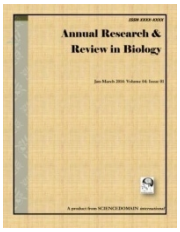

Annual Research & Review in Biology
4(24): 3707-3715, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Occurrence of two Types of Granule Cells with Different Excitability in Rat Dentate Gyrus Granule Cell Layer Following Pilocarpine-Induced Status Epilepticus

Nasrin Mehranfard[1,2*], Hamid Gholamipour-Badie[3], Fereshteh Motamedi[1], Mahyar Janahmadi[1,4] and Nima Naderi[1,5]

[1]Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Electrophysiology Research Center, Neuroscience institute, Tehran University of Medical Sciences, Tehran, Iran.
[3]Department of physiology and pharmacology, Pasteur institute of Iran, Tehran, Iran
[4]Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[5]Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author NM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author HGB wrote the protocol. Authors FM, MJ and NN managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 4th June 2014
Accepted 23rd June 2014
Published 1st July 2014

ABSTRACT

Aims: The evaluation of the neuronal excitability of dentate gyrus granule cells during the development of epilepsy in pilocarpine model of TLE in rats.
Place and Duration of Study: Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, between October 2012 and January 2013.
Methodology: Status epilepticus (SE) was induced by pilocarpine injection (350mg/kg; i.p) to male rats. Twenty minutes before pilocarpine injection, *N*-methyl scopolamine (1mg/kg; s. c) was injected to reduce peripheral effects of pilocarpine and after 3h, diazepam (4

**Corresponding author: Email: mehranfard_n@yahoo.com;*

mg/kg; i. p) was injected to stop seizures. Twenty four hour (acute phase) and 30 days (chronic phase) after inducing SE, the excitability of granule cells was evaluated using whole cell patch clamp recording.
Results: Fifty seven percent of granule cells in the acute phase were hyperexcitable and another 43% of cells were less excitable. In chronic phase, the majority of cells (71%) were hyperexcitable, while 29% of the cells had a low excitability. In both phases of epilepsy, membrane input resistance (Rin) in hyperexcitable cells was similar to that of control group, while Rin was significantly lower in less excitable cells compared to control group.
Conclusion: Results showed the presence of two groups of granule cells in pilocarpine-induced SE in rats: one group of cells with high excitability and another group of cells with less excitability. Although less excitability might, at least partly, protect granule cells from seizure-induced neurotoxicity, but hyperexcitability in majority of granule cells and the occurrence of spontaneous recurrent seizures during chronic phase suggest the existence of insufficient compensatory mechanisms, possibly facilitating the propagation of seizure activity.

Keywords: Dentate gyrus; epilepsy; pilocarpine; excitability; whole cell patch clamp.

1. INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common type of refractory epilepsy in adults [1]. Most features of TLE can be reproduced in animal models of TLE, specially kindling and SE models [2]. Systemic injection of pilocarpine can induces SE in rodents that is followed by three different periods including (i) Acute period (lasts 24 h), (ii) A seizure-free time interval known as latent period (lasts 4-44 days) and (iii) A chronic period which is associated with spontaneous recurrent seizures [3]. Epileptogenesis is a dynamic process during which progressively alters the neuronal excitability before the first spontaneous seizures appear [4].The dentate gyrus has long been a central point for the evaluation of involved mechanisms in epileptogenesis in TLE. There are different reports about the excitability of dentate gyrus granule cells (GCs) in epilepsy. For instance, Young and coworkers in 2009 reported a reduced excitability of GCs in a TLE model with Ammon's horn sclerosis [5]. In another study, Dietrich et al in 1999 reported two electrophysiologically different types in GCs from epileptic human hippocampus [6]. Nonetheless, there is little information regarding the neuronal excitability of dentate gyrus GCs during epileptogenesis process in pilocarpine-treated rats. In the present study, we evaluated the excitability of GCs during the epileptogenesis process in pilocarpine model of TLE using whole cell patch clamp recordings.

2. MATERIALS AND METHODS

2.1 Animals

Adult male wistar rats (150-230g) were used in this study. They were caged in groups of four with free access to food and tap water and under standardized housing conditions with a 12 h light–dark cycle (lights on at 7:00 a.m.), at a temperature of 22±1ºC. All experiments were carried out according to the National Institutes of animal care and use guidelines approved by the Institutional Ethic Committee (IEC) at Shahid Beheshti University of Medical Sciences.

