INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 9220023

**Modulation of excitability in hippocampal granule cells by
ethanol: The role of NMDA receptors**

Yuen, Geoffrey Lap-Fai, Ph.D.

Case Western Reserve University, 1992

U·M·I

**300 N. Zeeb Rd.
Ann Arbor, MI 48106**

**MODULATION OF EXCITABILITY IN HIPPOCAMPAL GRANULE
CELLS BY ETHANOL: THE ROLE OF NMDA RECEPTORS**

by

GEOFFREY LAP-FAI YUEN

Submitted in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Thesis Advisor: Dominique Durand, Ph.D.

Department of Biomedical Engineering
CASE WESTERN RESERVE UNIVERSITY

January 1992

CASE WESTERN RESERVE UNIVERSITY

GRADUATE STUDIES

We hereby approve the thesis of

GEOFFREY YUEN

candidate for the Ph D

degree.*

Signed:

Dominique DURAND
(chairman)
Patricia E. Cropper
Norman Rudy
Stephen W. Jones

Date December 5, 1991

*We also certify that written approval has been obtained for any proprietary material contained therein.

MODULATION OF EXCITABILITY IN HIPPOCAMPAL GRANULE CELLS BY ETHANOL: THE ROLE OF NMDA RECEPTORS

Abstract

by

GEOFFREY LAP-FAI YUEN

Although the chronic and acute behavioral effects of ethanol consumption are very well-known, the neuronal mechanisms and circuitry related to these effects are largely unknown. Previous unit recording has demonstrated that the hippocampus is susceptible to low dose of ethanol and is among the first brain regions to respond. Although other principal neurons in this brain region (pyramidal CA1 and CA3 neurons) have recently been shown to be sensitive to ethanol, dentate granule neurons, the first destination of incoming fibers from the entorhinal cortex, have not been systematically examined with respect to their susceptibility to low doses of ethanol. Through detailed intracellular recording, we demonstrated that ethanol indeed suppresses the excitability of these neurons in response to orthodromic stimulation while raising the excitability to somatic current injection. To clarify the mechanism that may underly the orthodromic firing threshold modulation, antagonist to

glutamate and gabaergic receptors were used to pharmacologically isolate the component(s) related to ethanol sensitivity. The results of this analysis suggest that a subtype of glutamate receptors, the NMDA-receptors are selectively involved in mediating the effects of ethanol. By combining information from a short-pulse electrotonic analysis and computer simulations, we were able to show that electrotonic changes also help to account for the orthodromic response depression, although the size of this contribution is probably smaller than that for the NMDA-receptors.

ACKNOWLEDGEMENTS

Perhaps the most important lesson I have learned from my graduate study was the fact that any small accomplishment on my part is dependent on the prior labor of many others. I would like to thank my research advisor, Dr. Dominique Durand, who never fails to surprise me with an unexpected viewpoint or idea and guided me through the uncharted course of my thesis research. Likewise, I wish to express my gratitude to Dr. Steven Jones, not only for the tremendous help he has provided in critically evaluating my thesis, but also for his careful attitude in conducting his science that often was a source of inspiration; to Dr. Patrick Crago for friendly conversations and to Dr. Yoram Rudy for his encouraging attitude.

I would like to thank many individuals in the Applied Neural Control Laboratory who for one reason or another have made my graduate experience all the more meaningful: Dr. Stewart Ferguson who will remain as one of the most inspiring individuals I have met in this life, Mr. Hani Kayyali who never loses the ability to laugh at my jokes, the soon Dr. Eddy Warman who never let a loose point

slide by. Don Atzberger and Hani also gave me much help with the Macs and the experimental setup. Madhavi Patil did most of the hard work in Chapter 2.

Special thanks go to WenJau Lee who made me proofread her term paper on NMDA-receptors at a time when I have absolutely no interest in the subject.

Last but not least I have to thank my sister, Gail, for all the support, help, chores and cooking during the last year and a half and my parents for all the confidence and patience they have in me during the seemingly endless years of my graduate education since 1982. This thesis is dedicated to both of my grandmothers, who made all of my hard work possible unknowingly.

TABLE OF CONTENTS

Abstract	ii
Acknowledgements	iv
Table of Contents	vi

Chapter 1. Introduction and Hypotheses

1.1 General introduction	1
1.2 Statement of research hypothesis and thesis organization	2
1.3 References	4

Chapte 2. Phenomenology of the acute action of ethanol on hippocampal granule neurons

2.1 Abstract	7
2.2 Introduction	8
2.3 Methodology	10
2.4 Results	15
2.4.1 Ethanol's effect on orthodromic EPSP	

2.4.2	Ethanol's effect on responses based on somatic current injection	27
2.5	Discussion	29
2.6	References	35
 Chapte 3. Cellular substrates of ethanol action		
3.1	Abstract	40
3.2	Introduction	41
3.3	Methods	42
3.4	Results	44
3.4.1	Effects of APV on control responses	44
3.4.2	The NMDA-receptor hypotheis	50
3.4.3	Effects of ethanol on APV -resistant responses	53
3.4.4	Effects of DNQX on control responses	54
3.4.5	Effect of ethanol on DNQX-resistant responses	55
3.4.6	Role of inhibition	67
3.4.6.1	General prevalence of inhibition.....	71
3.4.6.2	Effect of ethanol on BIC -resistant responses	76

3.4.6.3 Effect of ethanol on BIC/DNQX -resistant orthodromic responses	83
3.5 Discussion	
3.5.1 Basic issues related to the presence of NMDA- receptors in dentate gyrus cells	91
3.5.2 Involvement of NMDA receptors in acute depression of orthodromic response	98
3.5.3 The role of inhibition and electrotonus	101
3.6 References	104

Chapter 4. Modelling the effects of ethanol on hippocampal granule neurons

4.1 Abstract	114
4.2 Introduction	115
4.3 Methods	115
4.4 Results	121
4.4.1 General model properties and sensitivity of parameters	122
4.4.2 Fitting to experimental EPSPs	127
4.4.3 Contribution of electrotonic factors	138

4.5	Discussion	147
4.6	Conclusions	150
4.7	Glossary	154
4.8	References	155
 Chapter 5. Conclusions		 160
 Literature cited		 162

CHAPTER I

INTRODUCTION AND HYPOTHESES

1.1 General Introduction

Despite considerable past research, the neuronal circuits and mechanisms underlying the chronic and acute effects of ethanol are poorly understood. Instances of ethanol effects on subcellular mechanisms such as ionic channels or calcium concentrations as well as population or unit firing rates have been reported⁵. However, without identifying the specific neurons or cell types (in a relevant brain region) at which these effects can be monitored, there is no framework from which the biochemical, subcellular mechanisms can be evaluated from a cellular point of view, nor is it possible to understand how ethanol may produce consistent changes in the units and population responses. This situation is recently improved by the application of the in vitro hippocampal slice preparation, which permits direct identification of the principal cell types through location and their well-known electrophysiological properties⁸.

Furthermore, the hippocampus is a brain region widely implicated in both chronic (e.g. memory loss, epilepsy) and acute effects of ethanol. For example, both evoked and spontaneous unit activities in both CA1 and dentate molecular regions were depressed by acute ethanol^{2-4, 6}. Interestingly, however, intracellular recordings from CA1 and CA3 neurons have until recently failed to reveal consistent changes in firing threshold in response to ethanol⁷. It is also unclear whether the observed effects mediated are polysynaptic or local in origin. Since [i] dentate granule cells represent one of the three principal cells as well as the main 'input station' to the hippocampus¹ and [ii] both evoked and spontaneous activities in dentate gyrus were depressed in ethanol^{2,4} and [iii] comparable intracellular recording studies have not been carried out in dentate gyrus, a direct modulation of granule cell excitability by ethanol can in principle naturally account for some or all of the above observations.

1.2 Statement of Research Hypotheses and thesis organization

Assuming consistent electrical changes are induced by acute ethanol in specific cell types, the goal of this research is to initially identify a cellular locus at which such a consistent action of ethanol

can be monitored. Based on the well-known effects of ethanol on the hippocampus and the lack of consistent effects on pyramidal cells, we hypothesize that a change in the electrical properties of dentate granule cell may be observed intracellularly with acute ethanol treatment. This hypothesis was tested and confirmed experimentally in Chapter 2. The results of Chapter 2 and some pilot experiments involving selective glutamate receptor antagonists provides the basis for the second hypothesis, that a specific type of glutamate receptors (NMDA receptors) was primarily involved in the orthodromic threshold elevation of these neurons by ethanol. This hypothesis was evaluated experimentally with respect to both excitatory and inhibitory synaptic process in Chapter 3. As ethanol also appears to affect the electrotonic properties of these neurons, a model of these neurons was developed so that the relative (albeit average) contribution of the two factors can be assessed and evaluated with respect to the overall firing behavior of the neuron. The comparison of model and experimental responses reinforce the idea that inhibition of NMDA-receptors is primarily responsible for the orthodromic threshold elevation in ethanol.

1.3 References

- [1] Alger, B.E., Dhanjal, S.S., Dingledine, R., Garthwaite, J., Henderson, G., King, G.L., Lipton, P., North, A., Schwartzkroin, Sears, T.A., Segal, M., Whittingham, T.S. and Williams, John. Brain slice methods. In *Brain Slice*, Dingledine, R. (ed), 1984, Plenum, New York.

 - [2] Doller, H.J., Eckart, M.J. and Weight, F.F. Effects of ethanol on the hippocampal slice preparation. *Fed. Proc.* 309:281, 1980

 - [3] Durand, D., Corrigan, W. Kujtan, P., and Carlen, P.L. Effects of low-dose ethanol on CA1 hippocampal neurons in-vitro. *Canadian Journal of Physiology and Pharmacology*, 59:972-984, 1981

 - [4] Folger, W.R. and Klemm W.R., Unit responsivity in various Hippocampal layers and the depressive effects of ethanol" *Physiology & Behavior* 21: (1978) 169-176
-

- [5] Hunt, W.A. *Alcohol and Biological Membranes*. 1985. Guiford Press, New York.
- [6] Klemm, W.R. Effects of ethanol on nerve impulse activity. In Edward Majchrowicz and Ernest P. Noble, *Biochemistry and Pharmacology of Ethanol*, 2 (1980) pp. 243 - 267
- [7] Siggins, G.R., Bloom, F.E., French, E.D., Madamba, S.G., Mancillas, J., Pittman, Q.J. and Rogers, J. Electrophysiology of ethanol on central neurons. In Rubin Emanuel (Ed.) *Alcohol and the Cell: Annals of the New York Academy of Sciences*, 1987, 492 pp. 350-366.
- [8] Teyler, T.J. Brain slice preparation: Hippocampus. *Brain Res. Bull.* 5 (1980) 391-403.

CHAPTER II

PHENOMENOLOGY OF THE ACUTE ACTION OF ETHANOL ON HIPPOCAMPAL GRANULE NEURONS

In this chapter, the acute effects of ethanol on the intracellularly recorded responses of hippocampal granule cells are described. This chapter has been previously published, along with portions of the next chapter, in Brain Research with coauthors M. Patil and D. Durand. This work was supported in part by NIH Grant 1ROI-AA-06773-06 to Dr. D. Durand.

2.0 Abstract

The effects of low dose, acute ethanol treatment on neuronal firing threshold were studied in dentate granule neurons using standard intracellular recording techniques and the *in vitro* hippocampal slice preparation. Special precautions were made to avoid nonspecific effects related to solution switching and ethanol incubation. By stimulating the soma and orthodromically, the evoked responses in control and ethanol were studied. Based on a comparison of the EPSPs strength duration curve and somatic firing responses, we concluded that a higher threshold for orthodromic activation and lower threshold for somatic activation were induced by ethanol.

2.1 Introduction

Although the acute and chronic behavioral effects of ethanol are very well-known, the neuronal circuits and mechanisms underlying these effects are poorly understood. The results of many early studies are confounded by the use of extremely high doses of ethanol. For example, although ethanol has been observed to alter the shape of action potentials in squid axons, pacemaker activity and posttetanic potentiation (PTP) in *Aplysia* neurons, the concentration of ethanol necessary to produce these effects (400-1100 mM) are greater than lethal levels in mammals⁹. (For comparison, the legal intoxicated dose in humans corresponds to a nearly 10 mM of ethanol.) Progress in this field have until recently been limited by a lack of evidence that acute, low doses of ethanol (<200mM) produces specific and well-defined changes in identifiable single neurons of relevant brain regions. However, this type of information are needed to permit eventual identification of the neural pathways that are affected by ethanol and to allow subcellular level biochemical mechanisms to be related to a functional framework.

In this report, a robust, acute modulation of cellular excitability in dentate granule cells by low-doses of ethanol will be described. Although the hippocampus is known to be susceptible to ethanol⁶, earlier studies of the other principal neurons (CA1 and CA3 pyramidal neurons) in this structure using intracellular recording has revealed mixed effects of ethanol¹⁷. It is recently demonstrated that low dose of ethanol can suppress the N-methyl-D-aspartate (NMDA)-receptors mediated current in cultured hippocampal neurons as well as the synaptic responses in CA1 neurons^{15,16}. Although these results may account for the susceptibility of the hippocampus to ethanol, it is possible that a modulation of dentate granule cells can also contribute to the overall depression of the hippocampal unit activity, as dentate granule cells provide direct inputs to the pyramidal neurons and serves as the major "input station" to this brain region. Detailed intracellular recordings were therefore made to determine if ethanol exert any electrical changes that were observable in hippocampal granule cells.

2.2 Methodology

Hippocampal slices^{2,12,18} were prepared from adult Sprague-Dawley rats (300-350 gm) and maintained in a Haas-type interface-chamber (Medical Systems, Inc.) as documented previously⁵. Intracellular recordings were carried out with 3 M potassium acetate-filled microelectrodes (90 - 180 M Ω) at 35 °C using an oxygenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (aCSF) of (in mM): 124 NaCl, 3.75 KCl, 1.25 KH₂PO₄, 2.0 MgSO₄, 2.0 CaCl₂, 25 NaHCO₃ and 10 glucose. The microelectrodes were fabricated to maximize the holding duration for these neurons. This usually lead to higher input resistance electrodes until electrical noise started to interfere with current injection and baseline noise levels. It is often found that the use of electrodes with low input resistances resulted in cells with more depolarized resting potentials which may be due to impalement injury. None of the neurons included in this report were spontaneously active. All had acceptable resting potentials (-85 to -110 mv), input resistance (32 - 75 M Ω) and repetitive firing ability.

