Korean J Physiol Pharmacol
Vol 6: 143–147, June, 2002

Calcium Influx is Responsible for Afterdepolarizations in Rat Hippocampal Dentate Granule Cells

Won-Sun Park and Suk-Ho Lee

Department of Physiology and National Research Laboratory for Cellular Signalling, Seoul National University College of Medicine, Seoul 110–799, Korea

Granule cells in dentate gyrus of hippocampus relay information from entorhinal cortex via perforant fiber to pyramidal cells in CA3 region. Their electrical activities are known to be closely associated with seizure activity as well as memory acquisition. Since action potential is a stereotypic phenomena which is based on all-or-none principle of Na^+ current, the neuronal firing pattern is mostly dependent on afterpotentials which follows the stereotypic Na^+ spike. Granule cells in dentate gyrus show afterdepolarization (ADP), while interneurons in dentate gyrus have afterhyperpolarizaton. In the present study, we investigated the ionic mechanism of afterdepolarization in hippocampal dentate granule cell. Action potential of dentate granule cells showed afterdepolarization, which was characterized by a sharp notch followed by a depolarizing hump starting at about -49.04 ± 1.69 mV (n=43, mean±SD) and lasting 3~7 ms. Increase of extracellular Ca^{2+} from 2 mM to 10 mM significantly enhanced the ADP both in amplitude and in duration. A K^+ channel blocker, 4-aminopyridine (4-AP, 2 mM), enhanced the ADP and often induced burst firings. These effects of 10 mM Ca^{2+} and 4-AP were additive. On the contrary, the ADP was significantly suppressed by removal of external Ca^{2+}, even in the presence of 4-AP (2 mM). A Na^+ channel blocker, TTX (100 nM), did not affect the ADP. From these results, it is concluded that the extracellular Ca^{2+} influx contributes to the generation of ADP in granule cells.

Key Words: Afterdepolarization, Granule cell, Hippocampus

INTRODUCTION

As a part of limbic system, the hippocampus plays an important role in regulating neuronal excitability, plasticity, learning and memory (Alkon et al, 1991). Dentate gyrus granule cells in the hippocampus act as a relay, receiving input from the entorhinal cortex via the perforant pathway and sending output to CA3 and CA1 neuron via the mossy fiber projection (Witter et al, 1989; Lopes Da Silva, 1990).

When repolarization of neuronal action potential does not decline monotonically but is interrupted by additional depolarization, this prolonged depolarization phase is refered to as afterdepolarization (ADP). ADPs are known to have an important role in regulating neuronal excitability (Storm et al, 1990). Under physiological condition, a short current stimulus or a brief synaptic activation can evoke action potential firing. Repetitive firing evoked by long current pulses shows spike frequency adaptation. Dentate gyrus granule cells as well as hippocampal CA1 and CA3 pyramidal cells are regular spiking neurons and show ADPs (Zhang et al, 1993; Azouz et al, 1996).

The ionic mechanisms of ADP in these cells, however, still remain uncertain. It has been reported that Ca^{2+} and persistent Na^+ currents are responsible for ADPs in CA1 pyramidal cells. Others argue, however, that Ca^{2+} current rather suppressed ADP, and persistent Na^+ current may be involved. In septal nucleus neurons, ADPs are due to increase in Ca^{2+}-activated nonselective cation channels (Hasuo et al, 1990). In lateral amygdala, parasympathetic neurons and spinal sensory neurons, ADPs are mediated by activation of Ca^{2+}-activated Cl^- channels (Sugita et al, 1993).

In the dentate gyrus granule cell, though there is some evidence to suggest that Ca^{2+} currents are responsible for the ADP (Crunelli et al, 1983; Niesen et al, 1991), ionic mechanisms of ADP are not yet clearly known. In the present study, we investigated the ionic mechanisms of ADP observed in dentate granule cells, and found that the extracellular Ca^{2+} was essential for the generation of ADP, but Na^+ currents were not involved.

