate gyrus within one week after injury. As demonstrated in the paper, the input resistance of FPI SGCs was greater than in sham-injured SGCs. Firing frequency of FPI SGCs was significantly greater than that of sham-injured SGCs. The frequency of spontaneous IPSC as well as miniature IPSC was significantly decreased in semilular granule cells after FPI.

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 1, Fig 2).

As we proposed in Aim 2, 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 (Fig 3). Although, the **total dendritic length was not different between the two cell types (Fig. 4C), SGCs had more numerous first and second order branches and greater dendritic length in lower order branches than granule cells** (Fig. 4). SGCs show greater attenuation of proximal dendritic EPSP compared to GC (Fig. 5). SGC have greater axonal length, and greater higher order axons (Fig. 6). The results of these simulations are part of an abstract to annual meeting of American Epilepsy Society **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)** a manuscript including the structural and computational modeling data is under preparation.

Apart from the directly proposed studies, we have also examined and identified an **age dependant decrease in SGC tonic and synaptic GABAergic inhibition.** Recordings from SGCs 3 months post sham (4 month old rats) revealed a significant decrease in tonic GABA currents compared to SGCs from 1 month old rats (Fig. 7B,C; in pA, SGC 3months post sham: 4.5±3.1, n=5, SGC 1 week post sham: 16.65 ± 1.81, n=8; p < 0.05, t-test) whereas granule cells did not demonstrate a similar developmental change in tonic GABA currents (Fig. 7A,C; in pA, GC 3months post sham: 6.94±2.25, n=5, GC 1 week post sham: 8.35 ± 1.11, n=11; p > 0.05, t-test). Not only are SGCs from adolescent (1 month old) rats unique in having higher tonic GABA currents than SGCs form 4 month old rats, they also showed developmental decrease in sIPSC frequency. Moreover, since our attempts to identify a molecular marker and specifically tag SGCs was unsuccessful (see project challenges), we extended the study to include examination of molecular layer interneurons to identify the role of somato-dendric location in shaping network connectivity and post-traumatic plasticity. In morphologically identified molecular layer interneurons (MLI), application of a saturating concentration of the $GABA_AR$ antagonist BMI (100 µM) failed to decrease the holding current emphasizing that the changes in SGCs are unique among MLI. The magnitude of tonic GABA currents in molecular layer interneurons was not reduced after FPI (Fig. 8A, B). We also did not find any change in the intrinsic properties of MLIs including input resistance (in MΩ, sham injured MLI: 204.6 ± 24.27, n = 7 cells; FPI MLI: 172.6 ± 41.9, n = 4 cells, p > 0.05, t test), firing frequency and sag ratio (sham injured MLI, n = 7 cells; FPI MLI: n = 4 cells, p > 0.05, t test) after head injury. One week after FPI, the

frequency of sIPSC in MLIs was increased compared to that in sham injured MLIs (sham-injured MLI n=4 cells and FPI MLI n =3, p < 0.05, K–S test). A manuscript including the MLI studies is under preparation.

In addition to the above studies the research associate Dr. Li who started on the project after Dr. Gupta, has been examining the **involvement of <u>neuroimmune interactions in post-traumatic dentate hyperexcitability (Li et al., manuscript under review</u> –attached**).

3. PROJECT CHALLENGES
As mentioned above, our attempts to identify a cell-specific marker to distinguish SGCs from granule cells was not fruitful. Despite establishing single cell PCR in addition to immunolabeling, our examination did not identify neurochemical differences between granule cells and SGCs. Additionally, all attempts to selectively target SGCs via retrograde tracer techniques were unsuccessful. Since selective labeling of SGCs was a prerequisite to conducting the studies in aim 3, we were unable to undertake the experiments proposed in aim 3. Instead, we focused further on examining how morphological differences modify the integrative properties of SGCs using computational techniques (Fig. 5) and on physiological differences between MLI and SGC response to brain injury.