2.2 Animal Treatment

Pilocarpine hydrochloride (350 mg/kg; Sigma-Aldrich, St Louis, USA) was injected intraperitoneally into male rats for induction of SE. Twenty minutes before pilocarpine administration, N-methyl scopolamine (1mg/kg, s.c.) was injected in order to minimize the peripheral cholinergic effects of pilocarpine. Diazepam (4mg/kg, i. p.) was administered after 3 h to stop SE. Only motor seizures of grade 3 or greater on the Racine scale [7] were scored. Animals were then observed for the occurrence of spontaneous behavioural seizures. For the acute seizure group, rats were used 24h after inducing SE. Most rats indicated first spontaneous seizures 15-30 days (average 24 days) after SE. Hence, for the chronic seizure group, we used rats 30 days after pilocarpine-induced SE. A total of 37 and 52 animals were used for the acute and chronic periods, respectively.

2.3 Intracellular Recordings in Hippocampal Slices

2.3.1 Slice preparation

Adult rats were anesthetized with diethyl ether and decapitated. The brains were then quickly removed. Transverse hippocampal slices (350-400µm) were prepared from two groups of animals, acute and chronic seizure groups using a vibrating slicer (752 HA, Campden Instruments Ltd, UK) in a 4ºC ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 206 sucrose, 2.8KCl, 1 CaCl2, 2 MgSO4, 1.25 NaH_2PO4, 26 NaHCO3, 10 D-glucose and equilibrated to a pH of 7.4 oxygenated with 95% O2/5% CO2; the osmolarity of ACSF was adjusted to 295 mOsm. The slices were incubated in ACSF containing (in mM) 124 NaCl, 2.8 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 D-glucose at pH 7.4 and adjusted to 295 mOsm for 1 h at 32ºC and then stored at room temperature before being transferred to the recording chamber.

2.3.2 Patch-clamp recording

The slices were transferred to a submerged recording chamber and were perfused continuously with ACSF at 1–2 ml/min flow rate. Patch-clamp recordings were performed at room temperature. Dentate gyrus GCs were visualized by infrared videoimaging (Hmamatsu, ORSA, Japan) with a 40x water immersion objective. Recordings were made by borosilicate glass electrodes (1.2 mm O.D., 0.95 mm I.D. with inner filament; WPI, USA) which were pulled with a two-stage vertical puller (PC10, Narishige, Japan). The pipettes had a resistance of 3-6 MΩ and filled with an intracellular solution containing (in mM) 140 K-Gluconate, 10 HEPES, 2 MgCl2, 2 Na2-ATP, 1.1 EGTA, 0.1 CaCl2 and 0.4 Na2-GTP. The pH of the internal solution was set to 7.3 by KOH and the solution osmolarity was adjusted to 295 mOsm. Whole-cell patch-clamp recording was made from dentate gyrus GCs using Multiclamp 700B amplifier (Axon Instruments, Foster City, CA) equipped with Digidata 1320 A/D converter (Axon Instruments, Foster City, CA). Current signals were low-pass filtered at 3–5 kHz and sampled at 10 kHz and stored on a personal computer for offline analysis. The test seal function was frequently monitored throughout the recording to ensure that the seal was stable. Recordings were only obtained when seals of more than 1GΩ resistance were established. Recordings were accepted only if access resistance was less than 20 MΩ, and it did not change by 20% during the experiment. To investigate the neuronal excitability of GCs in current clamp mode, trains of action potentials (APs) were elicited by 1000 ms depolarizing current injections ranging 50-250pA from a holding potential of -75 mV. Electrophysiological parameters including the number of action potentials, resting membrane

potential (RMP), rheobase and input resistance (Rin) were measured. Rheobase was defined as required minimal current injection to elicit at least one action potential during 1000 ms. Rin was defined by the steepest slope of the current-voltage (I-V) curve based on steady-state responses to 300 ms hyperpolarizing current pulses (5 steps of 50pA).

2.4 Statistical Analysis

Data are shown as mean ± S.E.M. Primary measurements were made using Clampfit 10.3 software (Molecular device Inc.). Further statistical analysis was done using Graph Pad Prism 5 (GraphPad Software Inc.). Statistical comparisons were performed by one-way ANOVA and two-way ANOVA. P-value < 0.05 was considered as significant.