METHODS

Tissue preparation

Hippocampal slices (300 μm) were prepared from 2 to 3-wk-old Sprague-Dawley rats with Vibratome series 1000 (Technical Products International Inc.). Rats were decapitated, and their brains were removed quickly and rinsed

Corresponding to: Suk-Ho Lee, Department of Physiology, Seoul National University College of Medicine, Seoul 110-799, Korea. (Tel) 82-2-740-8222, (Fax) 82-2-763-9667, (E-mail) leesukho@snu.ac.kr

ABBREVIATIONS: ADP, afterdepolarization; 4-AP, 4-aminopyridine; TTX, tetrodotoxin; ACSF, artificial cerebrospinal fluid.

in ice-cold artificial cerebrospinal fluid (ACSF), which contains (mM): 125 NaCl, 3 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 10 glucose, 5 sucrose, 1.2 $MgSO_4$, 0.4 Na-Vit C. The ACSF was bubbled with mixture of 95% O_2 - 5% CO_2 at least for 1 hour before use, making the final pH 7.3 and osmolarity 300 mOsm.

Electrophysiology

Whole cell current-clamp recordings were obtained from granule cells located in dentate gyrus granule cell layer using a patch clamp amplifier (Swam II C, Life Science Resources, UK or Axoclamp-2A, Axon Instrument, USA). The voltage signals were filtered at 3 kHz and sampled at 6 kHz. The brain slices were constantly perfused at the rate of 3~5 ml/min with ACSF warmed at 31~34°C. Patch pipettes had resistances of 2~3 MΩ, when filled with the following solution (mM): 110 K-gluconic acid, 30 KCl, 15 *N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulfonic acid (HEPES), 4 K-ATP, 4 $MgCl_2$, 4 Na-Vit C, pH 7.3. The patch pipettes were pulled from borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using a Narishige puller (PP-83, Japan). Dentate granule cells were visually identified using an Olympus upright microscope (BX50WI) with Nomarski optic and 60X water-immersion objective. The series resistance in whole cell patch clamping neurons was within 15~25 MΩ. Cells were used for analysis if granule cells had a distinct afterdepolarization (ADP), a resting membrane potential lower than −65 mV. The liquid-junction potentials between ACSF and pipette solutions, calculated based on ionic mobilities, was −6 mV. Since they were not large and the liquid-junction potential between the pipette solutions and intracellular solution was not known, we did not make corrections for junction potentials in presenting and analyzing data.

Drug

All pharmacological compounds were prepared as aqueous stock solution of 100~1000 times the required final concentration and the stock solution was diluted in the ACSF solutions to make desired final concentration. The following compounds were purchased from Sigma (USA): 4-aminopyridine (4-AP), nickel chloride ($NiCl_2$). Tetrodotoxin (TTX) was obtained from Tocris (UK).

Statistics and presentation of data

The results in the text and in the figures are presented as means±SD, n=number of cells tested. Statistical analysis was carried out using the Student's *t* test. The difference between two groups was considered to be significant when $P<0.01$, and not significant when $P>0.05$.

RESULTS

Afterdepolarizations of dentate gyrus granule cells

We studied electrophysiological properties of the action potentials recorded on neuronal cells, whose soma were located in the granule cell layer of dentate gyrus and their diameters were about 8~12 μm in average (Freund & Buzsaki, 1996). Action potentials were evoked from the resting membrane potential (−70.72±4.54, n=54, means±SD) by injection of a brief depolarizing current pulse (3 ms in duration; 500~700 pA in amplitude). Fast repolarization phase after overshoot was followed by slow repolarization phase. It started at −49.04±1.69 mV (n=43, mean±SD) and lasted 3~7 ms. In 109 cells out of 123 granule cells examined in this study, the slow repolarization phase after action potentials were kept more depolarized than the response to subthreshold current injection. We considered the difference of the slow repolarization phase between suprathreshold response (marked by 1) and subthreshold response (marked by 2) as afterdepolarization (ADP) (Fig. 1).