The control responses of these neurons were also similar to those published previously for dentate granule cells (e.g. -85 - 90 mV¹³; -65 - 75 mV^{7,8,13}). The stimuli were delivered either extracellularly through a fine tungsten electrode positioned at the perforant path (*orthodromic stimulation*) at a point where the extracellular field potential is maximized or intracellularly through the recording glass micropipette at the dentate somatic layer (*somatic current injection*). The following responses were recorded in both control aCSF media and aCSF media with 50 mM ethanol: strength duration properties (described below), EPSPs for fixed-sized stimulus from orthodromic stimulation and repetitive firing response.

The firing threshold of the impaled neurons was characterized by finding the smallest current pulse amplitude ($I = Q/w$) necessary for eliciting an action potential at various (short as well as long) pulse widths (w). Each minimum current amplitude could then be plotted against its associated pulse width to give the strength duration curve (figure 2.2a). Separate strength duration curves were hence generated for (1) orthodromic extracellular current pulses and (2) somatic intracellular current pulses for each neuron. Orthodromic stimulation was carried out at a low frequency of 0.3 Hz (or less when

necessary) to avoid potentiation and/or habituation. This rate was based on paired pulse measurements by ourselves and others which suggested little to no pulse-to-pulse interaction beyond pulse delays of 300 msec¹.

To permit subsequent statistical analysis, each set of strength duration data was fitted to Lapicque's equation expressed in terms of charge¹⁰:

$$Q = \frac{Q_{rh} w}{T_m (1 - \exp(-w/T_m))}$$

where Q_{rh} and T_m , the rheobase charge and excitation time constant were estimated by nonlinear regression. These parameters can reflect changes in threshold such that larger values of Q_{rh} or smaller values of T_m indicate a higher firing threshold. These parameters reflect changes in firing threshold effectively at short pulse width of stimulation. This equation described the data well in most cases (9 out of 9 and 6 out of 9 cases {abbrev. 9/9 and 6/9} for orthodromic and somatic stimulation respectively with a criteria of $R^2 > 0.94$; fig 2.2b). The statistical comparisons were made using paired-t tests.

A solenoid-control valve system was used to produce the desired switching of media which in itself caused little or no

Figure 2.2a. The strength duration properties of a granule cell was shown by plotting injected current into soma against pulse width durations (upper figure) or vice versa (lower figure).

Figure 2.2b. The somatic strength duration data of another granule cell was described accurately by Lapicque's equation expressed in terms of charge (ordinate; $Q_{rh} = 1.00279$, $T_m = 0.3335$, $R^2 = 0.97$).

disturbances. Furthermore, the perfusion rate for control and ethanol aCSF were kept identical during each recording session, so that the degree of immersion of the slices in the chamber remained constant. With these precautions, consistent and precise data for the strength-duration relationship can readily be obtained from an individual neuron. The stability of this media switching method is illustrated in figures 2.2c & 2.2d with a cell that was apparently *insensitive* to 50 mM ethanol. Despite two solution changes, the repetitive firing responses remained steady while the resting potential fluctuated by less than 5 mV (between -89 and -84 mV) during the one and a half hour recording period.

2.3 Results

2.3.1 Effects of ethanol on orthodromically evoked response

Ethanol and control responses were compared in 16 neurons. The excitability changes induced by ethanol treatment differed for orthodromic and somatic excitation.

For *orthodromic* stimulation in control media, a small stimulus triggered an EPSP whose decay could be fitted with a simple

Figure 2.2c. Stability of the recording despite two solution changes in a neuron that was insensitive to ethanol. Stimulus = 0.4 nA, 100 ms. (Subdivisions in a and b: 20 mv and 25 ms).

Figure 2.2d. Ethanol media was introduced at 25 minutes and wash-out at 60 minutes. Stimulus = 1 nA, 100 ms. Calib.: 20 mv and 25 ms.

exponent using nonlinear regression (figure 2.3.1a; lower trace, $\tau = 9.1$ ms) whereas near threshold, EPSPs have much larger amplitudes, longer duration and often cannot be fitted with a single exponential (figure 2.3.1a, upper trace). In all but one cells, ethanol diminished the size of the EPSPs by 22.5 - 95 % ($p=0.0015$, mean difference= 10.94 mv; figure 2.3.1b), giving EPSPs with smaller amplitudes (if not close to zero) and shorter duration. These changes were often observed without concomitant changes in the resting potential (< 5 mv; figure 2.3.1c) and reversed upon returning to wash-out (figure 2.3.1d).

This depression of the EPSPs was accompanied by an increase in the orthodromic firing threshold. As illustrated in figure 2.3.1e, a threshold stimulus in control solution can no longer trigger an action potential in ethanol. This subthreshold response was not further increased by the application of a larger stimulus. The orthodromic strength duration properties demonstrated an apparent increase in the firing threshold across different combinations of stimulus strength and pulse widths of excitation (figure 2.3.1f). The rheobase charge and excitation time constant (Q_{rh} and T_m) were found to increase in 6/9 and 7/9 cases respectively (figs. 2.3.1g; $p =$

Figure 2.3.1a. Appearance of near threshold excitatory postsynaptic potential (EPSP; 2 V, 0.3 ms, upper trace) and low amplitude EPSP (2 V, 0.2 ms, lower trace) from orthodromic activation in control media.

Figure 2.3.1b Ethanol decreased the size of the EPSP evoked by subthreshold orthodromic stimulation.

figure 2.3.1c. Time course of the action of ethanol to the EPSP amplitude in a typical cell. Resting potential at A, B, C, D, E & F: -95, -100, -95, -94, -101 & -92 mV, Stimulus: 2 V, 0.3 ms.

Figure 2.3.1d. Reversible depression of EPSP by ethanol. The EPSP size in ethanol are often not increased by a larger stimulus.

Figure 2.3.1e. A suprathreshold stimulus which triggered an action potential in control media (upper) became subthreshold in ethanol (middle). Action potentials were clipped during acquisition.

Figure 2.3.1f. Ethanol increased orthodromic firing threshold in a neuron with a range of stimulus intensity.

0.013). The increases in threshold expressed by these parameters are limited to shorter pulse-widths, as the rheobase current is given by $I_{rh} = Q_{rh} / T_m$. Thus increasing Q_{rh} will tend to cancel the effect of a simultaneous increase in T_m (and vice versa). The experimental results support a threshold increase also up to a stimulus pulse-width of about 5 ms.

The switching of media alone cannot account for the observed drop in EPSP amplitude and increase in firing threshold as these changes can be reversed when switching back to control media. In particular, in 3 out of 5 cells the EPSPs recovered in amplitude when switching back to control after ethanol exposure (see figs. 2.3.1d & 2.3.1e). Although the recovery is usually incomplete, this was probably due to a short wash-out time (< 20 minutes) before the cells were eventually lost. Thus, the above results taken together suggested that the orthodromic firing response in granule cells was attenuated in 50 mM ethanol media.

In general, inhibitory postsynaptic potentials (IPSPs) were seldom observed in these neurons in control media except for the initial minutes after cell entry (when the resting potential would be stabilizing to more hyperpolarized values). This observation has also

Figure 2.3.1g. Increase orthodromic firing threshold due to ethanol expressed in terms of T_m and Q_{rh} .

been made by some workers who used the slice preparation^{7,8}. With respect to ethanol, there is no consistent correlation between the switch to 50 mM ethanol and the disappearance of the IPSP. Data from cells whose IPSP disappeared after switching to ethanol was not included in the analysis above. In two cells where the IPSPs persisted throughout the course of the experiment, no changes were observed in ethanol. Thus, it appeared that changes in IPSPs cannot account for the frequently observed depression of postsynaptic potentials in these neurons. (A more detailed examination of the role of inhibition can be found in Chapter 3.)

2.3.2 Effects on somatically evoked responses

For excitation with *intracellular somatic current injection*, ethanol had an opposite effect on the firing threshold. An excitatory effect is apparent in the changes in the strength-duration properties with ethanol treatment (8/9; figure 2.3.2a). Q_{rh} and T_m were found to *decrease* significantly in all 6 neurons examined where satisfactory fit were possible using the equation above (figure 2.3.2b; paired t-test, 95% confidence interval). This parameters suggests a lowering in the firing threshold of single action potential by ethanol primarily at

Figure 2.3.2a. Much shorter pulse-widths were necessary to evoke an action potential through somatic current injection in ethanol for various current amplitudes.

Figure 2.3.2b. Ethanol decrease somatic firing threshold expressed in terms of changes in T_m and Q_{rh} .

short pulse widths as changes in these parameters tended to cancel out at large pulse widths. However, this is partly due to the fact that Q_{rh} and T_m covary (see equation given in Methods). Experimentally, increase in threshold was observed up to a stimulus pulse-width of about 10 ms.

This lowering of the firing threshold of single action potentials was accompanied by a change in repetitive firing. For an identical current stimulus, the number of spikes evoked in ethanol was consistently higher than that in control (6/7). This effect was reversible in 3 out of 5 neurons (figure 2.3.2c). These observations suggest the somatic firing threshold is lowered by this dosage of ethanol. No uniform change, however, was observed with the morphology of single action potentials, afterhypolarization following repetitive firing or input resistance (figure 2.3.2d).

2.4 Discussion

The results described above are significant in several respects. First, a fairly uniform action of ethanol was observed in an identifiable group of neurons in the dentate somatic layer of the

Figure 2.3.2c. Ethanol induced a reversible increase in repetitive firing from direct somatic current injection. Stimulus: 0.6 nA, 100 ms.

Figure 2.3.2d. Lack of uniform effects of ethanol on input resistance of granule cells.

hippocampus. This is analogous to both earlier and recent reports that ethanol affect synaptic responses in CA1 pyramidal neurons¹⁵ and CA3 neurons¹⁷. The significance of a robust orthodromic depression in granule cells suggests that the previously reported depression in unit activity in various regions of the hippocampus should not be interpreted necessarily as local changes in neuronal properties (e.g. changes in CA1 and CA3 neurons only) but may be due to polysynaptic effects. Second, it was possible to show in detail that ethanol affects the firing threshold of individual granule cells. Although previous unit recording has shown somewhat similar elevation of threshold for activation, it was not possible to delineate what cellular elements or synaptic connections may be involved. The identification of an excitability depression intracellularly in granule cells will therefore permit one to further assess questions of mechanisms. Finally, dentate granule cells are widely considered to be the "input station" to the hippocampus through feedforward projections to CA1 and CA3 neurons and fires spontaneously *in vivo*¹¹, processing information arriving from the ipsilateral and contralateral entorhinal cortices. A depression in these neurons can therefore have profound consequences on the overall functioning of

the hippocampal neural subsystem, which presumably is closely related to place processing or recognition, epilepsy as well as memory.

The significance of an increase somatic excitability that is also reported here, however, is currently unclear. Likewise, the mechanisms of action of either of the effects described remained to be studied. Relative little is known about local synaptic interaction and neurotransmission in the dentate gyrus compared to the other regions of the hippocampus. Since no apparent changes in afterhyperpolarization, input resistance, inhibition or spike morphology were observed, however, it seems likely that some synaptic factors would probably be involved. Recent work has implicated the involvement of NMDA-receptors in the acute effect of ethanol in cultured hippocampal neurons and CA1 cells. It would therefore be of interest to determine if the reported effects of ethanol may be related to this type of glutamate receptors, since autoradiographic studies have also revealed a high density of NMDA-receptors in the inner molecular layer of the dentate gyrus similar to the outer molecular layer in the CA1 region⁴.

2.5 References

- [1] Abraham, W. and Bliss, T.V.P. An analysis of the increase in the granule cell excitability accompanying habituation in the Dentate Gyrus of the anesthetized rat. *Brain Res* 331 (1985) 303-313

 - [2] Alger, B.E., Dingledine, R., Henderson, A., Schwartzkroin, P., Whittingham, T.S. and Williams, John. Brain slice methods. In *Brain Slice*, Dingledine, R. (ed), 1984, Plenum, New York.

 - [3] Baskys, A., Berstein, N.K., Barolet, A.W. and Carlen, P.L. NMDA and quisqualate reduce a Ca-dependent K⁺ current by a protein kinase mechanism. *Neuroscience Letters* 112 (1990) 76-81

 - [4] Cotman, C.W., Monaghan, D.T., Ottersen, O.P. and Storm-Mathisen, J. Anatomical organization of excitatory amino acid receptors and their pathways. *Trends in Neuroscience* 10 (1987) 273 - 279

 - [5] Durand, D., Carlen, P., Gurevich, N., Ho, A. and Kunov, H.
-

Electrotonic parameters of rat Dentate Granule cells measured using short current pulses and HRP Staining" / *Neurophys.* 50: (1983) 1080-1097

[6] Folger, W.R. and Klemm W.R., Unit responsivity in various Hippocampal layers and the depressive effects of ethanol" *Physiology & Behavior* 21: (1978) 169-176

[7] Fournier, E., and Crepel, F., Electrophysiological properties of Dentate Granule cells in Mouse Hippocampal slices maintained in vitro. *Brain Research* 311: (1984) 75-86

[8] Fricke, R. A., and Prince, D. A. Electrophysiology of Dentate Gyrus Granule cells. / *Neurophysiol.* 51: (1984) 195-209

[9] Hunt, W.A. *Alcohol and Biological Membranes.* 1985. Guiford Press, New York.

[10] Jack, J., Noble, D., Tsien,R.W. *Electric Current Flow in Excitable Cells,* 1975, Oxford University Press.