3. RESULTS AND DISCUSSION

To investigate the effects of pilocarpine-induced seizures on the excitability of granule cells during the acute and chronic periods, we elicited the trains of APs by 1000 ms depolarizing current pulses ranging from 50 to 250pA at holding potential of -75 mV. Examples of number of action potentials in current injection of 250pA in different groups have been shown in Fig. 1A. During the acute phase, firing rate in 43% of GCs significantly decreased in depolarizing currents of 100pA ($p<0.001$), 150pA ($p<0.001$), 200pA ($p<0.001$) and 250pA ($p<0.01$). Firing rate also significantly decreased in 29% of cells during chronic phase in current injections of 100pA ($p<0.01$), 150pA ($p<0.001$), 200pA ($p<0.001$) and 250pA ($p<0.05$) (Fig.1B). However, the number of action potentials in 57% of cells markedly increased during the acute phase of SE in current injection of 250pA ($P<0.01$). Also, the number of action potentials in 71% of cells in chronic stage significantly increased in current injections of 100pA ($P<0.05$), 150pA ($P<0.001$), 200pA ($P<0.001$), and 250pA ($P<0.001$) compared to control group. Analysis was performed by two-way ANOVA followed by Bonferroni posttest.

Our results showed the presence of two types of GCs with different excitability following pilicarpine injection in rats. In agreement with our experiments, Dietrich et al also indicated a heterogeneity in GCs from hippocampus of epileptic human. They reported that the 80% of GCs in epileptic human hippocampus are hyperexcitable and a small fraction of these cells are not hyperexcitable (20%) [6]. Also, Parent et al in 1997 reported that there is a subset of newly born granule cells in dentate gyrus granule cell layer of adult rats after pilocarpine-induced seizures. They concluded that plasticity in hippocampal network may result from aberrant connections formed by newly generated GCs [8]. Our study indicates that change in the excitability of GCs occur in early stages of SE even when there is no aberrant connections of newly born GCs, suggesting changes in intrinsic properties of dentate gyrus GCs following pilocarpine- induced SE.

Resting membrane potential did not change in seizure groups (Acute seizure with high firing: -75.8±1.71 mV, N=10; Acute seizure with low firing: -76.67±2.15 mV, N=6; Chronic seizure with high firing: -73.05±0.68 mV, N=10; Chronic seizure with low firing: -74.75±2.65 mV, N=4) compared to control group (-76.3±1.59 mV, N=10).