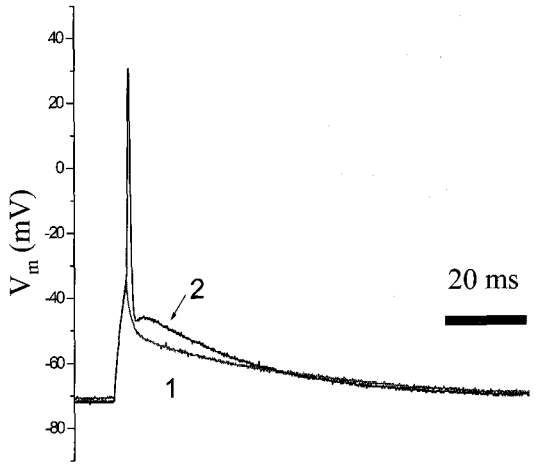

Fig. 1. Definition of an afterdepolarization (ADP). When a subthreshold depolarizing pulse (550 pA, 3 ms duration) was injected, only passive electrotonic response was observed (marked by '1'). When slightly higher current (600 pA, 3 ms duration) was injected, Na^+-dependent spike occurred on top of the passive depolarization phase. Under this condition, the sharp notch was observed between the spike and re-depolarizing hump (marked by '2'). We regarded the difference between suprathreshold and subthreshold responses as an ADP.

Ca^{2+} influx is involved in the generation of ADPs

In order to examine the possible involvement of inward Ca^{2+} currents, we studied the effects of change of extracellular Ca^{2+} concentration. When extracellular Ca^{2+} was increased from 2 mM to 10 mM, both the peak amplitude and the duration of ADP were enhanced (Fig. 2).

It is known that the isoforms of T-type Ca^{2+} channel expressed in hippocampus has low sensitivity for Ni^{2+} (IC_{50}=250 μM; Lee et al, 1999). Thus, there is no drug available which is able to differentiate T-type from HVA (high-voltage activated) Ca^{2+} channel. In order to block Ca^{2+} channel non-selectively, 2 mM $NiCl_2$ was added to 10 mM Ca^{2+} containing ACSF. $NiCl_2$ significantly suppressed the ADPs (Fig. 3A). Comparison of the action potential response in the presence of $NiCl_2$ with the subthreshold response demonstrated that ADPs were almost completely suppressed by 2 mM $NiCl_2$ (Fig. 3B). At lower concentration of $NiCl_2$ (100~200 μM), the ADPs were significantly reduced, however, the inhibition was not complete (data not shown). These results indicated that the Ca^{2+} influx, which was blocked by 2 mM $NiCl_2$, was involved in the generation of the ADPs.

A transient outward K^+ channel blocker, 4-aminopyridine (4-AP, 2 mM) added to 10 mM $CaCl_2$ containing ACSF solution, enhanced not only the ADP, but also evoked repetitive burst-like broad spikes on top of the ADP (Fig. 2, marked by 3; Fig. 4 marked by 2). The involvement of Ca^{2+} influx was further confirmed by the effect of Ca^{2+}-free ACSF on these ADPs: When Ca^{2+} was removed from the bathing ACSF, the ADP was gradually suppressed for over 7 min and was finally abolished, although 4-AP was still present in the bathing solution (Fig. 5). These results indicated that the extracellular Ca^{2+} influx was an essential requirment for the generation of ADP.

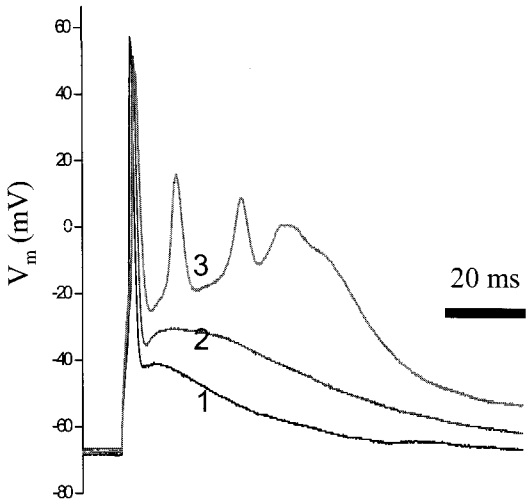

Fig. 2. Bath application of high Ca^{2+} (10 mM Ca^{2+}) ACSF, and/or 2 mM 4-aminopyridine (4-AP). The control spike shows a distinct re-depolarizing ADP (marked by '1'). The ADP was enhanced when 10 mM Ca^{2+} instead of 2 mM Ca^{2+} was added to superfusing ACSF (marked by '2'). Addition of 4-AP to the 10 mM Ca^{2+} ACSF further enhanced the ADP and subsequent burst firings were evoked on top of the ADP (marked by '3'). Depolarizing current pulse: 670 pA (amplitude) and 3 ms (duration).