- [11] Klemm, W.R. Effects of ethanol on nerve impulse activity. In Edward Majchrowicz and Ernest P. Noble, *Biochemistry and Pharmacology of Ethanol*, 2 (1980) pp. 243 - 267
- [12] Kelly, J.S. Intracellular recording from neurons in brain slices in vitro. In *Electrophysiology of Isolated Mammalian CNS Preparations*. Kerkut, G.A. and Wheal, H.V. (eds), Academic Press, 1981, New York.
- [13] Lambert, J.D.C. and Jones, R.S.G., Activation of NMDA receptors contributes to the EPSP at perforant path synapses in the rat dentate gyrus in vitro *Neurosci Lett.* 97 (1989) 323-328
- [14] Lima-Landman, M.T.R. and Albuquerque, E.X. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. *FEBS Letters* 247 (1989) 61-67.
- [15] Lovinger, D.M, White, G. and Weight, F.F. NMDA Receptor-mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. *J Neurosci* 10 (1990) 1372-1379

- [16] Lovinger, D.M, White, G. and Weight, F.F. Ethanol inhibits NMDA-activated ion current in Hippocampal neurons *Science* 243 (1989) 1721-1724
- [17] Siggins, G.R., Bloom, F.E., Mancillas, J. and Rogers, J. Electrophysiology of ethanol on central neurons. In Rubin Emanuel (Ed.) *Alcohol and the Cell: Annals of the New York Academy of Sciences*, 492 (1982) 350-366.
- [18] Teyler, T.J. Brain slice preparation: Hippocampus. *Brain Res. Bull.* 5 (1980) 391-403.
- [19] White, G., Lovinger, D.M, and Weight, F.F. Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Research* 507 (1990) 332-336
- [20] Yuen, G.L.F., Patil, M. and D. Durand. Effects of acute ethanol treatment on the firing threshold of hippocampal granule neurons. *Society for Neuroscience Abstr.* 16 (1990) 133.

CHAPTER III

CELLULAR SUBSTRATES OF ETHANOL ACTION

In this chapter, the possible basis for the observed effects of ethanol on granule cells are studied through the use of selective receptor antagonists. Portions of this chapter have been published as a short communication in Brain Research. The major results of this and the next chapter will be submitted to Journal of Neurophysiology. This work was supported in part by NIH Grants 1ROI-AA-06773-06 to Dr. D. Durand.

3.1 Abstract

Using standard intracellular recording techniques and selective antagonists to glutamate-receptor subtypes, we were able to show that a N-methyl-D-aspartate (NMDA) receptor mediated mechanism is implicated in the acute effects of ethanol on hippocampal granule neurons. In particular, the EPSPs were no longer sensitive to 50 mM ethanol when slices were pretreated with the antagonist for NMDA-receptors, 2-Amino-5-phosphonopentanoic acid (APV). This sensitivity to ethanol is not apparently affected, however, by other selective receptor antagonists to the nonNMDA glutamate and GABA_A receptor. This appears to be the primary factor (instead of electrotonic or inhibitory factors) that can account for the robust, suppressive effects of ethanol on the orthodromically evoked responses in these neurons. The linking of the effects of ethanol to the NMDA-receptors is extremely interesting as the latter are currently thought to relate to memory (e.g. LTP) and epilepsy, processes that are also affected by chronic ethanol exposure.

3.2 Introduction

We have previously demonstrated a robust, acute modulation of the excitability of hippocampal granule by low doses (50mM) of ethanol (Yuen et al 1991). In particular, whereas the firing threshold decreased for somatic current injection, the orthodromic responses (e.g. EPSPs) evoked from perforant path (extracellular) activation became suppressed in ethanol. Ethanol did not have any consistent effects on the morphology of single action potentials, spike adaptation, afterhyperpolarization following repetitive firing or input resistance. Thus it appears likely that the effects of ethanol may be mediated through changes in synaptic or electrotonic factors instead of ionic channels directly subserving spike and spike pattern generation. This interpretation is also bore out by a model of these neurons (Yuen and Durand, 1991) which suggests that the features of the action potential and repetitive firing mentioned above are determined primarily by somatic voltage-gated channels. The lack of effect of ethanol on these features therefore suggests that ethanol probably did not act on these channels directly.

Recent work have suggested that excitatory neurotransmission

in these neurons is mediated primarily by glutamate's action on two pharmacologically separable classes of receptors: one which is sensitive to NMDA (NMDA receptors) and another which is insensitive to NMDA (nonNMDA receptors, Lambert and Jones 1989). Thus, it would be of interest here to determine if any of the observed effects of ethanol can be related to these subclasses of glutamate receptors. Our strategy here was to pharmacologically block individual subtypes of receptors while examining the sensitivity of the resultant system to ethanol, focusing primarily on the orthodromic responses. The suppressive effect of ethanol on the orthodromic response will presumably be removed or altered given the appropriate receptor antagonists are applied. In the following, the experimental evidence relating the effects of ethanol to the glutamate receptor subtypes will be presented, followed by an assessment of the possible contribution of inhibitory synaptic conductances.

3.3 Methods

The techniques employed here for obtaining intracellular recordings from dentate granule cells in hippocampal slices have been described in detail previously (Alger et al 1984, Durand and

Carlen 1983, Fournier and Crepel 1984, Fricke and Prince 1984, Teyler 1980). 6,7-Dinitroquinoxaline-2,3-dione (DNQX), 2-Amino-5-phosphonopentanoic acid (APV) and bicuculline methchloride (BIC) were used as pharmacological blockers for the nonNMDA, NMDA and GABA-A receptors respectively (Research Biochemicals Incorporated). Due to the limited stability of long term intracellular recordings, it was often necessary to study the effects of the antagonists and ethanol separately (in distinct neurons). The concentrations for these drugs were chosen based on recent studies of these receptors either directly in hippocampal granule cells or pyramidal CA1 neurons: 10 μ M DNQX, 40 μ M APV and 15 μ M BIC (Baskys et al 1990, Collingridge et al 1988, Dale and Roberts 1985, Keller et al 1991, Lambert and Jones 1990, Urban et al 1990). These concentration of drugs were sufficient to block most or all of the corresponding receptors (DNQX, $IC_{50} = 2 \mu$ M). Since a significant number of neurons did not fire action potentials in response to orthodromic stimulation in these blockers, only cells that can response with action potentials to somatic current injection are considered acceptable for analysis. Also excluded are cells that exhibit

steady drifts.

Statistical analysis were performed on the amplitude of the EPSPs using the paired t-test. A summary of this analysis and related population description is provided in Table V.

3.4 Results

The effects of ethanol on each of the receptor antagonist-resistant orthodromic response are described below.

3.4.1 Effects of APV on control responses

The excitability changes induced by APV treatment resembled those induced by ethanol (n=10). In particular, the orthodromic responses were diminished (10 out of 10 cases) while the somatic excitability was enhanced in 40 μ M APV (4 out of 4 cases).

The action of 40 μ M APV on the subthreshold EPSP is shown for a neuron in figure 3.4.1a. On the average, the responses in APV stabilized about 10-15 minutes after perfusion. In 9/10 cases, the

amplitude of the EPSP was reduced 15-71% ($p=0.0004$, mean difference: 8.6 mV; see also Table V). The time course of APV action on the amplitude of the EPSP (for an identical stimulus) was shown in 3.4.1b. This effect was not attributable due merely to solution switching or insufficient drug incubation as in 3 out of 5 cells this effect of APV was reversed by returning to control aCSF solution (figure 3.4.1c).

The effects of APV on time course of the 'near-threshold' EPSP is clearly shown in terms of the rise and decay time of the EPSP (Table I). The EPSPs in some neurons were not included in this table as their amplitudes in APV were very close to zero, making the rise and decay time measurement unreliable. The results suggested that whereas the rise time remained similar, the decay observed in 40 μM APV often became faster than that in control (figure 3.4.1d; 5 out of 5 cells; see DISCUSSION).

The introduction of APV often impaired the ability of the cells to fire action potentials in response to an orthodromic stimulus (9/10). Thus it was not possible to obtain strength duration measurements as described in the last chapter. Nevertheless, in (3/3) cells where single spikes were evoked by a just threshold stimulus in

Figure 3.4.1a. Reduction of EPSP amplitude by 40 μ M APV.

Calibration: 5 mV, 10 ms.

Figure 3.4.1b. Time course of the action of APV on EPSP amplitude in a typical neuron.

Figure 3.4.1c. The reduction of the size of the EPSP by APV is reversible upon return to control media.

TABLE I

Rise and decay time of EPSP in control and APV media

<u>Cell</u>	rise time, t_r (ms)		decay time, t_d (ms)	
	<u>Control</u>	<u>APV</u>	<u>Control</u>	<u>APV</u>
1	2.7	2.55	66.9	49.2
2	2.5	2.2	84.4	11.6
3	3.6	3.2	39.6	9.4
4	3.4	4.4	42.8	25.6
5	3.0	3.0	31.6	22.4

Figure 3.4.1d. APV reduced EPSP decay time without apparent effects on rise time.

control media, the presence of 40 μM APV reduced these spikes to small subthreshold depolarization (figures 3.4.1e). The size of these depolarizations were not increased by a stronger stimulus (10 to 20 the stimulus strength in control).

The above changes were not due to a deterioration in the recording as APV also enhanced most cell's excitability in response to somatic current injection simultaneously (5/6) . This is illustrated in figure 3.4.1f for the repetitive firing response. Similar to the situation with ethanol media, APV decreased Q_{rh} and T_m for somatic current injection in all cases examined (4/4; $p=0.01$).

3.4.2 The NMDA-receptor hypothesis

As the pattern of excitability changes with APV matches closely with those of ethanol with respect to both orthodromic and somatic stimuli, it is reasonable to hypothesize that the effects of ethanol observed in these neurons are mediated at least in part through the NMDA subclass of the glutamate receptors. In fact, recent work have also provided much circumstantial evidence that are in favor of this hypothesis: [i] the EPSPs in dentate granule cells are mediated by glutamate receptors with a substantial contribution from the NMDA-

Figure 3.4.1e. Suprathreshold stimulus (2 V) in control evoked an action potential in APV (2 - 30 V). (Subdivisions: 20 mV, 20 ms).

Figure 3.4.1f. Repetitive firing with somatic injection is enhanced in the presence of APV. Subdivision: 20 mV, 25 ms.

subtype (Lambert and Jones 1989); [ii] a reversible depression of NMDA-related synaptic responses in adult hippocampal CA1 neurons can be produced with low doses of ethanol in the presence of GABA antagonists (Lovinger et al 1990, White et al 1990); [iii] moderate levels of NMDA receptors, second only to the highest concentrations seen in the CA1 region, are found in the inner molecular zone of the dentate gyrus (Cotman et al 1987) and [iv] low-doses of ethanol are capable of blocking NMDA-activated single-channel currents in rat hippocampal pyramidal cells (Lima-Landman and Albuquerque 1989).

To obtain experimental support for this hypothesis, the slices were first exposed to blockers of each of the relevant receptor subtypes and the sensitivity of the blocker-resistant EPSPs in these neurons to ethanol was examined.

3.4.3 Effects of ethanol on APV-resistant responses

In the presence of APV, ethanol produced mixed changes in the orthodromic responses (n=8). The effects of ethanol became evident between 10 to 15 minutes after the switching of media. Half of the cells examined did not show further depression upon the

introduction of ethanol (4/8; figure 3.4.3a).

Among the remaining cells, ethanol depressed the amplitude of the EPSP (33.1 %) in two cells and enhanced the EPSP in another (24.5%). Finally, in a cell whose EPSP was not reduced by by pretreatment with APV, ethanol also failed to produce any noticeable effects.

3.4.4 Effect of DNQX on control responses

In all cells examined, DNQX attenuated the orthodromic response consistently (9/9) without causing significant changes in somatic excitability.

The suppression of the subthreshold EPSP by 10 mM DNQX is illustrated in figures 3.4.4a. As is typical of most cells examined, the initial, fast phase of the EPSP was suppressed in the presence of DNQX (5/7). This is reflected in both the amplitude as well as the rise time of the EPSP. The size of the EPSP amplitude was reduced 10-39 % ($p=0.0004$, mean difference: 12.32 mv; see also Table V). A comparison of the rise and decay time of the EPSP in control and DNQX is given in figure 3.4.4b and TABLE II. Whereas the rise time was often increased in DNQX (6/7 cases), mixed changes were

Figure 3.4.3a Ethanol failed to further suppress the size of the EPSP in 50% of the cells examined. Calibration: 5 mv, 20 ms.

Figure 3.4.4a. Suppression of initial phase of the EPSP by DNQX.

Subdivisions: 5 mV, 40 ms.

TABLE II

Rise and decay time of EPSP in control and DNQX media

<u>Cell</u>	rise time t_r (ms)		decay time t_d (ms)	
	<u>Control</u>	<u>DNQX</u>	<u>Control</u>	<u>DNQX</u>
1	2.2	25	36	47
2	7.3	5.0	64	75
3	6.0	10	112	144
4	2.7	15	76	59
5	8.7	16	78	76
6	2.6	2.8	158	183
7	3.0	34	220	134

Figure 3.4.4b Changes in the EPSP rise and decay time duration with DNQX treatment. DNQX apparently increased rise time (up to 1100 %, cell number 1 and 8, off axis limit) while producing mixed effects in the decay time. Note difference in scale for the ordinate.

observed with the decay time. The decay time is defined as the duration from the peak of the EPSP to the time at 10 % of peak value, thus is presumably reflective of any slow component. Interestingly, in cells where the decay time was increased by DNQX, the asymptotic value of the EPSPs are somewhat larger than that in control.

The time course of the amplitude change due to the introduction of 10 μ M DNQX for a typical cell is shown in 3.4.4c. The responses in DNQX appeared to stabilize around 5 - 10 minutes after media switching. The effect of DNQX appeared to be reversible with respect to the amplitude and shape of the EPSP. Orthodromic stimulation, however, often failed to elicit action potentials in wash-out after exposure to DNQX (figure 3.4.4d, n=2). Among cells which retained their ability to fire action potentials orthodromically *in DNQX*, all exhibited a higher orthodromic firing threshold (3/5; figures 3.4.4e). Available data suggests that DNQX did not affect the somatic excitability of these neurons (3/3; figure 3.4.4f).

3.4.5 Effect of ethanol on DNQX-resistant responses

Most of the cells described in this section was pre-exposed to DNQX for 10 - 20 minutes before the recording session commenced.

Figure 3.4.4c. Time course of DNQX reduction of EPSP amplitude in a typical neuron showing stability of DNQX-resistant EPSP after 10 minutes.

Figure 3.4.4d. Recovery of EPSP in wash-out showing changes are not due to solution switching.

Figure 3.4.4e. Cells which were able to fire orthodromically in DNQX all exhibit a higher threshold.