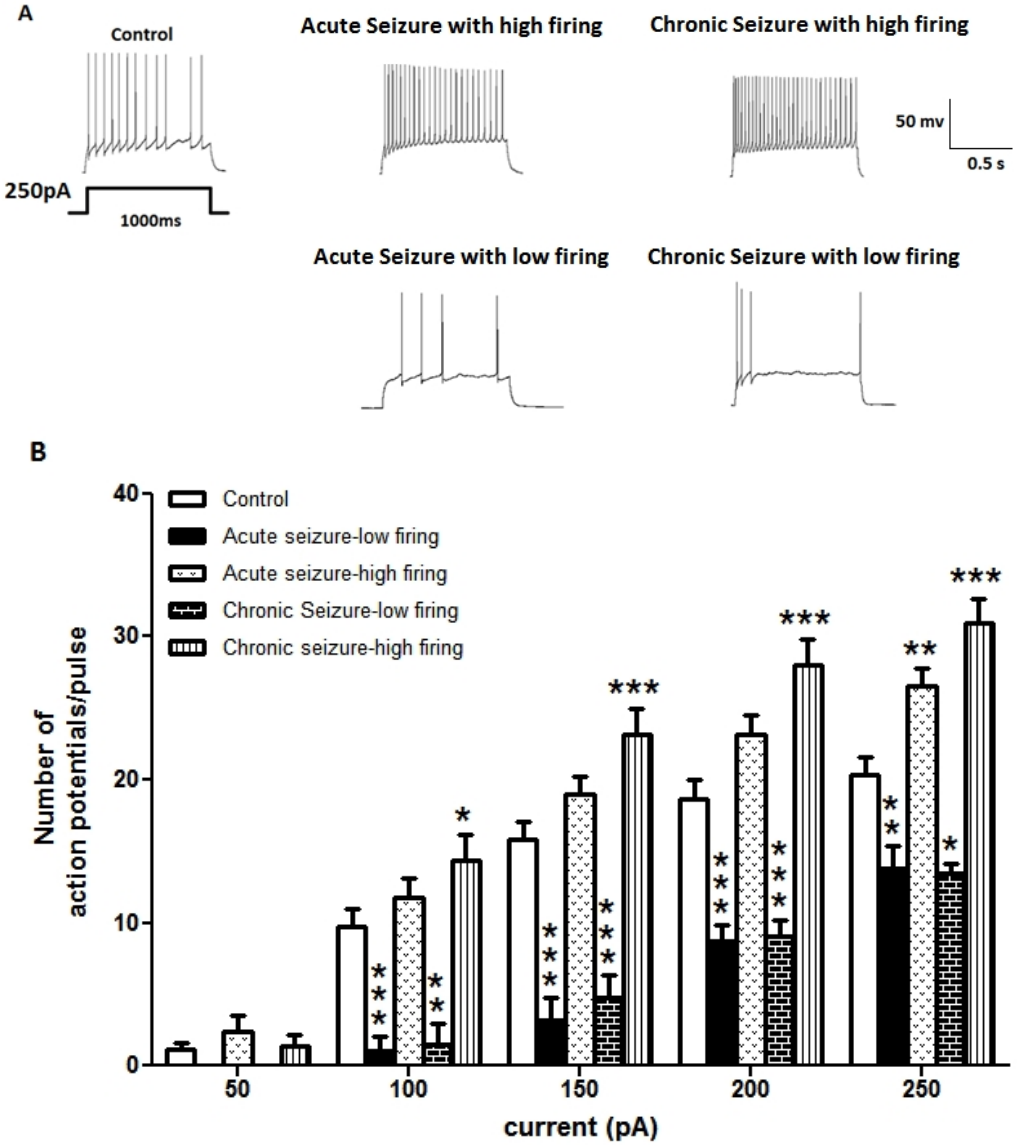

Fig. 1A. Examples of firing rate of GCs in the acute and chronic seizure groups. B) The presence of two types of GCs with different excitability in pilocarpine- induced SE. (N=10 for control group and seizure groups, N= 6 for acute seizure group with low firing rate, N= 4 for chronic seizure group with low firing). * p<0.05, ** p<0.01, *** p< 0.001, significant difference compared to control group

Input resistant significantly decreased in GCs with low firing rate both in acute (149.5±4.448 MΩ; p<0.001) and chronic (132.3±6.115 MΩ; p<0.001) seizure groups compared with controls (250.3±13.44 MΩ). However, Rin had no significant change in GCs with high firing rate both in the acute (263.1±14.16 MΩ) and chronic periods (295.5±14.44 MΩ) compared with that of control cells (Fig. 2). Analysis was performed by one-way ANOVA followed by Tukey's posttest [F (4,35)=21.45; p<0.0001].

Some studies indicated that GCs in epilepsy are more resistance than the other hippocampal cells. This feature of GCs could mediated by a change in active membrane properties including change in ion channels, resulting in hypoexcitability of cells [9-11]. However, Young et al in 2009 reported that passive membrane properties of dentate gyrus GCs can also essentially affect the excitability of cells in TLE. They showed a reduction in Rin and in the excitability of GCs that was associated with upregulation of inward rectifier K+ (Kir) channels in kainite model of TLE [5]. Also, Cameron and coworkers in 2000 reported an essential role of potassium conductance in determination of input resistance [12]. Moreover, Yarimar Carrasquillo et al in 2012 indicated that deletion of the Kv4.2 A-type K^+ channel increases input resistance in cortical pyramidal neurons compared to wild-type neurons [13]. Activation of $GABA_A$ receptors can also decrease input resistance without changes in resting membrane potential. For example, Akshay Gupta et al. in 2012 showed that a reduction in conductance of $GABA_A$ receptors is associated with an enhancement in input resistance as well as an increase in excitability of dentate semilunar granule cells 1 week after brain injury in rats [14]. In the present study, a reduction in Rin without a change in resting potential as well as hypoexcitability in a fraction of GCs suggest a possible role of leak channels including K^+ channels or $GABA_A$ receptors in these cells following pilocarpine-induced SE.

