ance of predator and prey, both of which are sub-annual species (presumably an adaptation of *Doridella* to exploit *Membranipora*); (ii) requirement of *Doridella* larvae for contact with *Membranipora* to trigger metamorphosis to the adult form (*7*); (iii) cryptic coloration of *Doridella* with respect to *Membranipora*; and (iv) a fast-acting, induced defense by *Membranipora* in response to predation by *Doridella* and the other, more generalized nudibranch predator.

Little is known about the chemical or physical arsenal available to most colonial organisms in the evolutionary arms race with their predators or about the patterns of spatial and temporal deployment of constitutive and induced defenses. As shown with *Membranipora*, induced or spatially variable patterns of colony defense may determine intermittent foraging patterns displayed by many predators on bryozoans (*13*) and other colonial prey.

Bryozoans are well represented in the fossil record (*14*). On the basis of information extracted from present-day interactions, paleontologists make inferences about events structuring interactions in "paleo-assemblages." However, predation has been a notoriously difficult process for paleontologists to quantify (*15*). If other species of recent and fossil anascan bryozoans respond to predators with the production of spines, then the existence of fossil bryozoans with bands of spined zooids would allow us to make inferences about the prevalence and incidence of predation in paleoseas.

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## Decreased Neuronal Inhibition in Vitro After Long-Term Administration of Ethanol

Abstract. *The pathophysiology of brain dysfunction was studied with an animal model of chronic alcoholism. Rats were fed a liquid diet with or without ethanol for 20 weeks and then the diet without ethanol for three more weeks. Hippocampal slices were prepared and intracellular recordings were obtained from dentate granule and CA1 cells. Significant depression of orthodromically elicited inhibitory postsynaptic potentials and postspike afterhyperpolarizations was observed in neurons from ethanol-exposed animals. No differences were observed in other active or passive membrane characteristics. These results suggest that a loss of neuronal inhibition could contribute to brain dysfunction in chronic alcoholism.*

Generalized learning deficits in rats and mice (*1*), loss of dendritic spines (*2*), decreased dendritic branching (*2, 3*), and cell death (*4*) have been reported in the hippocampus after three or more months of ethanol administration and several weeks of withdrawal on liquid diets. However, physiological changes in neurons have been little studied in animals with ethanol-induced brain damage. Augmentation of paired-pulse facilitation (*1, 5*) and changes in the distribution of synaptic current in CA1 cells (*6*) were measured extracellularly in the rat hip-

Table 1. Effects of long-term exposure to ethanol on intracellularly measured physiological parameters of granule cells. The amplitude and duration of the AHP were normalized to the number of spikes. Statistical analysis was done with a two-tailed *t*-test for all parameters except input resistance, subthreshold IPSP, and AHP amplitude and duration, for which a two-tailed Mann-Whitney *U* test was used because standard deviations for the two groups were significantly different by analysis of variance ($\alpha = 0.05$). N.S., not significant.

| Measure | Control group | | Ethanol group | | *P* |
|---|---|---|---|---|---|
| | Mean ± standard error | *N* | Mean ± standard error | *N* | |
| Resting potential (mV) | 55.65 ± 1.22 | 59 | 54.48 ± 1.23 | 49 | N.S. |
| Action potential (mV) | 73.70 ± 1.33 | 62 | 73.49 ± 1.54 | 53 | N.S. |
| EPSP (subthreshold) (mV) | 5.22 ± 0.49 | 32 | 4.87 ± 0.62 | 27 | N.S. |
| IPSP | | | | | |
| Subthreshold (mV) | 0.60 ± 0.13 | 34 | 0.39 ± 0.09 | 31 | < 0.05 |
| Maximum (mV) | 2.25 ± 0.19 | 54 | 1.51 ± 0.22 | 46 | < 0.025 |
| Maximum (second) | 0.580 ± 0.064 | 54 | 0.290 ± 0.046 | 46 | < 0.01 |
| Rheobase current (nA) | 0.090 ± 0.009 | 11 | 0.040 ± 0.010 | 9 | N.S. |
| AHP (mV per spike) | 0.84 ± 0.28 | 19 | 0.25 ± 0.05 | 17 | < 0.015 |
| AHP (second per spike) | 0.210 ± 0.055 | 19 | 0.08 ± 0.27 | 17 | < 0.02 |
| Input resistance (megohms) | 66.64 ± 9.71 | 22 | 51.84 ± 4.61 | 22 | N.S. |
| Time constant (msec) | 21.86 ± 1.32 | 22 | 20.17 ± 1.01 | 22 | N.S. |
| Rectification | 0.240 ± 0.023 | 27 | 0.220 ± 0.023 | 27 | N.S. |

Table 2. Effects of long-term exposure to ethanol on intracellularly measured physiological parameters of CA1 pyramidal cells. Fewer parameters were measured than for the granule cells because of insufficient data. Statistical analysis was done with a two-tailed *t*-test, except for maximum IPSP amplitude and AHP duration, for which a Mann-Whitney *U* test was used for the same reasons given in the legend to Table 1.