Figure 3.4.4f. Somatic firing threshold was not apparently affected in DNQX.

In contrast to the case with APV-resistant EPSPs, ethanol consistently diminished the size of the EPSP in all cases examined (9/9).

The suppressive effect of ethanol on the DNQX-resistant EPSP is illustrated in figure 3.4.5a. The amplitude of the EPSP was depressed between 26 to 72 % of control values ($p=0.0062$, mean difference: 9.04 mv; see also Table V). The dramatic degree of depression among all the neurons resembled that observed when control EPSPs were exposed to ethanol or APV but not DNQX (figure 3.4.5b). The time course of the EPSP amplitude modulation is shown in figure 3.4.5c for a typical neuron. It is unlikely that this effect of ethanol was due to nonspecific effects such as media switching or incomplete drug incubation since the size of the EPSP recovered to larger values than that in ethanol after returning to DNQX-only media (figure 3.4.5d; $n=2$).

During ethanol exposure, more intense orthodromic stimuli (up to 20 or 50 fold control levels) were also applied to determine if the diminished EPSPs could be enhanced or recruited simply by stronger excitation (so that the voltage dependent block of NMDA receptors may be presumably removed). Most neurons examined did not respond with a larger orthodromic response (5/6). Among

Figure 3.4.5a. DNQX-resistant EPSPs were suppressed by ethanol reliably. Subdivisions: 5 mv, 20 ms.

Figure 3.4.5b. Ethanol consistently suppressed the EPSP in all cells examined.

Figure 3.4.5c. Depression of DNQX-resistant EPSP by ethanol in a neuron.

Figure 3.4.5d. Recovery of EPSP in DNQX media after ethanol exposure.

neurons which were considered acceptable for analysis (6/9), ethanol increased the rise time and decreased decay times of the DNQX-resistant EPSPs (figure 3.4.5e; Table III). Available data suggests that somatic excitability was not sensitive to ethanol after exposure to DNQX (3/3; figure 3.4.5f).

Thus, there appears to be a clear difference between the sensitivity of DNQX-resistant and APV-resistant EPSPs to ethanol, which suggests that ethanol selectively affect the NMDA-receptors mediated current. The basis for the changes in somatic excitability, however, is currently unclear. A possibility not ruled out by our results is a lower level of tonic inhibition mediated by interneurons which possess NMDA-receptors. As NMDA-receptors are blocked by APV or ethanol, a decrease in tonic inhibition in turn leads to higher somatic excitability. However, this scenario does not account for the lack of sensitivity of somatic excitability to ethanol in DNQX.

3.4.6 Role of inhibition

The presence and role of inhibition in the effects of ethanol was assessed primarily through the monitoring of IPSPs. Analogous

Figure 3.4.5e. Ethanol increased EPSP rise time while decreased decay in DNQX media. In cell 3 and 6, the rise time increased to 544 and 647 % respectively (off axis limit).

TABLE III
Effects of ethanol on DNQX-resistant EPSPs

<u>Cell</u>	rise time t_r (ms)		decay time t_d (ms)	
	<u>DNQX</u>	<u>ETOH/DNQX</u>	<u>DNQX</u>	<u>ETOH/DNQX</u>
1	2.7	14.7	75.1	47.3
2	10.5	15.8	90	75
3	5.1	33	122.1	85.8
4	1.65	2.75	43.8	12.6
5	3.0	2.8	170	117
6	11	13	309	193.5

Figure 3.4.5f. Lack of obvious effects of ethanol on somatic excitability in DNQX.

to a number of previous reports (Fournier and Crepel, 1984, Crunelli et al 1981), IPSPs are infrequently observed intracellularly in the in vitro hippocampal slice preparation. Furthermore, ethanol appears to affect only a fraction of the cells that exhibit IPSPs (section 3.4.6.1). It is however possible that ethanol may introduce changes in a *shunting* IPSP which can participate in the postsynaptic potential and which does not present itself as a recordable IPSP. To examine if ethanol would be effective upon blockage of both types of inhibition, the GABA_A antagonist, bicuculline (BIC) was used to study the ethanol sensitivity of BIC- and BIC/DNQX-resistant orthodromic responses (sections 3.4.6.2 & 3.4.6.3). The results suggest that, although a role of inhibition in ethanol's orthodromic effects cannot be ruled out, changes in inhibition alone is insufficient to account for the orthodromic excitability changes described in the last chapter.

3.4.6.1 General prevalence of inhibition

13 of 37 cells sampled exhibited some form of inhibition as monitored by the presence of IPSPs (37.8 %). 15 of the 37 cells were recorded in DNQX media, which through blocking the fast non-NMDA component permits the fast IPSPs to be observed more

readily (if present).

The frequency of observing IPSPs in DNQX media was slightly higher than from that in control media (50 %), although the overall results from DNQX or control types of media were similar. The IPSPs from these neurons can be classified as in other neurons into two components: a fast component resembling the GABA_A response and a slow component similar to the GABA_B response, and were observed in 8 and 6 instances respectively (out of 37 cells). Often only one of the two components is observed in any individual neuron. The fast response, which is sometimes evoked in the absence of action potential firing and have a time course of less than 50 ms is illustrated in figure 3.4.6.1a. The slow component was more easily identified by a peak amplitude at around 200 ms from the stimulus.

Based on the theoretical equilibrium potentials of chloride or potassium ions (-90 to -98 mv), one would not expect to observe hyperpolarizing IPSPs at the normal resting potentials of these neurons (-90 to -100 mv) due to a lack of electrochemical gradient. However, we were able to observe IPSPs or paired pulse inhibition in some neurons that have resting potentials that were close to 100 mv (fig. 3.4.6.1b). The resting potential was experimentally determined as

Figure 3.4.6.1a. Typical appearance of the fast (upper) and slow (lower) IPSP in granule cells. Resting potentials: - 93 and -87 respectively. Subdivisions: 10 mV, 40 ms (upper); 4 mV, 100 ms (lower).

Figure 3.4.6.1b. IPSPs were observed at both depolarized as well as hyperpolarized resting potentials (cell number 23 to 30) .

the difference between the observed DC potential during intracellular recording and the extracellular voltage monitored immediately after the cell was dislodged. An average offset of 10 - 15 mv is commonly encountered, although values up to 20 mv have been observed. Thus it is possible that some of these IPSPs were actually measured at a true resting potential of about 90 mv. Similar large, negative resting potentials in these neurons have also been reported by others (Lambert and Jones 1989, Scharfman 1990). However, no direct correlation between IPSPs and resting potential was described. Mixed results have been reported in the literature for the presence of IPSPs in these neurons. Whereas Fricke and Prince (1984) reported 31 out of 34 cells exhibited a hyperpolarizing IPSP, Fournier and Crepel (1984) reported only 4 out of 32 showed any type of IPSPs (hyperpolarizing or shunting) and Crunelli et al (1981) reported that "on more than 90 granule cells tested we have never seen hyperpolarization induced by GABA application to the somatic region".

The ethanol sensitivity of the orthodromic responses were evaluated in 11 cells. In some neurons the IPSPs were observed immediately after cell entry, but disappeared a few minutes later as the resting potential stabilized to a more negative value (e.g. -75 to -90

mv). In these cases, ethanol did not introduce any observable changes in inhibition (2/11; figs. 3.4.6.1c). The 'hump' seen in the middle record at about 20 ms from the spike was sensitive to both APV and ethanol and cannot be interpreted as a depolarizing IPSP revealed by a negative resting potential. In the remaining cells where IPSPs could be observed after ethanol infusion (9/11), ethanol did not seem to affect the fast component and produced mixed effects on the slow component (figure 3.4.6.1d).

Based on our measurements, a possible interpretation is that ethanol only produced noticeable changes in the inhibition of a small percentage of granule cells. However, the results could also reflect our inability to monitor the inhibitory currents, as would be the case if a shunting instead of a hyperpolarizing inhibition is involved. This hypothesis is therefore further assessed by experiments using bicuculline (BIC), which is capable of blocking either type of inhibition.

3.4.6.2 Effect of ethanol on bicuculline-resistant responses

Bicuculline (BIC), a specific blocker of GABA_A receptors, was used to assess the effects of ethanol on the excitability of these cells in

Figure 3.4.6.1c. Effect of ethanol on fast IPSP. Calib.: 15 mV, 20 ms.

Figure 3.4.6.1d. Non-uniform effects of ethanol on IPSP amplitudes.

the absence of fast inhibition. In these experiments, the slices were usually exposed to 15 μ M bicuculline (BIC) for 15 - 20 minutes before the data from any cell was acquired (in order to obtain stabilized responses). The responses of granule cells in 15 μ M BIC are qualitatively similar to those in control media, except when a sufficiently large orthodromic stimulus is imposed, some cells fired multiple action potentials (instead of firing single action potentials as in control). In all cases examined, ethanol diminished the orthodromic excitability through reducing the size of the EPSP and/or raising the threshold for burst-firing.

The depression of the subthreshold responses is illustrated in figure 3.4.6.2a for a typical cell and figure 3.4.6.2b for the whole population of neurons with acceptable data (n=7). Ethanol depressed the amplitude of the EPSP by 13.7 - 86.5 percent ($p=0.0078$, mean difference: 11.32 mv; see Table V). The decrease in EPSP amplitude is somewhat smaller than that in control/ethanol media. For example, less incidence of close to zero size EPSP are observed in BIC. An analysis of the rise time and decay of the EPSP showed that ethanol decreased both of these parameters (4/5, figs. 3.4.6.2c; Table IV). A higher firing threshold in ethanol is observed in most cases in terms

Figure 3.4.6.2a. Ethanol suppressed EPSP in the presence of 15 μ M bicucullinep. Subdivisions: 10 mv, 20 ms.

Figure 3.4.6.2b. Ethanol depressed the size of the EPSP in all neurons examined.

Figure 3.4.6.2c. Ethanol decreased both the rise and decay time of BIC-resistant EPSPs.

TABLE IV
Rise and decay time of EPSPs in BIC and
ETOH/BIC media

<u>Cell</u>	rise time, t_r (ms)		decay time, t_d (ms)	
	<u>BIC</u>	<u>ETOH/BIC</u>	<u>BIC</u>	<u>ETOH/BIC</u>
1	6.3	4.4	56.6	58.8
2	5.7	4.8	51.3	27.8
3	8.2	5.2	67.2	48.0
4	4.8	4.2	47.7	40.2
5	4.2	5.4	63.6	41

of a failure of a previously suprathreshold stimulus to evoke an action potential (3/6; figure 3.4.6.2d) or a smaller number of spikes evoked in 15 μ M BIC (3/6; figure 3.4.6.2e).

A summary of the effects of various drugs on the orthodromically evoked EPSP is given in Table V.

3.4.6.3 Effects of ethanol on BIC/DNQX-resistant responses

The stabilized responses of granule cells in 15 μ M BIC and 10 μ M DNQX differed from that in 15 μ M BIC in that the neurons were in general much more excitable, more prone to firing bursts while action potentials are generated even at very low levels of stimulation (figure 3.4.6.3a). In addition, the action potentials tended to have lower amplitudes. Due to the all-or-none nature of the firing response, subthreshold EPSPs were not obtainable for making comparisons as was done before for other treatments. Ethanol nonetheless exerted a depressive effect on the orthodromic spiking response in all but one neuron examined (7/8). In particular, ethanol reduced both multiple firing or single action potentials to a subthreshold response (figure 3.4.6.3b). Similar to the situation in

Figure 3.4.6.2d. Suprathreshold stimulus in BIC can no longer trigger an action potential in ethanol, regardless of a larger applied stimulus. Subdivisions: 20 mV, 20 ms.

Figure 3.4.6.2e. Ethanol reduced the number of spikes evoked from an identical orthodromic stimulus. Subdivisions: 20 mV, 20 ms.

TABLE V
Effects of various drugs on EPSP

<u>Drug (n):</u>	<u>Mean Amp</u>	<u>Std. Dev.</u>	<u>Range</u>	<u>Paired t-test</u>	<u>Mean Diff.</u>
aCSF (12)	26.943	8.081	11 - 39		
ETOH	12.06	7.427	0.8-30	0.0015	10.937
aCSF (10)	16.0567	7.843	8.6 - 29.5		
APV	8.067	4.273	3.6 - 15.2	0.0004	8.6
APV (10)	8.45	4.4	3.6 - 15.2		
ETOH/APV	6.85	5.533	0.8 - 16.3	NS	1.287

TABLE V

Effects of various drugs on EPSP (ctn'd)

<u>Drug (n):</u>	<u>Mean Amp</u>	<u>Std. Dev.</u>	<u>Range</u>	<u>Paired t-test</u>	<u>Mean Diff.</u>
aCSF (9)	23.11	11.677	13 - 43		
DNQX	10.79	6.437	4.4 - 22.4	0.0004	12.324
DNQX (9)	13.46	9.616	4.4 - 33.6		
ETOH/DNQX	4.425	3.75	0.4 - 11.2	0.0062	9.036
BIC (7)	21.866	11.419	7.86 - 36		
ETOH/BIC	10.534	6.044	1.87 - 16.8	0.0078	11.32

Figures 3.4.6.3 a & b. Only bursting type orthodromic responses were obtained in BIC/DNQX media (a, upper). These 'bursts' are suppressed to subthreshold response in ethanol (b, lower). Results obtained from same cell; resting potentials : -76 & - 69 mV. Subdivisions: 20 mV, 40 ms.

control or DNQX media, slow inhibition was observed infrequently (1/8; range of resting potentials: -58 to -95 mv). In one neuron where a slow IPSP can be observed, ethanol did not affect the amplitude or time course of the IPSP (fig. 3.4.6.3c).

Figure 3.4.6.3c. Lack of apparent effects of ethanol on slow inhibitory postsynaptic potentials. Resting potential : -85 & -89 (ethanol) mV; subdivisions: 10 mV, 50 ms.

3.5 Discussion

3.5.1 Basic issues related to the conductances underlying the PSPs in hippocampal granule cells

Although the focus of this work is on the effect of ethanol on the electrical responses of hippocampal granule cells, the results of our experiments raised several basic questions that were not addressed explicitly in previous discussions: [i] Does NMDA-receptors play a significant role in the orthodromically evoked EPSPs of dentate granule cells ? [ii] Why is the time decay of the NMDA-receptors mediated EPSPs reported here apparently shorter than those reported originally in culture mouse and other neurons (e.g. 40 ms compared to 150 ms; Mayer and Westbrook 1987, Lester et al 1990) ? [iii] Why was the magnesium block apparently ineffective in preventing the NMDA-receptors from participating in the EPSP ? Issues more closely related to the effects and mechanism of ethanol action in these cells are discussed in the next section.