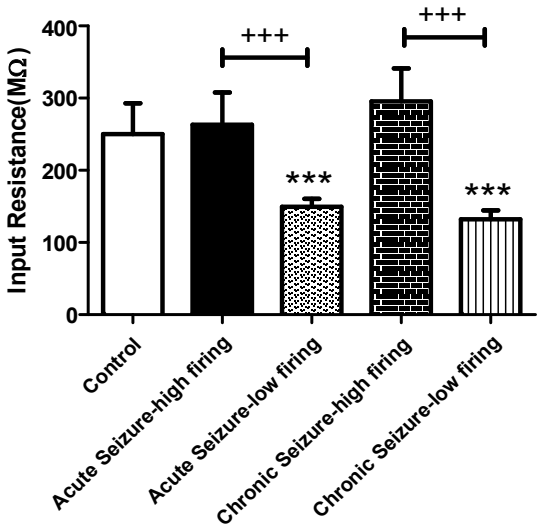

Fig. 2. Reduction in input resistance of GCs with low firing rate during acute and chronic stages of pilocarpine-induced seizure. *** p<0.001, significant difference compared to control group; +++ p<0.001 significant difference compared to acute seizure group

Based on Ohm's law (R =U/I) it is predicted that a reduction in Rin of GCs should result in a decreased excitability. Hence in the next step, we evaluated rheobase in GCs by measuring the minimal current required to elicit a single action potential in cells during 1000 ms (Fig. 3A). Results indicated that the rheobase current markedly increased in GCs with low firing rate both in the acute (201.7±39.70 pA, N=6; p<0.001) and chronic phases (260±39.37pA, N =4; p<0.001) compared with control granule cells (62.86±5.44pA, N= 10), confirming the predicted decrease in the excitability of GCs with low firing (Fig. 3B). In other

words, greater current was needed to elicit a single AP in the GCs with low firing rate compared to control cells. Rheobase in the GCs with high firing rate had no significant change in both the acute (60.02±4.271 pA, N=10) and chronic (58.12±2.841pA, N= 10) seizure groups compared with control group. Analysis was performed by one-way ANOVA and Tukey's posttest [F(4,35)=26.11; p<0.0001].