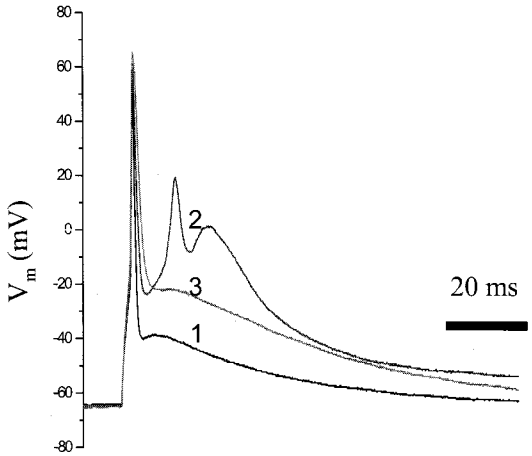

Fig. 4. $NiCl_2$ (2 mM) abolished the bursting spikes induced by high external [Ca^{2+}] (10 mM) and 4-AP (2 mM). The granule cell had a normal action potential under the control condition (ACSF containing 2 mM Ca^{2+}, marked by '1'). Burst-like broad spikes were newly evoked when ACSF containing 10 mM Ca^{2+} and 2 mM 4-AP was superfused on the slice (marked by '2'). Addition of 2 mM $NiCl_2$ to the bathing solution containing 10 mM $CaCl_2$ and 2 mM 4-AP abolished the bursting spikes (marked by '3'). All spikes were evoked by a depolarizing pulse of 500 pA and 3 ms.

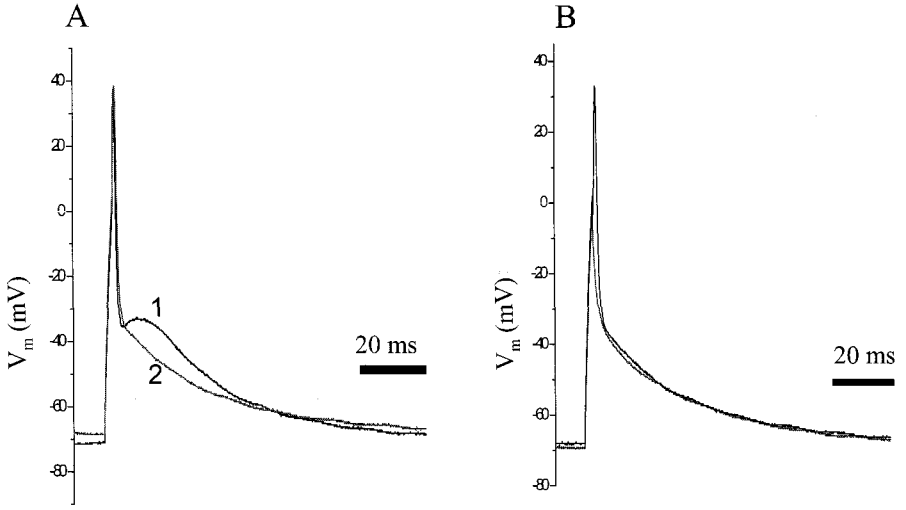

Fig. 3. Effect of bath application of 2 mM $NiCl_2$ on ADP. A. Addition of 2 mM $NiCl_2$ to the perfusate, which was ACSF containing 10 mM $CaCl_2$, suppressed the ADP. The action potential evoked under the control condition (10 mM Ca^{2+} ACSF), and that in the presence of 2 mM $NiCl_2$ were marked by 1 and 2, respectively. B. The action potential evoked in the presence of 2 mM $NiCl_2$ was superimposed on the subthreshold response. All spikes were evoked by a depolarizing pulse of 700 pA and 3 ms.