[i] NMDA-receptors mediated EPSPs in dentate granule cell ?
Although the existence of NMDA-receptors in hippocampal granule

cells has been disputed in the past (e.g. Mody and Heinemann 1987, Lambert and Jones 1989), recent work using autoradiography, intracellular recording, whole-cell as well as single channel patch-clamp recording has repeatedly confirmed their presence in these neurons (Keller et al 1991, Urban et al 1990). Several factors which may have made the identification of these channels in current clamp difficult include : (1) the efficacy of the antagonist used previously for NMDA-receptors appeared to be inadequate (Mayer and Westbrook 1987); (2) a low ratio of the NMDA-receptors and nonNMDA-receptors may result in a short instead of a long decay time course (see below and Chapter 4) and (3) the normal slow decay of the NMDA-conductance is probably masked by inhibition. This last factor was observed most clearly in the presence of DNQX, when the duration of the EPSP became much longer than that in control. Therefore much current evidence supports a role of the NMDA-receptors in the EPSP in dentate granule cells.

It remains unclear, however, why the fraction of the EPSP that is sensitive to APV (on the average 50%) in our preparation appears to be significantly larger than that reported by others. The proportion of NMDA to nonNMDA receptors does vary among dentate granule

neurons, however, since APV tended to block the EPSPs to different extent even among granule cells.

[ii] NMDA-receptors mediated 'fast' EPSPs ? Although the decay time constants of the component blocked by ethanol in our preparation is somewhat shorter than the values reported originally in culture mouse and other neurons (e.g. 40 compared to 150 ms; Mayer and Westbrook 1987, Lester et al 1990), this does not invalidate the assumption that these components are related to the NMDA-receptors for a number of reasons. [a] The slow components of the EPSP are sensitive to APV (see figure 3.5.2a). [b] Similar 'short-duration' EPSPs pharmacologically related to the NMDA-receptors have been observed in rat granule cells (Lambert and Jones 1989, Mody and Heinemann 1987), human hippocampal granule cells (Urban et al 1990), CA1 (Collingridge et al 1988, Lovinger et al 1990, Poolos and Kocsis 1990) and other neurons (Dale and Roberts 1985, White et al 1990). [c] The intracellular recording experiments related to this study was carried out at a temperature of 35 C, which can lead to faster rates of activation and decay than the values obtained from room-temperature voltage or current -clamped experiments (performed at 25 C). In accordance with this, all of the studies cited in

[b] which reported 'fast' NMDA-receptors mediated EPSPs except the study by Dale and Roberts (1985) were conducted at 35 °C. [d] Computer simulations suggest that a smaller ratio of NMDA compared to nonNMDA receptors (<1) can also lead to an underestimation of the decay time constant from peeling analysis (figure 3.5.1a). This underestimation of the slow EPSP decay is accentuated at the temperature of our experiments (figure 3.5.1b, assumed $Q_{10}=3.0$, 35 °C; see Chapter 4 for details of the simulation). The validity of a peeling analysis in this context is illustrated in figure 3.5.1c where a late linear phase is clearly seen in the $\log(\text{amplitude})$ plot for the decay of the EPSP. This is due to the fact that the time constant of the NMDA-receptors mediated current is well separated from the other time constants in the system (NMDA-receptor decay time constant: >100 ms, nonNMDA-receptor decay time constant: < 5 ms, electrotonic time constant: 15 ms). Not all acceptable cells were used in this analysis as the amplitudes of some EPSPs are very close to zero in ethanol with a significant noise level, thus making the logarithmic operation involved difficult.

Thus the ratio of receptors and the effect of temperature (factors [c] and [d]) may together account for the wide range of time

Figure 3.5.1a Extracted decay from model varies with receptor ratio.

Figure 3.5.1b Extracted decay from model with Q_{10} correction.

Figure 3.5.1c Examples of degree of fit obtained from peeling analysis of decay. Waveforms are from same cells as in figures 4.9 - 4.12.

constants reported for the NMDA-receptors mediated EPSPs currently found in the literature.

[iii] Apparent lack of magnesium block: It is unclear why the presence of 2.0 mM of magnesium failed to block completely the NMDA-receptors mediated EPSP in our preparation. However, this observation is not unique to this study. For example, Lambert and Jones (1989) reported similar APV-sensitive EPSPs in dentate granule cells at a resting potential of -85 mv and identical concentration of extracellular magnesium. Similar results have also been reported by others (Mody and Heinemann 1987, Urban et al 1990, Collingridge et al 1988, Lovinger et al 1990). Computer simulations using an experimental unblocking function described by Ascher and Nowak (1988) and typical equations for a synaptic conductance also reliably generated significant synaptic potentials (see Chapter 4). Based on the parameters and results of these simulation, it is likely that the lack of magnesium block observed was due to both [a] nonzero resting levels/opening of NMDA-receptors and [b] the relative positioning of the blocking curve relative to the resting potential (see figure 4.7). A recent single-channel patch clamp study of the NMDA-receptors isolated from granule cells supported the notion that a significant

population of these receptors is active at rest (Keller et al 1991).

3.5.2 Involvement of NMDA receptors in the acute depression of orthodromic response by ethanol

Although a role of the NMDA-receptors in the postsynaptic potential (PSP) of hippocampal granule cells is apparent, other candidates such as nonNMDA receptors and GABA_A receptors may also participate in the effects of ethanol. The inhibitory postsynaptic potential related to the GABA_B receptors, IPSP_B, however, is in general small in amplitude and effective at a later time than that concerned most of the reported results here. We have demonstrated previously that the PSP is clearly suppressed by 50 μ M of ethanol. This effect of ethanol persisted to different degrees in the presence of the three receptor antagonists. The resemblance to the results in aCSF control media can be roughly ranked in the order: 10 μ M DNQX > 15 μ M BIC > 40 μ M APV, based on a comparison of the consistency and percentage of suppression of the PSP amplitude and decay times. The specific role of the NMDA receptors in this effect is suggested by: [i] the extracted decay time constant of the EPSPs in ethanol are similar

to those in APV in that slower components than 30 ms are no longer observed (figure 3.5.2a and b); [ii] the obvious difference between the ability of APV and DNQX to impair the orthodromic suppression by ethanol. The similarity between the consistency with which ethanol blocks the PSPs in DNQX and aCSF control media suggests that the reduction of nonNMDA receptors failed to reduce the efficacy of ethanol. This suggests that these receptors are less important in the effects of ethanol in granule cells; [iii] if the effect of ethanol on the orthodromic response is due purely to a change in passive properties (e.g. attenuation due to dendritic tree), then one may expect that a larger responses would be elicited if a larger size stimulus is applied. The failure of more intense orthodromic stimulation to elicit larger postsynaptic responses in both DNQX and aCSF control media therefore suggests that dendritic electrotonic factors cannot in itself account for the small PSPs observed in ethanol; [iv] the relative lack of effect of the reduction of GABA_A receptors by BIC on ethanol's action; [v] previous studies have demonstrated dose-dependent effects of ethanol on isolated NMDA-receptors (Lima-Landman and Albuquerque 1989) in terms of both enhancement and depression of channel opening. At the concentration level used in our experiment,

Figure 3.5.2a Extracted decay time constants in control and ethanol.

Figure 3.5.2b Extracted decay time constants in various media.

a similar depression of the receptor opening is predicted from the patch-clamp results.

3.5.3 The role of inhibition and electrotonus

The results presented above, however, produce a somewhat mixed picture for the involvement of GABA_A receptors. Whereas the analysis based on the occurrence of fast IPSPs suggests they are infrequently observed and not consistently affected by ethanol, the amplitude reduction of the EPSP in BIC appeared to be weaker than that in aCSF control media. This suggests that our results can also be interpreted alternatively by the presence of a shunting inhibition, which may not show up as a negative potential in the intracellular recordings. According to Lambert and Jones (1990), however, "GABAergic inhibition did not seem to play a major role in shaping the synaptic response since picrotoxin (up to 20 mM) only occasionally caused a slight prolongation of the falling phase of the EPSP". Other evidence suggesting a probable involvement of inhibition in the normal orthodromic response of these neurons include: [i] repetitive firing are produced from orthodromic stimulation in BIC; [ii] a computer model using only glutamate

receptors in the dendrites also show tend to discharge repetitively; [iii] a peeling analysis of the EPSP decay revealed that better fit using single exponential models can often be achieved for the data in BIC in comparison to control and DNQX media, thus suggesting that a GABA_A component may be present in the normal PSP of granule cells; [iv] GABAergic synaptic boutons are observed to encircle granule cell soma and proximal dendrites (Kosaka et al 1984). Thus the somewhat reduced efficacy of ethanol in BIC in terms of EPSP amplitude reduction may perhaps be explained by effects of ethanol on this component.

The possible role of inhibition does not invalidate, however, the role of the NMDA-receptors in ethanol's suppression of the orthodromic response as the results in BIC media are nevertheless similar to that in aCSF control media. Similarly, neither should changes in passive properties be dismissed as a factor that may be affected by ethanol in our preparation (see Chapter 4).

It is also apparent from the preceding discussion that the basic mechanisms for inhibition in granule cells are poorly understood at present and demands more systematic investigation in the future.

A significant perspective gained from this work is the fact that

NMDA-receptors are involved in the modulation of these neurons' basic threshold properties during normal cellular functions. This is in contrast to much prevalent work that views these receptors as factors (or 'switches') that are induced (or activated) in special processes such as learning and memory (e.g. LTP and epilepsy).

The linking of the acute effects of ethanol to NMDA-receptors also presents the intriguing opportunity to study the development of the neuronal effects of ethanol. It is well known that the chronic effects of ethanol include impairment of memory as well as predisposition towards seizures upon withdrawal. Both of these conditions have recently been linked to the NMDA-receptors and the hippocampus (see for example, Mody and Heinemann 1987, Collingridge and Bliss 1987). Furthermore, acute or ethanol treatment blocks long term potentiation (LTP), a probable candidate for long-term memory (Sinclair and Lo 1986, Durand and Carlen 1984). Ethanol is also well-known for its ability to alleviate seizures. Thus it may prove possible to follow the development of chronic ethanol responses through a monitoring of the NMDA-receptors along with others factors that actively interact with it.

3.6 References

- [1] Abraham, W. and Bliss, T.V.P. An analysis of the increase in the Granule cell excitability accompanying habituation in the Dentate Gyrus of the anesthetized rat. *Brain Res* 331 (1985) 303-313

- [2] Alger, B.E., Dhanjal, S.S., Dingledine, R., Garthwaite, J., Henderson, G., King, G.L., Lipton, P., North, A., Schwartzkroin, Sears, T.A., Segal, M., Whittingham, T.S. and Williams, John. Brain slice methods. In *Brain Slice*, Dingledine, R. (ed), 1984, Plenum, New York.

- [3] Ascher, P. and Nowak, L. The role of divalent cations in the N-methyl-d-aspartate responses of mouse central neurones in culture. *J Physiol.* 399 (1988) 247-266

- [4] Baskys, A., Berstein, N.K., Barolet, A.W. and Carlen, P.L. NMDA and quisqualate reduce a Ca-dependent K⁺ current by a protein kinase mechanism. *Neuroscience Letters* 112 (1990) 76-81

- [5] Collingridge, G.G., Herron, C.E. and Lester, R.A.J. Synaptic activation of N-methyl-d-aspartate receptors in the Schaffer Collateral-commissural pathway of rat hippocampus. *J Physiol.* 399 (1988) 283-300
- [6] Cotman, C.W., Monaghan, D.T., Ottersen, O.P. and Storm-Mathisen, J. Anatomical organization of excitatory amino acid receptors and their pathways. *Trends in Neuroscience* 10 (1987) 273 - 279
- [7] Crunelli, V, Assaf, S. Y. and Kelly, J. S. Intracellular recordings from Granule cells of the Dentate Gyrus in vitro. In *Electrophysiology of Isolated Mammalian CNS Preparations*. Kerkut, G.A. and Wheal, H.V. (eds), 1981, Academic Press, New York.
- [8] Dale, N. and Roberts, A. Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in *Xenopus* embryos. *J Physiol* (1985) 35-59.
-

- [9] Durand, D., Carlen, P., Gurevich, N., Ho, A. and Kunov, H. Electrotonic Parameters of Rat Dentate Granule cells measured using short current pulses and HRP Staining" *J Neurophys.* 50: (1983) 1080-1097

- [10] Durand, D. and Carlen, P. Impairment of long term potentiation in rat hippocampus following chronic ethanol treatment. *Brain Research* 308: (1984) 325-332

- [11] Folger, W.R. and Klemm W.R., Unit responsivity in various Hippocampal layers and the depressive effects of ethanol" *Physiology & Behavior* 21: (1978) 169-176

- [12] Fournier, E., and Crepel, F., Electrophysiological properties of Dentate Granule cells in mouse Hippocampal slices maintained in Vitro. *Brain Research* 311: (1984) 75-86

- [13] Fricke, R. A., and Prince, D. A. Electrophysiology of Dentate Gyrus Granule cells. *J Neurophysiol.* 51: (1984) 195-209

- [14] Griller, S. Neural Networks Models of Vertebrate Sensorimotor Systems. *Annual Meeting, Society for Neuroscience, Session 138.1*, New Orleans, Louisiana, November 10-15, 1991.

- [15] Hunt, W.A. *Alcohol and Biological Membranes*. 1985. Guiford Press, New York.

- [16] Jack, J., Noble, D., Tsien, R.W. *Electric Current Flow in Excitable Cells*, Oxford University Press, Cambridge, 1975.

- [17] Keller, B.U., Konnerth, A. and Yaari Y. Patch clamp analysis of excitatory synaptic currents in granule cells of rat hippocampus. *J Physiol.* 435: 275 - 293 (1991)

- [18] Kelly, J.S. Intracellular recording from neurons in brain slices in vitro. In *Electrophysiology of Isolated Mammalian CNS Preparations*. Kerkut, G.A. and Wheal, H.V. (eds), 1981, Academic Press, New York.