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 22 publications and highlighted on <u>Psychologyprogress.com</u> 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. With respect to clinical treatment, <u>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.</u>

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 (*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. (*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 (*submitted to Annual Neurology attached*) Toll-like receptor 4 signaling contributes to early increase in dentate excitability after concussive brain injury by NMDA receptor independent mechanisms
4. 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. <u>The Journal of Neuroscience</u> 32(7): 2523-2537.

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, Swietek B, Santhakumar V. Structural differences between granule cells and semilunar granule cells (Society for Neuroscience), 2014
2. Korgaonkar A, Li Y, Santhakumar V, Differential toll-like receptor 4 modulation of dentate excitability in the normal and injured brain (American Epilepsy Society), 2014
3. 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
4. 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
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 INHIBITRORY 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 3 years. A detailed financial report will be directly communicated by the grants office.
Briefly, during the 4 year period, granted funds of $209,808 have been spent in accordance with the original budget on the following major categories as needed for the research progress: ~ $148,716 has been spent on research personnel salary including fringes. Funds amounting to ~ $3,301 were used to purchase and house animals needed for the studies and $17,960 was used to obtain experimental supplies. A total of $4,868 was use to travel to scientific meetings and present the research findings at scientific meetings by the PI(s). In addition to the above direct costs, the indirect cost was $34,967.
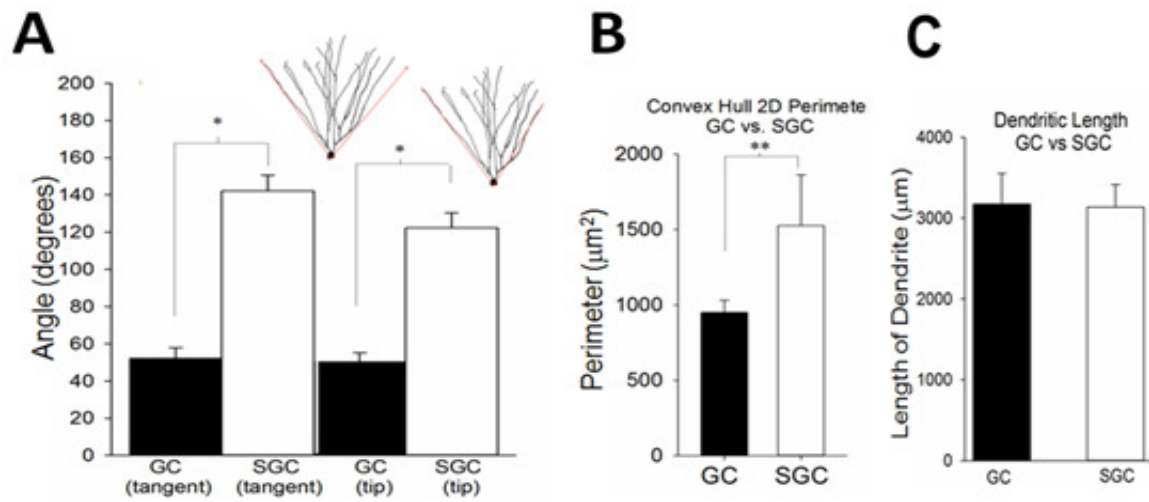
Figures
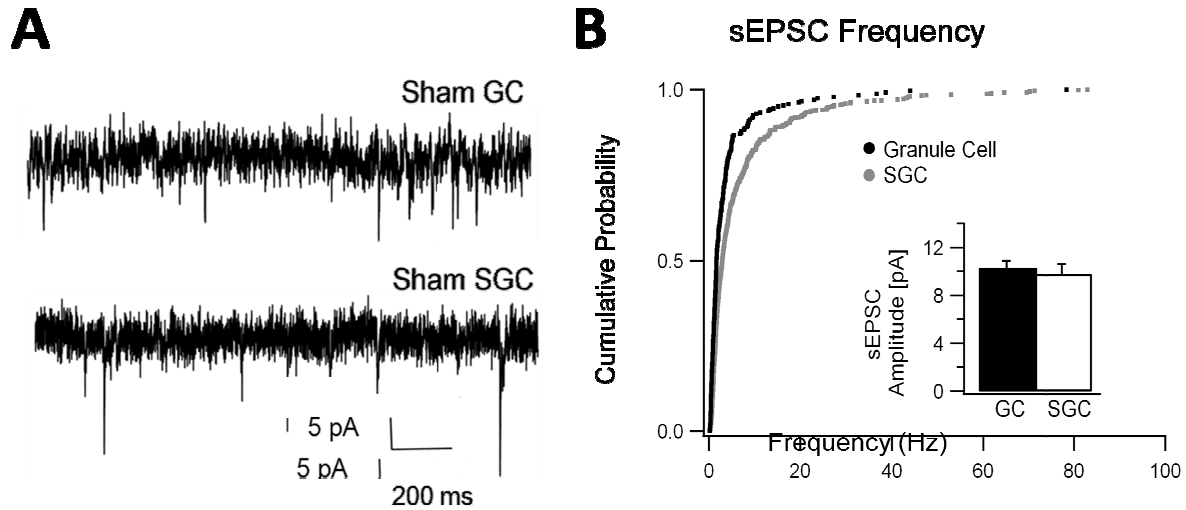
FIGURE 1: SGCs receive more excitatory synaptic inputs than GCs. A. Representative current traces show sEPSCs in granule cells and SGCs. B. Cumulative probability plot demonstrates the higher frequency of sEPSCs in SGCs. *Inset:* Summary of sEPSC amplitude in GCs and SGCs.
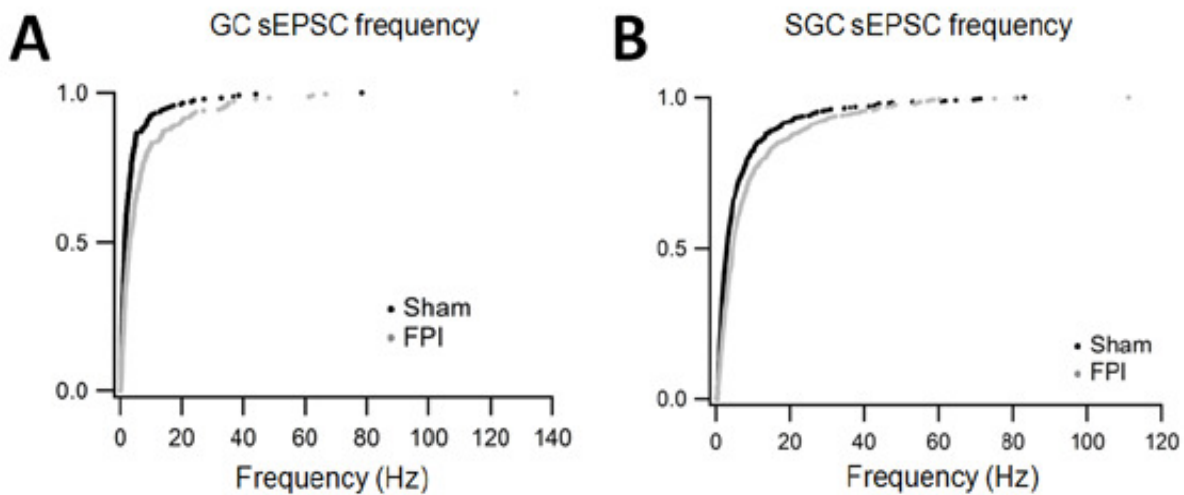