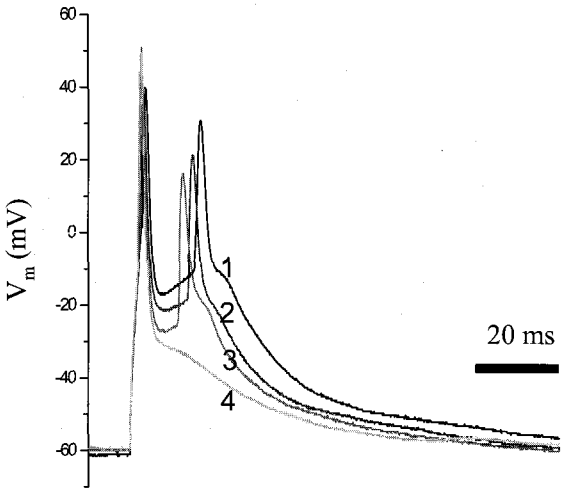

Fig. 5. The gradual change of the ADP during wash-out of external Ca^{2+}. Removal of $CaCl_2$ from the bathing solution containing 10 mM Ca^{2+} and 2 mM 4-AP gradually suppressed ADPs. The AP traces before the replacement and those at 2, 4, and 7 min after the replacement were superimposed, and marked by '1', '2', '3' and '4', respectively. Depolarizing current pulse: 610 pA, 3 ms.

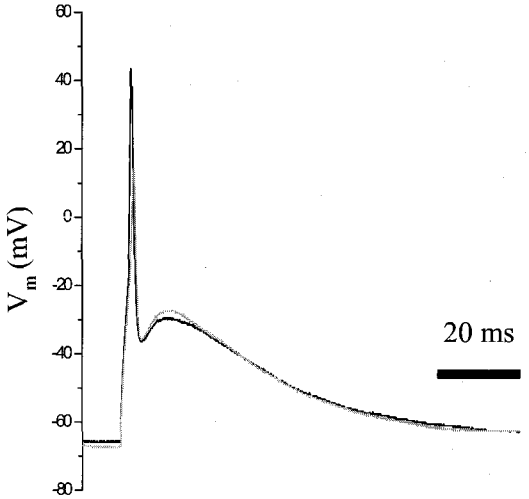

Fig. 6. Tetrodotoxin (TTX) has no effect on the ADP. TTX (100 nM) was added to the perfusate containing 10 mM Ca^{2+}. TTX did not affect the ADP, though it reduced the amplitude of spike action potential (gray trace). Depolarizing current pulse: 630 pA, 3 ms.

TTX has no effect on ADPs

Based on the observation that low concentration of TTX (<500 nM) suppressed the magnitude of ADPs, Azouz et al. (1996) proposed persistent Na^+ current as an underlying ionic mechanism of ADPs observed in CA1 pyramidal cells. In order to test the possible role of persistent Na^+ current in the generation of ADPs in dentate granule cells, we examined the effect of TTX on ADPs. When 100 nM TTX was applied in the bathing solution, the spike overshoot decreased from +45 mV to +13 mV, indicating a partial block of fast Na^+ current, however, the ADPs in dentate granule cells were not affected (Fig. 6). This result suggested that, unlike in CA1 pyramidal neurons, TTX-sensitive Na^+ currents were not responsible for the ADPs in dentate granule cell.

DISCUSSION

The main findings in the present study were 1) that the ADPs in the soma of dentate gyrus granule cells were dependent on extracellular Ca^{2+}, and 2) that TTX-sensitive Na^+ channel was not involved in the generation of the ADPs. These findings were different from those observed in pyramidal cells, where TTX-dependent Na^+ channel is known to be involved in their ADP generation (Azouz et al, 1996).

Most studies indicate that the generation of ADPs provide a prolonged somatic depolarization which is required for the initiation of multiple spikes (Jensen et al, 1996; Stuart et al, 1997; Ildiko & William, 1999; Williams & Stuart, 1999). Ionic mechanisms of ADP in hippocampal pyramidal cells have been studied by many groups, and it has been suggested that the active component of somatic ADPs in CA1 pyramidal cells is associated with Ca^{2+} current (Wong & Prince, 1981). It has also been suggested that Na^+ current is responsible for a major part of ADPs in CA1 pyramidal neurons (Azouz et al, 1996), and contrary to the earlier reports, the external Ca^{2+} rather suppresses ADPs. Subsequently, Magee and Carruth (1999) suggested that the active membrane properties of the dendritic arborization are involved in the generation of ADPs. These contradictory results might have been due to methodological differences between studies. However, the most plausible explanation of the different results might be that there are multiple mechanisms of ADP in CA1 pyramidal neurons. Compared with CA1 pyramidal neurons, dentate granule cells are not well studied as far as the ionic mechanisms of their ADP generation are concerned.