| Measure | Control group | | Ethanol group | | *P* |
|---|---|---|---|---|---|
| | Mean ± standard error | *N* | Mean ± standard error | *N* | |
| Resting potential (mV) | 51.62 ± 2.22 | 12 | 52.50 ± 2.86 | 12 | N.S. |
| Action potential (mV) | 69.17 ± 2.21 | 12 | 72.50 ± 2.92 | 12 | N.S. |
| IPSP (maximum) (mV) | 5.54 ± 1.21 | 11 | 2.37 ± 0.61 | 12 | N.S. |
| IPSP (maximum) (second) | 0.440 ± 0.078 | 11 | 0.210 ± 0.060 | 12 | < 0.05 |
| AHP (mV per spike) | 1.72 ± 0.39 | 9 | 0.98 ± 0.29 | 7 | N.S. |
| AHP (second per spike) | 0.48 ± 0.14 | 9 | 0.16 ± 0.06 | 7 | < 0.05 |

pocampus in vivo after 5 months of ethanol treatment and 2 months of withdrawal. We used the mammalian hippocampal slice preparation (Fig. 1A) (*7*) because this preparation is well suited for stable intracellular recordings, permitting measurement of neuronal membrane and synaptic properties.

Male Wistar rats ($N$ = 22) were randomly divided into two groups receiving a liquid diet with or without ethanol (*8*). The rats, which weighed 200 g at the beginning of the experiment, were housed singly and given free access to their liquid diet for 23 weeks. Ethanol-exposed animals received 35 percent of their caloric intake as ethanol for 20 weeks and were then switched to the control diet for 3 weeks. In the control diet maltose dextrins were used in place of ethanol. Animals exposed to ethanol did not show signs of intoxication during treatment nor evidence of hyperexcitability during withdrawal. For each recording session, an animal from one of the two groups (the investigator did not know which group) was anesthesized with ether and then decapitated. Hippocampal slices were prepared and maintained in a viable condition on a nylon mesh subperfused with artificial cerebrospinal fluid (*9*). Recordings were made at 33° to 35°C with electrodes filled with horseradish peroxidase (*10*). Unipolar stimulating tungsten electrodes were placed in the stratum radiatum or in the perforant path for orthodromic stimulation of CA1 or dentate granule cells, respectively (Fig. 1A). Granule cells ($N$ = 115) or CA1 pyramidal cells ($N$ = 24) exhibiting action potentials greater than 60 mV and input resistances of greater than 20 megohms were studied. Usually six or seven cells were successfully studied per animal. Resting membrane potential, excitatory postsynaptic potentials (EPSP's) subthreshold for spike genesis (inset in Fig. 1B), maximum inhibitory postsynaptic potentials (IPSP's) (Fig. 1B), subthreshold IPSP, rheobase current, postspike afterhyperpolarizations (AHP's) (Fig. 1C), membrane time constant, and cell input resistance were recorded and later measured (*11–13*).

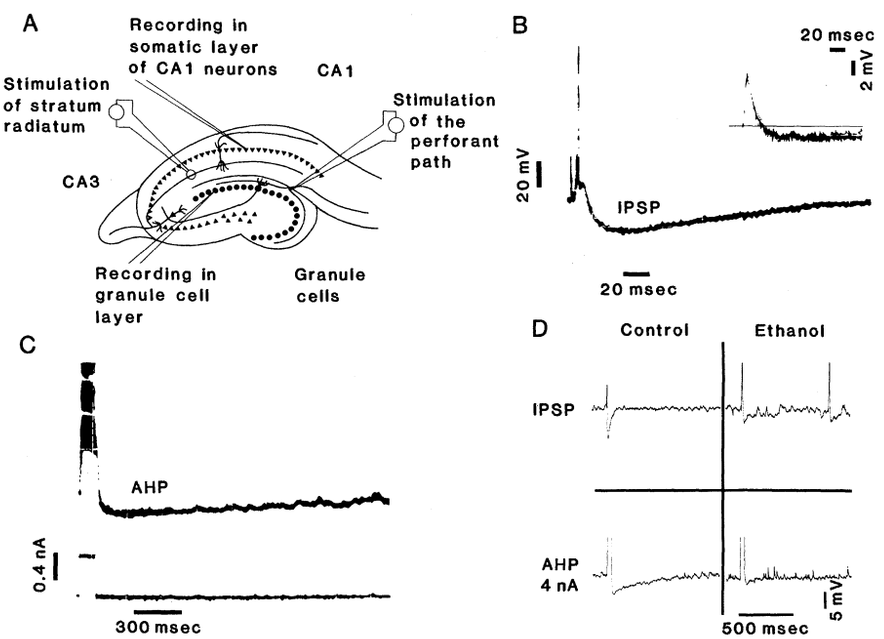


Fig. 1. (A) Schematic of a hippocampal slice, showing stimulating and recording sites for intracellular experiments in the dentate gyrus and the CA1 region. (B) An IPSP following an EPSP with an action potential triggered by orthodromic stimulation of the perforant path (150 μA). Inset: subthreshold EPSP-IPSP sequence in another granule cell (50-μA stimulation). (C) An AHP following a train of action potentials triggered by a 0.4-nA depolarizing current pulse injected into the granule cell through the recording electrode. (D) IPSP's and AHP's recorded at low speed on a chart recorder in granule cells from control and ethanol-treated animals. Note the marked depression of both potentials in the cell from the ethanol-treated animals. The IPSP's were elicited by orthodromic stimuli of 110 μA. The AHP's were triggered by a 0.4-nA depolarizing current pulse (100 msec) injected intracellularly. The increased depolarizing noise in the records for ethanol-treated animals was not a feature that consistently differentiated the two groups.