- [18] Klemm, W.R. Effects of Ethanol on Nerve Impulse Activity. In Edward Majchrowicz and Ernest P. Noble, *Biochemistry and Pharmacology of Ethanol*, 2 (1980) pp. 243 - 267
- [19] Kosaka, T., Hama, K. and Wu, J. GABAergic synaptic boutons in the granule cell layer of rat Dentate Gyrus. *Brain Research* 29 (1984) 353 -359
- [20] Lambert, J.D.C. and Jones, R.S.G., Activation of NMDA receptors contributes to the EPSP at Perforant Path synapses in the rat Dentate Gyrus in vitro *Neurosc Lett.* 97 (1989) 323-328
- [21] Lambert, J.D.C. and Jones, R.S.G., A reevaluation of excitatory amino acid-mediated synaptic transmission in rat Dentate Gyrus. *J Neurophysiology.* 64 (1990) 119-132
- [22] Lester, R.A.J., Clements, J.D., Westbrook, G.L. and Jahr, C.E. Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* 346 (1990) 565-567

- [23] Lima-Landman, M.T.R. and Albuquerque, E.X. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat Hippocampal Pyramidal cells. *FEBS Letters* 247 (1989) 61-67.
- [24] Lovinger, D.M, White, G. and Weight, F.F. NMDA Receptor-mediated synaptic excitation selectively inhibited by ethanol in Hippocampal slice from adult rat. *J Neurosci* 10 (1990) 1372-1379
- [25] Lovinger, D.M, White, G. and Weight, F.F. Ethanol inhibits NMDA-activated ion current in Hippocampal neurons *Science* 243 (1989) 1721-1724
- [26] Mayer, M.L. and Westbrook, G.L. The action of N-methyl-D-aspartic acid on Mouse Spinal neurones in culture. *J Physiol.* 361 (1985) 65-90.
- [27] Mayer, M.L. and Westbrook, G.L. The physiology of excitatory amino acids in the vertebrate central nervous system. *Progress in Pharmacology* 28 (1987) 197-276.

- [28] Mody, I and Heinemann, U. NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature* 326 (1987) 701-704
- [29] Patil, M., Durand, D., Yuen, G.L. and Chintalacharuvu, R. Estimation of passive electrotonic parameters using the inverse Fourier transform technique to study ethanol effects on Granule cells. *Soc. Neuroscience Abst.* 17 (1991) 1336
- [30] Poolos, N.P. and Kocsis, J.D. Dendritic action potentials activated by NMDA receptor-mediated EPSPs in CA1 Hippocampal Pyramidal cells. *Brain Research* 524 (1990) 342-346
- [31] Scharfman, H. Personal communication, 1990.
- [32] Siggins, G.R., Bloom, F.E., French, E.D., Madamba, S.G., Mancillas, J., Pittman, Q.J. and Rogers, J. Electrophysiology of Ethanol on Central Neurons. In Rubin Emanuel (Ed.) *Alcohol and the Cell: Annals of the New York Academy of Sciences*, 1987, 492 pp. 350-366.

- [33] Sinclair, J. G. and Lo, G. F. Ethanol blocks tetanic and calcium-induced long-term potentiation in the Hippocampal slice. *Gen Pharmac* 17: (1986) 231-233.
- [34] Teyler, T.J. Brain slice preparation: Hippocampus. *Brain Res. Bull.* 5 (1980) 391-403.
- [35] Urban, L., Aitken, P.G., Friedman, A. and Somjen, G.G. An NMDA-mediated component of excitatory synaptic input to Dentate Granule cells in 'epileptic' human hippocampus studied in vitro. *Brain Research* 515: 319-322 (1990).
- [36] White, G., Lovinger, D.M, and Weight, F.F. Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Research* 507 (1990) 332-336
- [37] Yuen, G.L.F., Patil, M. and D. Durand. Effects of acute ethanol treatment on the excitability of hippocampal granule neurons. *Brain Research* 563 (1991) 315-320.

- [38] Yuen, G.L.F., and D. Durand. Reconstruction of Hippocampal granule cell electrophysiology. *Neuroscience* 41 (1991) 411-423.

CHAPTER IV

MODELLING THE EFFECTS OF ETHANOL ON HIPPOCAMPAL GRANULE NEURONS

A model is developed in this chapter which describes ethanol's modulation of the orthodromic responses. This chapter will be submitted to the Journal of Neurophysiology for review and publication. This work was supported in part by NIH Grants 1ROI-AA-06773-06 to Dr. D. Durand.

4.1 Abstract

A model of the effects of ethanol on the orthodromic responses of hippocampal granule cells was developed for comparison with experimental results and functional exploration. The model incorporates voltage dependent NMDA receptors and generates results that are consistent with the hypothesis of a reduction of the current mediated by these receptors by ethanol. Further simulations also suggests that: [i] varying ratios of NMDA to nonNMDA receptors may account for the widely observed differences in NMDA-receptors related EPSP decay times in different preparations; [ii] although ethanol also affected the electrotonic properties of these neurons, the contribution of this factor is on the average less important than that of the NMDA-receptors; [iii] an inhibitory potential may be inherent in the orthodromic firing responses of these neurons; [iv] in the absence of NMDA receptors, a much higher frequency of synaptic bombardment is necessary to generate similar firing rates as in control conditions.

4.2 Introduction

Ethanol has been observed to suppress the firing and subthreshold responses of hippocampal granule cells in response to perforant path stimulation²⁴. Furthermore, receptor antagonists studies suggest that this depression is related to the NMDA-receptors²⁴. In order to assess the ability of this hypothesis to account for the experimental observations and investigate the contributions of other factors (e.g. electrotonic properties), we have developed a model of the granule cells with dendritic glutamate receptors. Our eventual goal is to summarize the effects of ethanol on single granule cells in terms of a mathematical model.

4.3 Methods

The basic philosophy of the modelling has been described previously in the context of a model of the firing behavior of hippocampal granule cell²⁵. This previous model consists of four active conductances in the soma and a passive dendrite and was

capable of accounting for most the firing behavior of these neurons including strength duration properties, action potential morphology and repetitive firing. Our objective here is to obtain the simplest model of the NMDA and nonNMDA glutamate receptors that captures the most significant features for our experimental results (i.e. subthreshold EPSPs in various media). In most of the simulations of the subthreshold orthodromic response below, a passive model was used to facilitate comparison with the data. This is a good first approximation as the I-V curve of granule cells are highly linear around resting potentials^{6,7}. Typical passive properties were assumed from previous studies^{5,25}. The fitted parameters will be integrated into a four-conductance model of the granule cells²⁵ in a later section to estimate the effect on the orthodromic firing properties.

The nonNMDA receptor is treated as a typical synaptic conductance^{10,11} described by the reaction:

where X represents the released neurotransmitter (i.e. glutamate), R

the postsynaptic receptor, C the bound receptor and H the bound conducting receptor. To simplify the equations involved and reduce the number of free parameters irrelevant for our objective, it was assumed that : [i] the binding of the neurotransmitters to the receptor reaches equilibrium rapidly and [ii] the activation of the bound receptors to the conducting state as well as the decay of H follows a simple exponential model. The equation describing the population of the conducting receptors H is then governed by,

$$dh/dt = c/\tau_o - h/\tau_d$$

where h and c are the fraction of bound conducting and bound nonconducting receptors respectively and τ_o and τ_d the time constant for H activation and removal respectively. h and c are obtained by dividing each respective population by the total number of receptors, R_0 . The synaptic current due to the nonNMDA receptors is then given by,

$$G_{syn} = g_{syn} * R_0 * h$$

and

$$I_{\text{syn}} = G_{\text{syn}} (V - E_{\text{rev}})$$

where g_{syn} and E_{rev} are the unitary synaptic conductance and reversal potential and G_{syn} the synaptic conductance.

Unlike the nonNMDA receptors, the NMDA receptors are voltage dependent due to a magnesium blockage mechanism¹. The equation for the voltage dependence of the magnesium block has been described experimentally by Ascher and Nowak¹ and adapted to give a sigmoidal function of the unblocked fraction k_{unblock} by Holmes and Levy⁸ and Zador et al²⁶ as

$$k_{\text{unblock}} = 1 / (1 + \eta [\text{Mg}^{2+}] \cdot \exp(-V/12.5))$$

where η (eta) controls the influence or gain of the magnesium block (figure 4.1). This fraction is incorporated into our model to describe the NMDA receptors through :

$$G_{\text{syn}} = g_{\text{syn}} \cdot R_0 \cdot h \cdot k_{\text{unblock}}$$

The assumptions and equations for the NMDA-receptors are

otherwise identical to that of the nonNMDA receptors, except for the slower rise and decay time constant of the former compared to the latter. The steady state I-V curve for this conductance is illustrated in figure 4.2, assuming a resting potential of -70 mv in the experimental results. The resting potential of the model was fixed at 0 mv.

The initial parameters adopted were similar to those from Holmes and Levy⁸ and Keller et al¹². These parameters were based on single channel and whole-cell patch clamp studies in culture mouse central neurons^{1,15,18}, performed at room temperature. Other simulation issues such as algorithm and hardware can be found in Yuen and Durand²⁵. The location of the NMDA-receptors and non-NMDA receptors used in the model (0.3 electrotonic length from soma) was constrained by autoradiographic studies which shown the highest density of labeling in the inner molecular layer (150 - 200 μ from soma; cf space constant 400 - 500 μ)³.

The basic passive structure of the model was constructed based on typical parameters from these neurons^{5,25}. A total of eight dendritic compartments, each at a length of 0.11λ was used to

Figure 4.1 Unblocking function with a resting potential = 0 mV.

Figure 4.2 Number of NMDA-receptors determines current amplitude.

simulate the dendritic tree. The compartmental resistance and capacitances were then determined by the membrane and cytoplasmic resistivities, membrane capacitance, cross-sectional and surface area. Further details of this model can be found in Yuen and Durand²⁵.

4.4 Results

In the following, the general properties of the model and sensitivity of the model parameters will be first described, followed by a fitting of selected experimental EPSPs in control and ethanol from the last chapter. This comparison of the model and experimental responses in turn led to a study of the behavior of the EPSP decay time constant in which the ratio of NMDA and nonNMDA receptors is varied. The impact of specific electrotonic parameter changes (as revealed by short-pulse measurements) is then evaluated. Finally, the functional impact of these changes (NMDA-related and electrotonic) is illustrated in a model of granule cell orthodromic firing response.

4.4.1 General model properties and sensitivity of parameters

Each synaptic conductance is controlled by 3 parameters: number of receptors, rise and decay time constants, while the NMDA-receptors have an additional three parameters regulating the voltage dependence: half unblocking voltage of the Mg^{2+} -blocking curve, extracellular magnesium concentration and gain factor for the magnesium block, η (eta). The sensitivity of the EPSP to these parameters will be illustrated in the following, although in general fixed values were assumed for the voltage dependence parameters. A comparison of the remaining six synaptic parameters revealed that the shape of the EPSP is regulated mostly by the number of receptors and decay time constant. Although these two latter parameters are somewhat correlated, they affect the shape of the EPSP dissimilarly so that useful values can be arrived at with relatively few trials.

The sensitivity of the EPSP to number and time constants of the nonNMDA synapses are illustrated in figures 4.3. These simulated waveforms were obtained by varying the parameter in question by a factor between one-tenth to ten times the average value. The average value for each parameter was in turn based on fitted values to experimental waveforms which are presented in the

Figure 4.3 Sensitivity of EPSP to nonNMDA-receptor parameters.

next section. The "average" EPSP contains approximately equal number of NMDA and nonNMDA receptors (see Table I for parameter values). A comparison of the three parameters for this synapse show that the EPSP shape is affected mostly by changes in the number of receptors and decay time constant. Furthermore, as expected, the number of receptors has a more significant effect on the amplitude than decay time constant, which affect primarily the decay of the EPSP.

The sensitivity of the EPSP to NMDA-receptor parameters are illustrated in figure 4.4. The most significant factors affecting the shape of the EPSP are similarly, the number of receptors and decay time constant. However, the observed changes now has much less effects on the peak amplitude. Furthermore, while the number of receptors affect drastically the width of the response, decay time constant affects primarily the rate of decay.

A distinguishing property of the two types of glutamate receptors related to voltage dependence can be observed as DC-currents are injected prior to synaptic activation. When nonNMDA receptors are activated at different initial voltage levels through DC-current injection, the EPSP response is diminished with more

Table I
Parameters for a 'typical' dual component synapse

	<u>nonNMDA</u>	<u>NMDA</u>
channel conductance	5 pS	50 pS
number of channels	1250	1000
rise time constant	0.5 ms	4 ms
decay time constant	4.0 ms	140 ms
η (blocking gain)	-	0.2
$[Mg^{2+}]$	-	2.0 mM

Figure 4.4 Sensitivity of EPSP to NMDA-receptor parameters.

depolarized voltages (figure 4.5). This is typical of most synapses in general and is caused by a diminished driving force for the synaptic current. An opposite pattern of enhanced EPSP with depolarized voltages is observed with the NMDA receptors due to the unblocking of the channels at more positive voltages (figure 4.6). This effect is accentuated if the half voltage for unblocking is moved to more negative voltages, so that less blocking is in effect at resting potential (figure 4.7). Although this shift (+15 mV) was not assumed in our model, it may account for the significant levels of NMDA-conductance at rest in granule cells reported recently ¹¹. The effect of changing extracellular magnesium concentration is qualitatively the same and is illustrated in figure 4.8.

These properties of the NMDA-receptors mediated EPSP have been described experimentally in both granule cells (cf. fig. 2 in Lambert and Jones¹³) and other neurons⁹.

4.4.2 Fitting to experimental EPSPs

The model described in the last section was fitted to typical EPSP responses in control, ethanol, DNQX and DNQX/ethanol media so as to assess the contribution of the NMDA (and nonNMDA)

Figure 4.5 Depolarization decreases the size of nonNMDA EPSP.

figure 4.6 Depolarization increases the size of NMDA EPSP.

Figure 4.7 Reduced blocking at rest accentuated EPSP voltage dependence.