Analogous to CA1 pyramidal neurons, it is highly possible that many different ionic mechanisms are involved in ADPs in dentate gyrus granule cells. However, our present study mainly focused on the role of extracellular Ca^{2+} and Na^+ current in the ADP genereation, and the results clearly showed that ADPs in granule cell were dependent on extracellular Ca^{2+}. Evidence favoring the fact that Ca^{2+} influx is essential for the generation of the ADP is as follows: 1) more pronounced ADPs were generated in higher external $[Ca^{2+}]$; 2) ADPs were completely abolished by 2 mM $NiCl_2$.

The bursting spikes observed in the presence of 10 mM Ca^{2+} and 2 mM 4-AP were clearly broader than the preceeding Na^+-spikes (Fig. 2 and 4). In addition, they were abolished by the addition of 2 mM $NiCl_2$ to the bath solution (Fig. 4) or by the removal of external Ca^{2+} (Fig. 5). It has earlier been shown that the density of the transient K^+ channel increased linearly with distance from the soma, and blockage of the dendritic K^+ channel with 4-AP induced dendritic Ca^{2+} channel activation in CA1 pyramidal neuron (Hoffman et al, 1997). Subsequently, Magee & Carruth (1999) showed that application of 4-AP to the distal dendrites increased the ADP duration and bursting spikes in CA1 pyramidal cells, while no such effect was elicited by proximal or soma application of 4-AP. Consistent with these reports, our results suggest that the spikes evoked

in the presence of 2 mM 4-AP and 10 mM Ca^{2+} might be the results of Ca^{2+} influx mediated by activation of dendritic Ca^{2+} channels.

In hippocampal granule cells, several types of voltage-activated Ca^{2+} currents have been described (Blaxter et al, 1989; Eliot et al, 1994). In this study, we did not characterize the type of Ca^{2+} channels involved in ADP. Since the mean V_m to start ADP was -49.04 ± 1.69 mV in this study, where steady state inward currents via HVA Ca^{2+} channels were already deactivated, it seems to be quite likely that low-threshold transient (T-type) Ca^{2+} channels are involved in the generation of ADP, as suggested by Zhang et al (1993). Recently, however, it has been reported that voltage-gated Ca^{2+} channels deactivate slowly enough during action potential repolarization to produce an ADP in subicular pyramidal cells (Jung et al, 2001). It is not an easy task to prove whether the T-type Ca^{2+} channel is solely responsible for the ADP, due to a lack of blocker specific for the channel. Zhang et al (1993) presented only the indirect evidence for the involvement of T-type Ca^{2+} channel; such as similarity between T-type Ca^{2+} channel and ADP in the frequency response and in the dependence on prepulses. Thus, it remains to be elucidated whether Ca^{2+} tail current mediated by multiple Ca^{2+} channel subtypes is involved in producing the ADP.

Moreover, the dependence of ADP on extracellular Ca^{2+}, however, gives rise to two possible mechanisms of ADP generation: Ca^{2+} influx itself and/or intracellular Ca^{2+} dependent inward currents (for example, Na^{+}-Ca^{2+} exchange current and Ca^{2+} dependent nonselective cation current). We did not elucidate here the role of intracellular Ca^{2+} played in the generation of ADPs. However, it seems quite plausible that intracellular Ca^{2+} might enhance the ADP in some degree, since chelation of intracellular Ca^{2+} reduced the ADP remarkably (data was not shown), in disaggrement with the result of Zhang et al. (1993). They showed that chelation of intracellular Ca^{2+} enhanced the ADP by about 5~10 %, and the inhibition of Ca^{2+} activated K^{+} currents was suggested to be a mechanism of this enhancement. In the present study, we could not observe any contribution of Ca^{2+} activated K^{+} currents to the action potentials of granule cells. This point needs to be further investigated in the future study.