Ethanol-exposed animals did not show any significant differences from control animals in the amplitude of resting membrane potentials and action potentials in granule and CA1 cells or in EPSP heights, rheobase current, membrane time constant, input resistance, and membrane rectification in granule cells (Tables 1 and 2). However, maximum IPSP amplitude and duration were decreased in granule cells from ethanol-fed animals, as was IPSP duration in CA1 cells (Fig. 1D and Tables 1 and 2) (*13*). The amplitude of the subthreshold IPSP was also significantly depressed in granule cells (data on CA1 cells were insufficient). Similarly, the duration and amplitude of the AHP (*13*) in granule cells and the duration of the AHP in CA1 cells were significantly reduced.

Such depression of neuronal inhibition has also been suggested by results obtained with extracellular recordings in vivo (*1*, *5*). The significant decrease in the amplitudes of IPSP's and AHP's in two different types of cells strongly supports the hypothesis that neuronal inhibition is depressed after prolonged exposure to ethanol. Such results could be attributed to a hyperexcitable withdrawal phase lasting more than 3 weeks after treatment. However, in rats this hyperexcitability reaches a peak 8 hours after ethanol is withdrawn and is characterized by convulsions, tremors, and other signs of heightened excitability of the central nervous system (CNS) (*14*). The observed depression of inhibitory potentials may reflect a pathological state associated with ethanol-induced brain damage rather than withdrawal. Howev-

er, brain damage and withdrawal could have certain mechanisms in common, including depression of inhibition.

The decrease in neuronal inhibition could be attributable to altered concentrations of neurotransmitters, loss of inhibitory interneurons, or changes in receptors. For example, repeated exposure to ethanol is associated both with decreased concentrations of γ-aminobutyric acid (GABA) in the CNS and with a reduced density of low-affinity GABA receptor binding sites (*15*). IPSP's in CA1 and granule cells were thought to be generated solely by a GABA-mediated increase in chloride conductance until it was shown that only the early phase of the orthodromically elicited IPSP is chloride-mediated and that the later phases are due to an increase in potassium conductance (*16*) which is partly or entirely calcium-mediated. Calcium-mediated potassium conductance, presumably activated by a transient increase in the concentration of intracellular free $Ca^{2+}$ (*17*), also mediates the long-lasting AHP that follows action potentials in CA1 cells (*18*). With suprathreshold stimulation, the IPSP that follows an action potential is actually a combination of an IPSP and an AHP. Our data show that subthreshold and suprathreshold IPSP's and AHP's were reduced in ethanol-exposed animals (Fig. 1D). Therefore, these intracellular recording experiments support the hypothesis that an impairment in calcium-mediated potassium conductance could be responsible for the depression of the IPSP and AHP, although an effect on potassium conductance alone cannot be ruled out. There is to our knowledge no evidence directly linking impairment of potassium channel function with exposure to ethanol.

The role of calcium in the actions of ethanol has been extensively investigated. Low concentrations of ethanol have been shown to hyperpolarize CA1 cells with a conductance increase—even in perfusate containing no $Ca^{2+}$—and to enhance calcium-mediated AHP's and IPSP's (*19*). These observations are compatible with the suggestion that a single dose of ethanol increases the concentration of intracellular free $Ca^{2+}$. Heavy exposure to ethanol might lead to chronically raised levels of intracellular free $Ca^{2+}$, which can diminish inward calcium currents (*20*). A specific increase in $Ca^{2+}$ in synaptic membranes with a concomitant decrease in $Ca^{2+}$ binding has been noted after prolonged exposure to ethanol (*21*), suggesting saturation of intracellular calcium buffering or impairment of a $Ca^{2+}$ pump leading to increased intracellular $Ca^{2+}$. Significantly elevated intracellular $Ca^{2+}$ is highly toxic to cells (*22*). For example, ethanol (100 m*M*) kills rat hepatocytes by increasing intracellular $Ca^{2+}$ (*23*).

Depressed neuronal inhibition has been suggested as a factor in impaired CNS function in ethanol-exposed animals (*1*) and in chronic alcoholics (*24, 25*). Our results lend further support to this idea.

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10. Intracellular electrodes were pulled on a Brown-Flaming electrode puller with thin-walled glass capillary tubes (Haer No. 30.30.0) and were filled with 4 percent horseradish peroxidase (Sigma IV) diluted in 0.05*M* tris-HCl buffer with 0.2*M* KCl at *p*H 7.4. After physiological measurements were made, the cells were stained for morphological evaluation. Electrode resistance varied from 40 to 120 megohms.
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