Figure 4.8 NMDA-receptor EPSP size dependence on $[Mg^{2+}]$.

synaptic conductance in each case. The fast and slow time courses of the two selected EPSPs represent the two major EPSP types observed experimentally. The simulation results are consistent with a reduction in the number of NMDA receptors in ethanol containing media.

Control and ethanol media EPSP responses from a typical cell are compared to the model fitted responses in figures 4.9 and 4.10 and the parameters summarized in Table II. The closeness of fit suggests that a NMDA-receptor suppression hypothesis is consistent with the observed data. The ethanol related EPSP response, for example, is generated simply by setting the number of NMDA receptors to zero (and a 6 % reduction in the number of nonNMDA receptors).

Comparisons between model and DNQX-resistant EPSP responses are shown in figures 4.11 and 4.12 (Table II). A close fit can be achieved primarily for the peak and decay portion of the EPSP, suggesting some other process may be operative during the upstroke of the EPSP. Nevertheless, the decay in DNQX/ethanol can be simulated by a 50% reduction in the number of NMDA receptors. The slightly larger number of nonNMDA receptors in DNQX/ethanol probably reflects a fast component inherent in the

Figure 4.9 Comparison of fitted model and control EPSP. Parameters are given in Table II.

Figure 4.10 Comparison of fitted model and ethanol EPSP. Parameters are given in Table II.

Table II

Parameters for model EPSPs fitted to experimental data

	<u>Control</u>	<u>Ethanol</u>	<u>DNOX</u>	<u>ETOH/DNOX</u>
<u>NonNMDA receptors:</u>				
number of channels	1750	1650	380	390
τ_{rise} (ms)	0.95	0.95	0.01	0.5
τ_{decay} (ms)	2.0	2.0	7.5	0.5
<u>NMDA receptors:</u>				
number of channels	130	0	1900	1000
τ_{rise} (ms)	2.5	2.5	3.0	3.0
τ_{decay} (ms)	135	135	145	135

Figure 4.11 Comparison of fitted model and DNQX-resistant EPSP.

Figure 4.12 Comparison of fitted model response and EPSP in ETOH/DNQX.

experimental response that is not well represented in the model at this stage (e.g. spike generating sodium channels). Furthermore, as shown in the last chapter, the model is relative insensitive to changes in this parameter (i.e. rise time constant).

As discussed in the last chapter, the decay time constants of some of the NMDA-receptors mediated EPSP observed in our preparation are shorter than the values reported originally for culture spinal cord and other neurons (e.g. > 150 ms)^{15,17,18} (see Discussions, Chapter 3), but are similar to those in hippocampal or similar preparations^{9,13,14,16,19,21,22}, mostly studied at a temperature of 35 C. The intracellular recording experiments related to this study were carried out at a temperature of 35 C, which leads to faster rates of activation and decay than the values obtained from room-temperature voltage clamped experiments (25 C). Furthermore, peeling analysis of model-generated waveforms suggested that a variable ratio of NMDA and nonNMDA receptors can lead to an underestimation of the decay time constant when more nonNMDA receptors are present (figure 4.13). This effect is accentuated at the temperature of our experiments (figure 4.14, assumed $Q_{10}=3.0$, 35 C). A more or less constant decay constant is obtained only if more

Figure 4.13 Extracted decay constant varies with ratio of receptors.

Figure 4.14 Extracted decay constants with Q_{10} correction made to receptor kinetics.

NMDA receptors are present instead. An examination of the parameters in Table II and the difference in EPSP duration in two neurons (see figures 4.9 and 4.11) illustrate the fact that difference in shape and duration of the EPSP could arise simply from the ratio of the two types of glutamate receptors (and not necessarily from different synapse decay time constant). These considerations can in principle account for the wide range of time constant reported for the NMDA-receptors mediated EPSPs currently found in the literature 4,9,13,16.

4.4.3 Contribution of electrotonic parameters

Although measurement of input resistances did not support changes in the passive properties of these neurons in ethanol (see Chapter 2), subsequent short-pulse analysis of another group of granule cells revealed specific changes in the electrotonic structure¹⁹. These changes are described below simply as an introduction to the simulations to follow (Table III). The details of the inverse-Fourier transform method for analyzing short pulse data has been described previously and was developed independently of this thesis²³. The short pulse analysis results reported here were performed by

Table III

Electrotonic parameters in control and ethanol

<u>Parameter:</u>	<u>Control</u>	<u>Ethanol</u>	<u>Statistical Significance</u>
T_m (ms)	15.4	10.0	$p < 0.0002$
T_s (ms)	0.763	1.04	$p < 0.02$
L	1.011	1.32	$p < 0.007$
R_{in}^S (M Ω)	120.05	137.04	NS
R_{in}^D (M Ω)	632.33	552.55	NS
R_{in}^N (M Ω)	81.8	82.18	NS
C_s (pF)	7.63	7.14	NS
ρ	0.186	0.212	NS

Madhavi Patil²⁰.

As shown in Table III, whereas the input resistances for the neuron, the soma or dendritic tree did not vary significantly in ethanol, the dendritic time constant (τ_m) and the electrotonic length (L) were affected up to 30 % in ethanol. Furthermore, whereas the former decreased by 30%, the latter increased by 30 % in ethanol. It was assumed that there is no significant change in the size of the soma, dendritic diameter and length (d & l). A change in the dendritic time constant can be attributed to either changes in the dendritic membrane resistivity (R_m^D) or the dendritic membrane capacitance (C_m), or both, since ($\tau_m = R_m^D * C_m$). The impact of these changes on the EPSP can be therefore be evaluated by examining two extreme cases : [i] 30 % decrease in dendritic membrane resistivity (resistive case) and [ii] 30 % decrease in dendritic capacitance (capacitive case). (A table of the symbols used here can be found in the **Glossary** at the end of **Conclusions**).

Since the dendritic input resistance, electrotonic length (L), membrane resistivity (R_m^D) and cytoplasmic resistivity (R_i^D) are related by the following equation for a finite cable with sealed ends

(Jack et al 1975),

$$R_{in}^D = \sqrt{\left[\frac{4 R_m^D * R_i^D * \coth^2 L}{\pi^2 d^3} \right]}.$$

Assuming typical values of granule cells (Table IV; see also Durand 1987, Yuen and Durand 1991), it is easily verified that to maintain a fixed R_{in}^D when L and R_m^D are increased and decreased 30% respectively, R_i^D must increase by 11 % in ethanol, as the ratio of dendritic input resistances in control and ethanol must remain close to unity:

$$\frac{R_{in}^{D'}}{R_{in}^D} = \sqrt{\frac{R_m^D * R_i^{D'} * \coth^2 L'}{R_m^D * R_i^D * \coth^2 L}} = 1$$

where the numerator contains the relevant parameters in ethanol and the denominator contains the parameters in control. Thus, the resistive effect can be simulated by creating a set of parameters for the ethanol response with identical input resistance to control and appropriate changes in the electrotonic length, membrane and cytoplasmic resistivities (Table IV; a more extreme increase of 50% in

Table IV

**Model electrotonic parameters used
for generating control and ethanol EPSPs**

<u>Parameter:</u>	<u>Control</u>	<u>Ethanol</u>
R_m^D ($\Omega \cdot \text{cm}^2$)	4400	3100
R_m^S ($\Omega \cdot \text{cm}^2$)	874	874
R_{in}^D ($M\Omega$)	128	112
R_{in}^S ($M\Omega$)	185	185
R_{in}^N ($M\Omega$)	76	70
R_i ($\Omega \cdot \text{cm}$)	170	265
L	0.88	1.144
d (μm)	3.4	3.4
λ (μm)	490	363
C_m (pF/cm ²)	3500	3500

R_i^D was assumed). The capacitive effect can be represented conveniently by keeping control values for all parameters except for the dendritic membrane capacitance (C_m).

The effect of the changes in the electrotonic properties are shown in figures 4.15 and 4.16. The results of either resistive or capacitive changes are similar. For the 'fast' EPSP, a decrease in the resistivity or capacitance leads to a decrease in the duration of the EPSP (half peak-amplitude duration reduction: 21 and 23 % for change in R_m^D and C_m respectively), without significant changes in the peak amplitude. For the 'slow' EPSP in DNQX, these passive changes lead to a decrease in the peak amplitude as well as half peak-amplitude duration: 21 and 24 % decrease in maximum amplitude for change in R_m^D and C_m respectively). Therefore, although apparent changes in the EPSP are introduced in all cases, these changes are in themselves insufficient to account for the average depression of the orthodromic response by ethanol, which was about 50%. Furthermore, if the observed changes were due to resistive instead of capacitive changes, then the expected effect would be smaller than the extent reported above (as a large, 50 % increase in R_i^D was assumed).

Figure 4.15 Upper: resistive properties were changed according to values in Table IV. Lower: effect on EPSP when membrane capacity was lowered by 30 %. (Smooth solid line = modified model response).

Figure 4.16 Upper: resistive properties were changed according to values in Table IV for a neuron's EPSP in DNQX. Lower: effect on EPSP when membrane capacity was lowered by 30 %.

Figure 4.17 Changes induced by ethanol on passive properties tended to raise somatic firing threshold in model.

The effect of these passive changes on the somatic strength duration properties was also studied by means of a four-channel model of these neurons. The results, however, are opposite to the experimental observation²⁴ (see figure 2.3.2a), suggesting a higher threshold for firing in ethanol (figure 4.17). Thus, the specific observed changes in the electrotonic structure cannot account for changes in somatic excitability.

4.5 Discussion

The degree and ease of fitting of the experimental EPSPs (in control and ethanol media) by a simple NMDA/nonNMDA receptor model supports a reduction of the NMDA-receptors as a possible explanation of the experimental depression of the EPSP by ethanol. Although the extent to which this factor (instead of passive and other factors) contributes varies from cell to cell, we have shown above that electrotonic changes cannot account for the average degree of depression that was observed with ethanol. This is particularly true if the electrotonic changes are due to a resistive component, since the simulations presented here represented an extreme case of its

possible contribution. Ultimately, simultaneous experimental measurements of both EPSP and short pulse response in the same cells will be necessary to clarify this point.

Since we have demonstrated previously that blockage of nonNMDA receptors or GABA_A receptors does not disrupt significantly the effect of ethanol on the EPSP²⁴, our results here reaffirm the central importance of NMDA-receptors for understanding the acute effects of ethanol on the orthodromic responses in granule cells. These results also suggest that the difference in shape and duration of the EPSPs in granule cells may be due in fact to varying ratios of NMDA to nonNMDA receptors, so that when the NMDA-component is removed by APV or ethanol, components with different decay time constants are observed to be affected.

The implication of the combined effect of changes in passive properties and NMDA-mediated conductance is explored through incorporating the parameters found in the last sections into a model of granule cells that contains four somatic active conductances (figure 4.18). This four-channel model was capable of reproducing most of the firing responses observed in these neurons²⁷. With the

Figure 4.18 When the NMDA-receptor mediated current is set to zero at the same time the electrotonic properties were modified, an identical synaptic input generated a smaller number of spikes.

glutamate receptors incorporated, the model tended to show repetitive firing analogous to the responses in BIC or BIC/DNQX media. This is consistent with the notion that an inhibitory process is latent in the normal control response, which is not included in our model. Nevertheless, the relative effect of the changes in the two factors was to reduce repetitive firing, as is observed experimentally in BIC/DQNX/ethanol media (see fig. 3.4.6.3c, Ch.3). Since both the reduction of the slow NMDA-receptor mediated conductance and the dendritic time constant both tended to lower temporal summation, it can be expected that higher frequency of synaptic bombardment would now be necessary to produce the same firing pattern (figure 4.19).

4.6 Conclusions

By developing a model of the NMDA/nonNMDA receptors in hippocampal granule cells, we were able to show that a selective reduction of the NMDA-receptors is consistent with : [i] the shape of the EPSPs in control and ethanol and [ii] the relatively short observed

Figure 4.19 When the NMDA-receptor mediated current is set to zero at the same time the electrotonic properties were modified, a larger or more frequent stimulus is necessary to evoke a similar firing pattern.

values of the EPSP decay time constants. The model suggested that the latter may be due to varying ratio of NMDA and nonNMDA receptors. The model also suggests that there is a significant contribution from changes in the electrotonic properties (up to 24 % in some cases). However, the size of this modulation of the EPSP size is in general smaller than that was observed with ethanol (>50%). Thus, passive changes cannot in itself account for the effects of ethanol on the orthodromic responses. Further simulation results also suggest that these factors (electrotonic and NMDA-receptors modulation) cannot account for the decrease in somatic firing threshold in ethanol that has been reported for these neurons.

4.7 Glossary of electrotonic parameters used in text

<u>Symbol:</u>	<u>Term represented:</u>
R_m^D	dendritic membrane resistivity ($\Omega \cdot \text{cm}^2$)
R_m^S	somatic membrane resistivity ($\Omega \cdot \text{cm}^2$)
R_{in}^D	dendritic input resistance ($M\Omega$)
R_{in}^S	somatic input resistance ($M\Omega$)
R_{in}^N	neuronal input resistance ($M\Omega$)
R_i^D	cytoplasmic resistivity ($\Omega \cdot \text{cm}$)
L	electrotonic length
d	dendritic diameter (μm)
λ	space constant (μm)
C_m	specific membrane capacitance (pF/cm^2)
τ_m	membrane time constant (ms)

4.8 References

- [1] Ascher, P. and Nowak, L. The role of divalent cations in the N-methyl-d-aspartate responses of mouse central neurones in culture. *J Physiol.* 399 (1988) 247-266

- [2] Collingridge, G.G., Herron, C.E. and Lester, R.A.J. Synaptic activation of N-methyl-d-aspartate receptors in the Schaffer Collateral-commissural pathway of rat hippocampus. *J Physiol.* 399 (1988) 283-300

- [3] Cotman, C.W., Monaghan, D.T., Ottersen, O.P. and Storm-Mathisen, J. Anatomical organization of excitatory amino acid receptors and their pathways. *Trends in Neuroscience* 10 (1987) 273 - 279

- [4] Dale, N. and Roberts, A. Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in *Xenopus* embryos. *J Physiol* (1985) 35-59.