FIGURE 2: Both SGCs and GCs show increase in sEPSC frequency after FPI. A-B. Cumulative probability plots show an increase in sEPSC frequency in both GCs (A) and SGCs (B)

FIGURE 3: SGCs and GCs differ in dendritic contraction angle and 2D perimeter

A-C. Summary plots of dendritic morphometric analysis compare the dendritic contraction angle (A) 2D perimeter (B) and total dendritic length (C) of GCs and SGCs (n=6 GCs and n=9 SGCs, *indicates p<0.05, t-test).
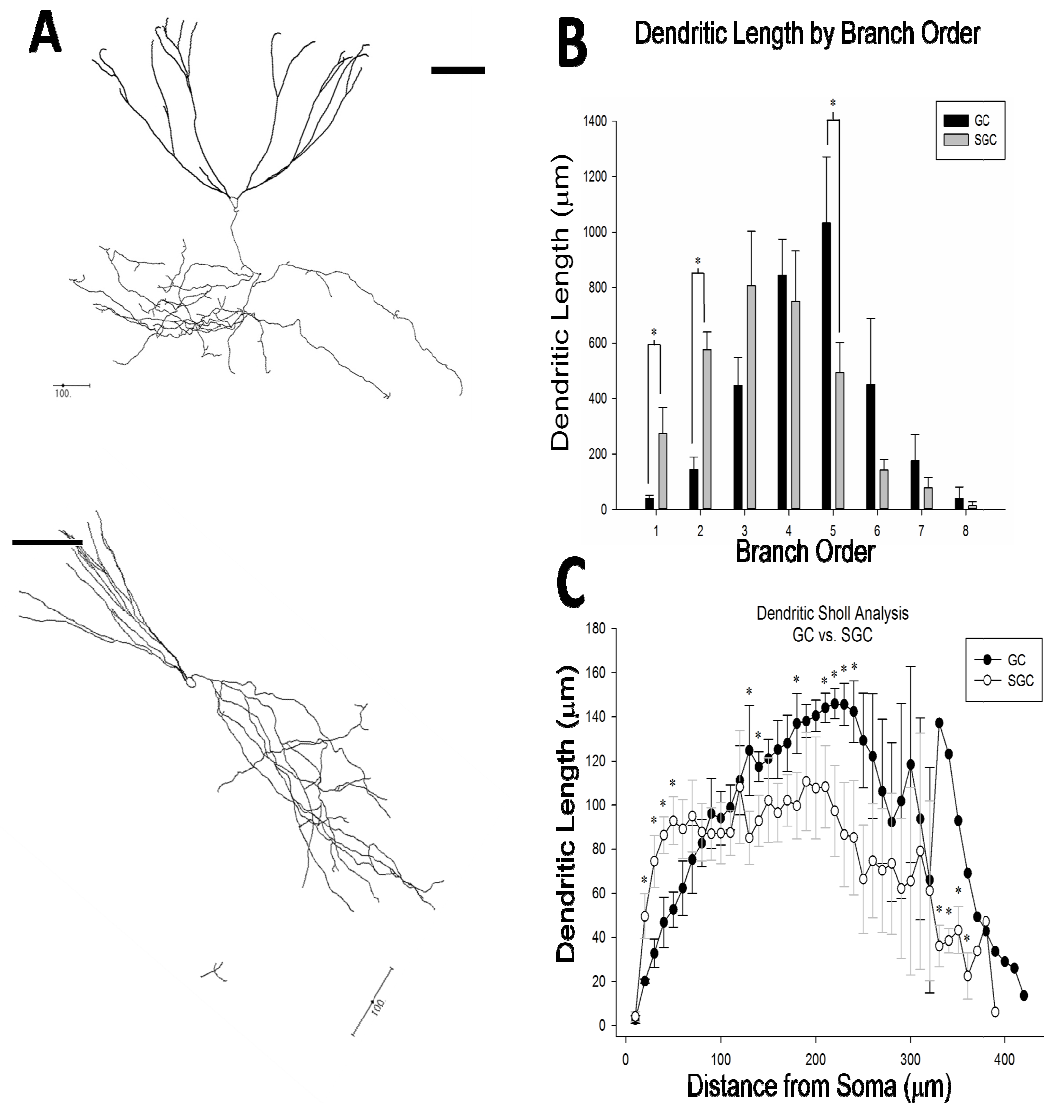