ACKNOWLEDGEMENT

This work was supported by the Research Fund for Newly Appointed Faculty Members and the SNU Research Center Fund from Seoul National University. Park WS is a postgraduate student supported by BK21 program from the Ministry of Education.

REFERENCES

Alkon DL, Amaral DG, Bear MF, Black TJ, Cohen NJ, Disterhoft JF, Eichenbaum H, Golski S, Gorman LK, et al. Learning and memory. *Brain Research Reviews* 16(2): 193-220, 1991

Azouz R, Jenson MS, Yarri Y. Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *J Physiol* 492.1: 211-223, 1996

Blaxter TJ, Carlen PL, Niesen C. Pharmacological and anatomical separation of calcium currents in rat dentate granule neurons *in vitro*. *J Physiol* 412: 93-112, 1989

Crunelli V, Assaf SY, Kelly JS. Intracellular recordings from granule cells of the dentate gyrus *in vitro*. In *Neurobiology of the Hippocampus*. Edited by Seifert W. New York, Academic, p 197-214, 1983

Eliot LS, Johnston D. Multiple components of calcium current in acutely dissociated dentate gyrus granule neurons. *J Neurophysiol* 72(2): 762-777, 1994

Freund TF, Buzsaki G. Interneuron of the hippocampus. *Hippocampus* 6: 347-470, 1996

Hasuo H, Gallagher JP. Facilitatory action of muscarine on the slow afterdepolarization of rat dorsolateral septal nucleus neurons *in vitro*. *Neuroscience Letters* 112: 234-238, 1990

Hoffman DA, Jeffrey CM, Costa MC, Johnston D. K^{+} channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* 387: 869-875, 1997

Ildik A, William RH. Role of multiple calcium and calcium- dependent conductances in regulation of hippocampal dentate granule cell excitability. *J Computational Neuroscience* 6: 215-235, 1999

Jensen MS, Azouz R, Yaari Y. Spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *J Physiol* 492(1): 199-210, 1996

Jung HY, Staff NP, Spruston N. Action potential bursting in subicular pyramidal neurons in driven by a calcium tail current. *J Neuroscience* 21(10): 3312-3321, 2001

Lee JH, Gomora JC, Cribbs LL, Perez-Reyes E. Nickel block of three cloned T-type calcium channels: low concentrations selectively block alpha1H. *Biophysical J* 77(6): 3034-3042, 1999

Lopes DA, Silva FH, Witter MP, Boeijinga PH, Lothman AHM. Anatomic organization and physiology of the limbic cortex. *Physiological Reviews* 70: 453-511, 1990

Magee JC, Carruth M. Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. *J Neurophysiol* 82(4): 1895-1901, 1999

Nissen C, Charlton MP, Carlen PL. Postsynaptic and presynaptic effects of the calcium chelator BAPTA on synaptic transmission in rat hippocampal dentate granule neurons. *Brain Research* 555: 319-325, 1991

Storm JF. Potassium currents in hippocampal pyramidal cells. *Progress in Brain Research* 83: 161-187, 1990

Stuart G, Schiller J, Sakmann B. Action potential initiation and propagation in rat neocortical pyramidal neurons. *J Physiol* 505.3: 617-632, 1997

Sugita S, Tanaka E, North RA. Membrane properties and synaptic potentials of three types of neuron in rat lateral amygdala. *J Physiol* 460: 705-718, 1993

Williams SR, Stuart GJ. Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. *J Physiol* 521.2: 467-482, 1999

Witter MP, Groenewegen HJ, Lopes Da Silva FH, Lothman AHM. Functional organization of the extrinsic and intrinsic circuitry of the hippocampal region. *Progress in Neurobiol* 33: 161-253, 1989

Wong RK, Prince DA. Afterpotential generation in hippocampal pyramidal cells. *J Neurophysiol* 45(1): 86-97, 1981

Zhang L, Valiante TA, Carlen PL. Contribution of the low-threshold T-type calcium current in generating the post-spike depolarizing afterpotential in dentate granule neurons of immature rats. *J Neurophysiol* 70(1): 223-231, 1993