- [5] Durand, D., Carlen, P., Gurevich, N., Ho, A. and Kunov, H. Electrotonic Parameters of Rat Dentate Granule cells measured using short current pulses and HRP Staining" *J Neurophys.* 50: (1983) 1080-1097

- [6] Fournier, E., and Crepel, F., Electrophysiological Properties of Dentate Granule Cells in Mouse Hippocampal Slices Maintained in Vitro. *Brain Research* 311: (1984) 75-86

- [7] Fricke, R. A., and Prince, D. A. Electrophysiology of Dentate Gyrus Granule Cells. *J Neurophysiol.* 51: (1984) 195-209

- [8] Griller, S. Neural Networks Models of Vertebrate Sensorimotor Systems. *Annual Meeting, Society for Neuroscience, Session 138.1*, New Orleans, Louisiana, November 10-15, 1991.

- [9] Holmes, W.R. and Levy, W.B. Insights into associative Long-term potentiation from computational models of NMDA receptor-

ediated calcium influx and intracellular calcium concentration changes. *J Neurophysiol.* 63 (1990) 1148-1168

[10] Jack, J., Noble, D., Tsien, R.W. *Electric current flow in excitable cells*, Oxford University Press, Cambridge, 1975.

[11] Junge, D. *Nerve and Muscle Excitation*. Sinauer Press, New York, 1991

[12] Keller, B.U., Konnerth, A. and Yaari Y. Patch clamp analysis of excitatory synaptic currents in granule cells of rat hippocampus. *J Physiol.* 435: 275 - 293 (1991)

[13] Lambert, J.D.C. and Jones, R.S.G., Activation of NMDA receptors contributes to the EPSP at perforant path synapses in the rat dentate gyrus in vitro *Neurosc Lett.* 97 (1989) 323-328

[14] Lambert, J.D.C. and Jones, R.S.G., A reevaluation of excitatory amino acid-mediated synaptic transmission in rat dentate gyrus. *J Neurophysiology.* 64 (1990) 119-132

- [15] Lester, R.A.J., Clements, J.D., Westbrook, G.L. and Jahr, C.E. Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* 346 (1990) 565-567
- [16] Lovinger, D.M, White, G. and Weight, F.F. Ethanol Inhibits NMDA-Activated Ion Current in Hippocampal Neurons *Science* 243 (1989) 1721-1724
- [17] Mayer, M.L. and Westbrook, G.L. The action of N-methyl-d-aspartic acid on mouse spinal neurones in culture. *J Physiol.* 361 (1985) 65-90.
- [18] Mayer, M.L. and Westbrook, G.L. The physiology of excitatory amino acids in the vertebrate central nervous system. *Progress in Pharmacology* 28 (1987) 197-276.
- [19] Mody, I and Heinemann, U. NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature* 326 (1987) 701-704

- [20] Patil, M., Durand, D., Yuen, G.L. and Chintalacharuvu, R. Estimation of passive electrotonic parameters using the inverse Fourier transform technique to study ethanol effects on granule cells. *Soc. Neuroscience Abst.* 17 (1991) 1336
- [21] Poolos, N.P. and Kocsis, J.D. Dendritic action potentials activated by NMDA receptor-mediated EPSPs in CA1 hippocampal pyramidal cells. *Brain Research* 524 (1990) 342-346
- [22] Urban, L., Aitken, P.G., Friedman, A. and Somjen, G.G. An NMDA-mediated component of excitatory synaptic input to dentate granule cells in 'epileptic' human hippocampus studied in vitro. *Brain Research* 515: 319-322 (1990).
- [23] Ali-Hassan, W., Saidel, G., Durand, D. Estimation of electrotonic properties using an inverse Fourier Transformation technique, *Trans. in Biomedical Engineering*, 1991, in print.
- [24] White, G., Lovinger, D.M, and Weight, F.F. Ethanol Inhibits

NMDA-activated Current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Research* 507 (1990) 332-336

[25] Yuen, G.L.F., Patil, M. and D. Durand. Effects of acute ethanol treatment on the excitability of hippocampal granule neurons. *Brain Research* in press (1991).

[26] Yuen, G.L.F., and D. Durand. Reconstruction of Hippocampal Granule Cell Electrophysiology. *Neuroscience* 41 (1991) 411-423.

[26] Zador, A., Koch, C. and Brown, T.H. Biophysical model of a Hebbian synapse. *Proc. Natl. Acad. Sci. USA.* 87 (1990) 6718-6722

CHAPTER V

CONCLUSIONS

This research was carried out based on the premise that acute ethanol can produced consistent and specific changes in single, neuronal properties. We have successfully identified such a cellular locus in hippocampal granule neurons, in which the excitability of these neurons were reduced orthodromically. Such an identification permitted a further isolation of the subcellular substrate responsible primarily for the modulation, in particular, the NMDA-receptors. Whereas a reduction in this synaptic conductance can account for ethanol's effects on the orthodromic responses both experimentally and conceptually (in terms of a model), it does not rule out the potential contributions of inhibitory synaptic conductances or electrotonic factors, nor does it account for the heightened somatic excitability observed in ethanol.

Further experimentation using whole-cell patch recording should provide more reliable data both for precise estimation of the reduction in NMDA-mediated synaptic currents as well as possible

isolation of the substrates underlying the somatic excitability elevation.

The confirmation of our first hypothesis that granule cells are consistently suppressed orthodromically by ethanol implies that the previously observed depression of cell firing in this region may be accounted for, at least in part, by a suppression of the input neurons. The role of NMDA-receptors in this effect confirms our second hypothesis and opens up the possibility of conducting a developmental study of the chronic effects of ethanol as these receptors are presumably also related to epilepsy and memory. It is remarkable that although input resistances measurement did not support any changes in passive properties induced by ethanol, the contribution of this factor is not negligible on the average (up to 24 % reduction in amplitude), as shown by our computer simulations. Further experimentation in which both short pulse and EPSP measurements are carried out in control and/or DNQX media will provide more direct experimental information of the relative role of these factors in individual granule cells.

LITERATURE CITED

- [1] Abraham, W. and Bliss, T.V.P. An analysis of the increase in the granule cell excitability accompanying habituation in the Dentate Gyrus of the anesthetized rat. *Brain Res* 331 (1985) 303-313
- [2] Alger, B.E., Dhanjal, S.S., Dingledine, R., Garthwaite, J., Henderson, G., King, G.L., Lipton, P., North, A., Schwartzkroin, P.A., Sears, T.A., Segal, M., Whittingham, T.S. and Williams, John. Brain slice methods. In *Brain Slice*, Dingledine, R. (ed), 1984, Plenum, New York.
- [3] Ali-Hassan, W., Saidel, G., Durand, D. Estimation of electrotonic properties using an inverse Fourier Transformation technique, *Trans. in Biomedical Engineering*, 1991, in print.
- [4] Ascher, P. and Nowak, L. The role of divalent cations in the N-methyl-d-aspartate responses of mouse central neurones in culture. *J Physiol.* 399 (1988) 247-266
- [5] Baskys, A., Berstein, N.K., Barolet, A.W. and Carlen, P.L. NMDA and quisqualate reduce a Ca-dependent K⁺ current by a protein kinase mechanism. *Neuroscience Letters* 112 (1990) 76-81
- [6] Collingridge, G.G., Herron, C.E. and Lester, R.A.J. Synaptic activation of N-methyl-d-aspartate receptors in the Schaffer Collateral-commissural pathway of rat hippocampus. *J Physiol.* 399 (1988) 283-300
- [7] Cotman, C.W., Monaghan, D.T., Ottersen, O.P. and Storm-Mathisen, J. Anatomical organization of excitatory amino acid receptors and their pathways. *Trends in Neuroscience* 10 (1987) 273 - 279
- [8] Crunelli, V, Assaf, S. Y. and Kelly, J. S. Intracellular recordings from granule cells of the Dentate Gyrus in vitro. In *Electrophysiology of Isolated Mammalian CNS Preparations*. Kerkut, G.A. and Wheal, H.V. (eds), 1981, Academic Press, New York.

- [9] Dale, N. and Roberts, A. Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in *Xenopus* embryos. *J Physiol* (1985) 35-59.
- [10] Doller, H.J., Eckart, M.J. and Weight, F.F. Effects of ethanol on the hippocampal slice preparation. *Fed. Proc.* 309:281, 1980
- [11] Durand, D., Carlen, P., Gurevich, N., Ho, A. and Kunov, H. Electrotonic parameters of rat Dentate Granule cells measured using short current pulses and HRP Staining" *J Neurophys.* 50: (1983) 1080-1097
- [12] Durand, D. and Carlen, P. Impairment of long term potentiation in rat hippocampus following chronic ethanol treatment. *Brain Research* 308: (1984) 325-332
- [13] Durand, D., Corrigan, W. Kujtan, P., and Carlen, P.L. Effects of low-dose ethanol on CA1 hippocampal neurons in-vitro. *Canadian Journal of Physiology and Pharmacology*, 59:972-984, 1981
- [14] Folger, W.R. and Klemm W.R., Unit responsivity in various Hippocampal layers and the depressive effects of ethanol" *Physiology & Behavior* 21: (1978) 169-176
- [15] Fournier, E., and Crepel, F., Electrophysiological properties of Dentate granule cells in Mouse Hippocampal slices maintained in vitro. *Brain Research* 311: (1984) 75-86
- [16] Fricke, R. A., and Prince, D. A. Electrophysiology of Dentate Gyrus granule cells. *J Neurophysiology* 51: (1984) 195-209
- [17] Griller, S. Neural networks models of vertebrate Sensorimotor systems. *Annual Meeting, Society for Neuroscience, Session 138.1*, New Orleans, Louisiana, November 10-15, 1991.
- [18] Holmes, W.R. and Levy, W.B. Insights into associative long-term potentiation from computational models of NMDA receptor-mediated calcium influx and intracellular calcium concentration changes. *J Neurophysiol.* 63 (1990) 1148-1168

- [19] Hunt, W.A. *Alcohol and Biological Membranes*. 1985. Guiford Press, New York.
- [20] Jack, J., Noble, D., Tsien, R.W. *Electric Current Flow in Excitable Cells*, Oxford University Press, Cambridge, 1975.
- [21] Junge, D. *Nerve and Muscle Excitation* 2nd ed. Sinauer Press, New York, 1991
- [22] Keller, B.U., Konnerth, A. and Yaari Y. Patch clamp analysis of excitatory synaptic currents in granule cells of rat hippocampus. *J Physiol*. 435: 275 - 293 (1991)
- [23] Kelly, J.S. Intracellular recording from neurons in brain slices in vitro. In *Electrophysiology of Isolated Mammalian CNS Preparations*. Kerkut, G.A. and Wheal, H.V. (eds), Academic Press, 1981, New York.
- [24] Kosaka, T., Hama, K. and Wu, J. GABAergic synaptic boutons in the granule cell layer of rat Dentate Gyrus. *Brain Research* 29 (1984) 353-359
- [25] Lambert, J.D.C. and Jones, R.S.G., Activation of NMDA receptors contributes to the EPSP at perforant path synapses in the rat dentate gyrus in vitro *Neurosc Lett*. 97 (1989) 323-328
- [26] Lambert, J.D.C. and Jones, R.S.G., A reevaluation of excitatory amino acid-mediated synaptic transmission in rat Dentate Gyrus. *J Neurophysiology*. 64 (1990) 119-132
- [27] Lester, R.A.J., Clements, J.D., Westbrook, G.L. and Jahr, C.E. Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* 346 (1990) 565-567
- [28] Lima-Landman, M.T.R. and Albuquerque, E.X. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. *FEBS Letters* 247 (1989) 61-67.
- [29] Lovinger, D.M., White, G. and Weight, F.F. NMDA Receptor-

mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. *J Neurosci* 10 (1990) 1372-1379

[30] Lovinger, D.M, White, G. and Weight, F.F. Ethanol inhibits NMDA-activated ion current in Hippocampal neurons *Science* 243 (1989) 1721-1724

[31] Mayer, M.L. and Westbrook, G.L. The action of N-methyl-d-aspartic acid on mouse spinal neurones in culture. *J Physiol.* 361 (1985) 65-90.

[32] Mayer, M.L. and Westbrook, G.L. The physiology of excitatory amino acids in the vertebrate central nervous system. *Progress in Pharmacology* 28 (1987) 197-276.

[33] Mody, I and Heinemann, U. NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature* 326 (1987) 701-704

[34] Patil, M., Durand, D., Yuen, G.L. and Chintalacharuvu, R. Estimation of passive electrotonic parameters using the inverse Fourier transform technique to study ethanol effects on granule cells. *Soc. Neuroscience Abst.* 17 (1991) 1336

[35] Poolos, N.P. and Kocsis, J.D. Dendritic action potentials activated by NMDA receptor-mediated EPSPs in CA1 hippocampal pyramidal cells. *Brain Research* 524 (1990) 342-346

[36] Siggins, G.R., Bloom, F.E., French, E.D., Madamba, S.G., Mancillas, J., Pittman, Q.J. and Rogers, J. Electrophysiology of ethanol on central neurons. In Rubin Emanuel (Ed.) *Alcohol and the Cell: Annals of the New York Academy of Sciences*, 1987, 492 pp. 350-366.

[37] Sinclair, J. G. and Lo, G. F. Ethanol blocks tetanic and calcium-induced long-term potentiation in the Hippocampal slice. *Gen Pharmac* 17: (1986) 231-233.

[38] Teyler, T.J. Brain slice preparation: Hippocampus. *Brain Res.*

Bull. 5 (1980) 391-403.

[39] Urban, L., Aitken, P.G., Friedman, A. and Somjen, G.G. An NMDA-mediated component of excitatory synaptic input to Dentate Granule cells in 'epileptic' human hippocampus studied in vitro. *Brain Research* 515: 319-322 (1990).

[40] White, G., Lovinger, D.M, and Weight, F.F. Ethanol Inhibits NMDA-activated Current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Research* 507 (1990) 332-336

[41] Yuen, G.L.F., Patil, M. and D. Durand. Effects of acute ethanol treatment on the excitability of hippocampal granule neurons. *Brain Research* 563 (1991) 315-320.

[42] Yuen, G.L.F., and D. Durand. Reconstruction of Hippocampal granule cell electrophysiology. *Neuroscience* 41 (1991) 411-423.

[43] Zador, A., Koch, C. and Brown, T.H. Biophysical model of a Hebbian synapse. *Proc. Natl. Acad. Sci. USA.* 87 (1990) 6718-6722