FIGURE 4: SGCs have more low-order branches and greater dendritic length in proximal branches. A. Representative reconstructions of a GC (bottom) and a SGC (top). Scale 100 mm (thick lines: dendrites). B. Summary histogram of average dendritic length by branch order in each cell type (n=6 GCs and n=8 SGCs, *p<0.05, two-way RM ANOVA). C. Summary of dendritic Sholl analysis (n=5 GCs and SGCs each, *p<0.05, t-test).
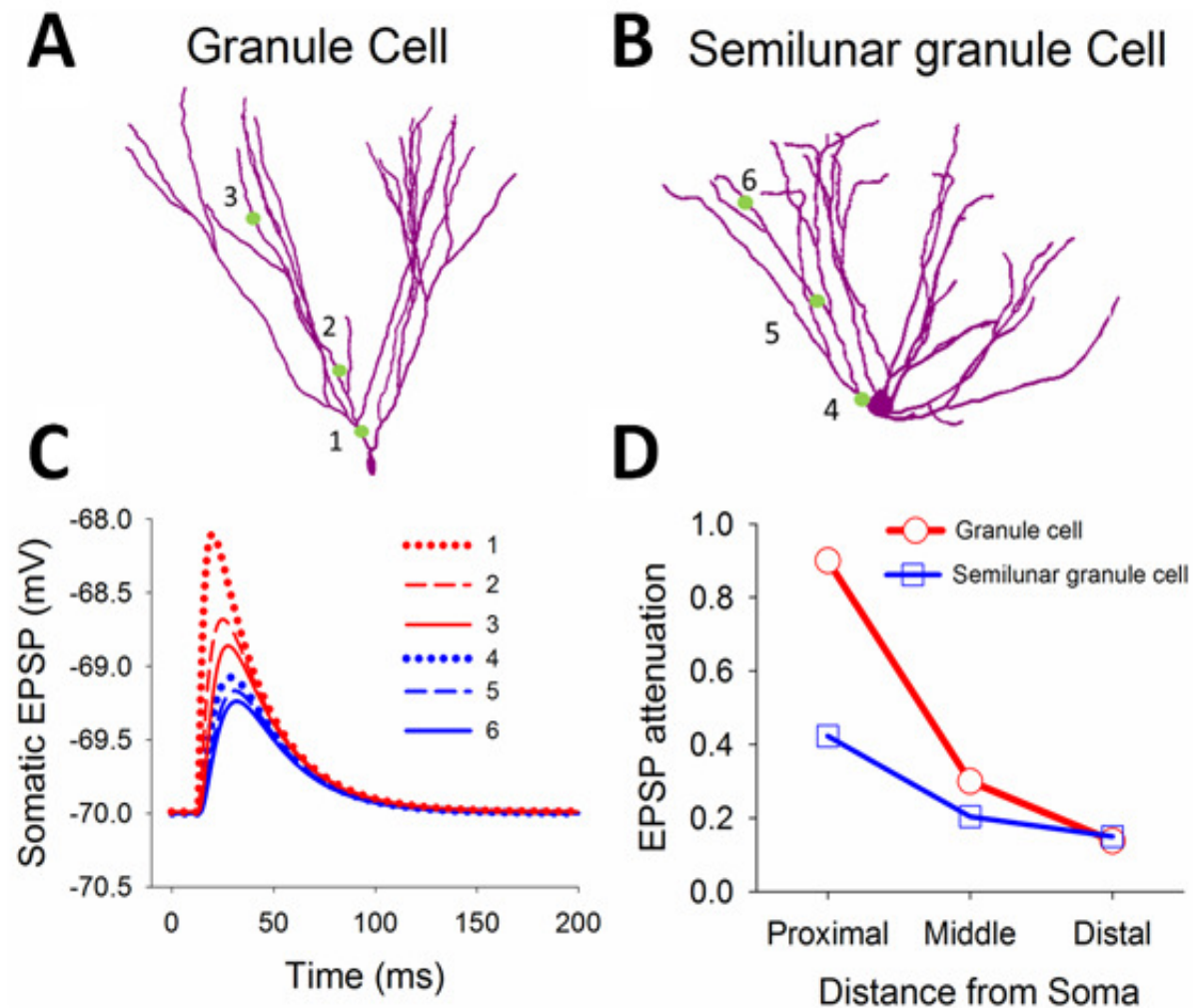
FIGURE 5: SGCs show greater attenuation of proximal dendritic EPSP compared to GC.