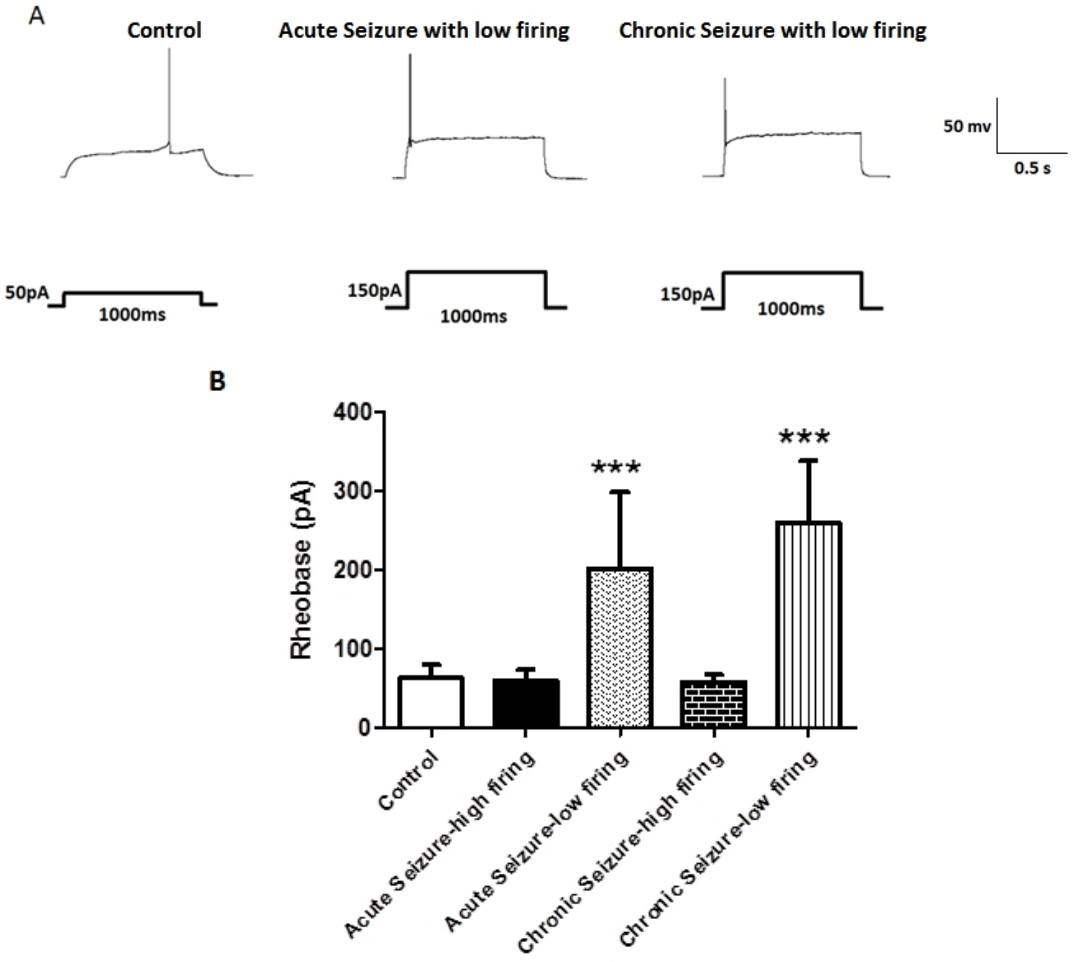

Fig. 3A. Examples of rheobase current to elicit a single AP in the GCs of control and the GCs with low firing rate in seizure groups (50 pA elicited a single AP in control group, while the GCs with low firing rate in seizure groups produced a single AP in greater current injection of 150 pA). B) Increase in Rheobase of the granule cells with low excitability during the acute and chronic stages of pilocarpine-induced seizure. *** p<0.001, significant difference compared to control group

4. CONCLUSION

The present study indicated that granule cells of dentate gyrus in pilocarpine-induced SE fall into two groups of cells: one group of cells with high excitability and another group of cells with less excitability during both the acute and chronic stages of epilepsy. Reduction in the excitability of GCs in seizure groups was associated with a significant reduction in input

resistance of cells suggesting an increase in membrane leak currents in less excitable granule cells following pilocarpine induced seizure. This condition might, at least partly, protect granule cells from seizure-induced neurotoxicity in early phase of SE, however, hyperexcitability in majority of GCs and the occurrence of spontaneous recurrent seizure during chronic phase suggest insufficient compensatory mechanisms in dentate gyrus, possibly facilitating propagation seizure activity.

ACKNOWLEDGMENTS

This work was supported by a grant from Neuroscience Research Center, Shahid Beheshti University of Medical Sciences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chang BS, Lowenstein DH. Epilepsy. N. Engl. J. Med. 2003;349:1257–1266.
2. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: Rewiring the brain. Prog Neurobiol. 2004;73:1–60.
3. Leite JP, Bortolotto ZA, Cavalheiro EA. Spontaneous recurrent seizures in rats: An experimental model of partial epilepsy. Neurosci. Biobehav. Rev. 1990;14:511–517.
4. Engel Jr J, Pedley TA. What is epilepsy? In: Epilepsy: A comprehensive textbook. Philadelphia: Lippincott-Raven. 2005;1–11.
5. Young C.C, Stegen M, Bernard R, Muller M, Bischofberger J, Veh RW, et al. Upregulation of inward rectifier K+(Kir2) channels in dentate gyrus granule cells in temporal lobe epilepsy. J Physiol. 2009;587:4213-4233.
6. Dietrich D, Clusmann H, Kral T, Steinhauser C, Blumcke I, Heinemann U, et al. Two electrophysiologically distinct types of granule cells in epileptic human hippocampus. Neuroscience. 1999;90:1197-1206.
7. Racine R.J. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972;32:281-294.
8. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J. Neurosci. 1997;17:3727–3738.
9. Beck H, Steffens R, Elger CE, Heinemann U. Voltage-dependent Ca2+ currents in epilepsy. Epilepsy Res. 1998;32:321–332.
10. Isokawa M, Mello LE . NMDA receptor-mediated excitability in dendritically deformed dentate granule cells in pilocarpine-treated rats. Neurosci Lett. 1991;129:69–73.
11. Selke K, Muller A, Kukley M, Schramm J, Dietrich D . Firing pattern and calbindin-D28k content of human epileptic granule cells. Brain Res. 2006;1120:191–201.
12. Cameron WE, et al. Role of potassium conductances in determining input resistance of developing brain stem motoneurons. J Neurophysiol. 2000;84:2330-9.
13. Yarimar Carrasquillo, Andreas Burkhalter, and Jeanne M Nerbonne. A-type K^+ channels encoded by Kv4.2, Kv4.3 and Kv1.4 differentially regulate intrinsic excitability of cortical pyramidal neurons. J physiol. 2012;590:3877-3890.
14. Akshay Gupta, Fatima S. Elgammal, Archana Proddutur, Samik Shah, Vijayalakshmi Santhakumar. Decrease in Tonic Inhibition Contributes to Increase in Dentate

Semilunar Granule Cell Excitability after Brain Injury. The Journal of Neuroscience. 2012;32:2523-2537.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=582&id=32&aid=5152