A-B. Morphologically reconstructed granule cell (A) and semilunar granule cell (B) used in simulations. Green dots indicating location of synaptic input resulting in a 2mV local EPSP. C. Somatic membrane voltage traces show EPSP in response to synaptic inputs at dendritic locations (1, 2, 3) in GC (in red) and (4, 5, 6) in SGC (in blue). D. Summary plot show EPSP attenuation (ratio of somatic EPSP amplitude/dendritic EPSP amplitude). Note the greater attenuation of proximal dendritic EPSP in the SGC compared to GC.

FIGURE 6: SGCs have greater axonal length, and greater higher order axons.

A. Representative reconstructions of a GC (left) and an SGC (right). Scale 100 mm. **B.** Summary of average axonal length (n=4 GCs and n=7 SGCs, *p<0.05, t-test). **C.** Summary of number of axonal branches by branch order (n=3 GCs and n=7 SGCs, *p<0.05, two-way RM ANOVA).
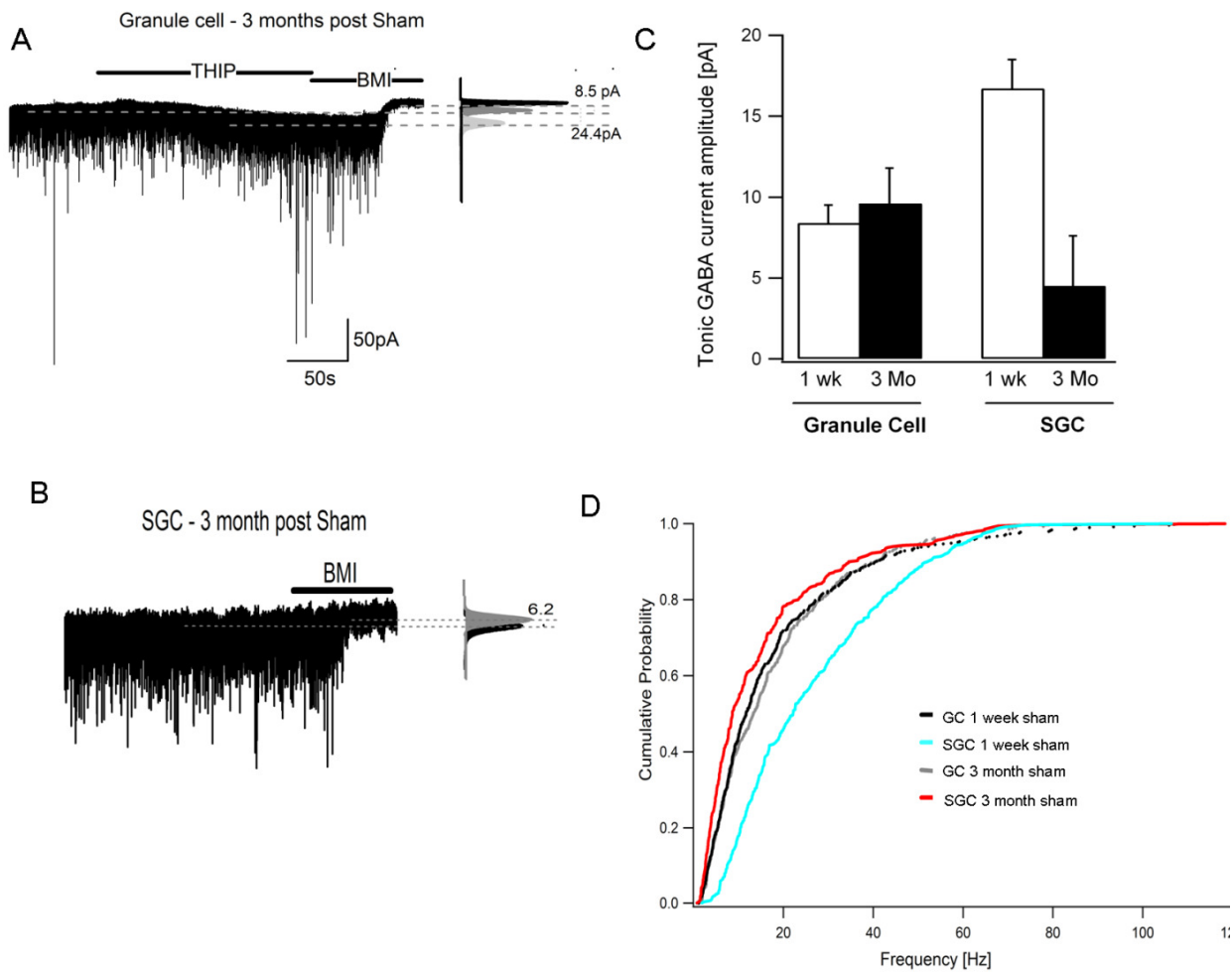
Figure 7. Selective age dependant decrease in SGC tonic and synaptic inhibition.

A-B. Recordings show tonic IGABA in granule cells (A) and SGC (B) from 3 month post sham adult rats. C. Histogram depicting no age related change in tonic current amplitude in granule cells and SGCs at 3 months have significantly lower tonic current than adolescent rats. D. Cumulative probability graph showing lower sIPSC frequency in sham adult SGCs compared to SGCs in sham adolescent rats.
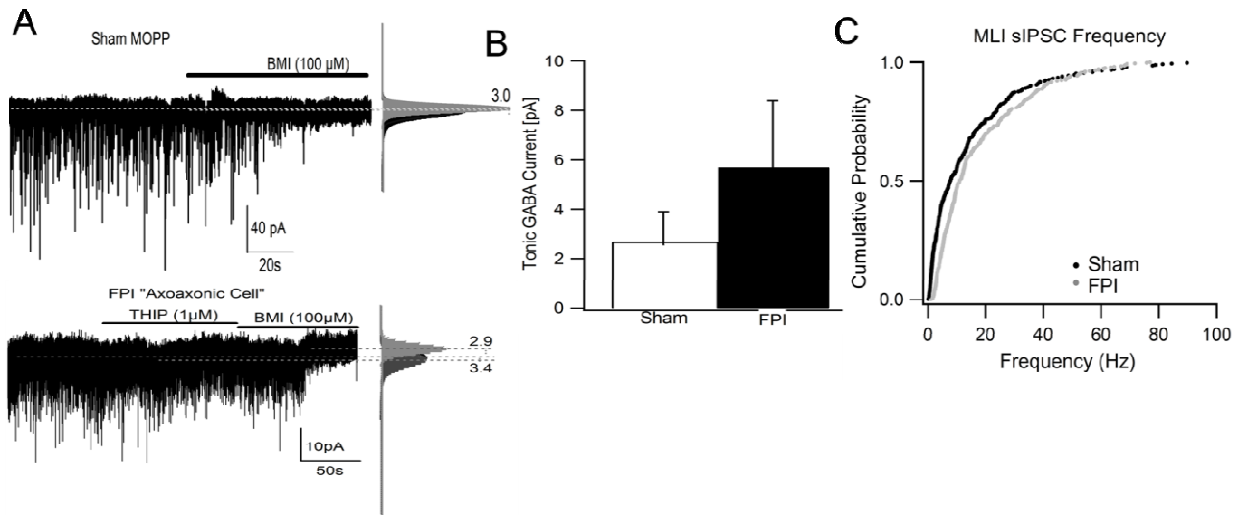
Figure 8. MLI tonic GABA currents are not decreased after FPI.
A. Voltage-clamp recordings from a sham-(above) and FPI-(below) MLI illustrate the magnitude of tonic I_{GABA} blocked by BMI. *B.* Summary plot of MLI tonic I_{GABA} . *C.* Cumulative probability plot shows an increase in sIPSC frequency in MLI after the injury.