low- and high-frequency slopes are reduced; (iii) the peak is broadened; (iv) the peak is shifted to a lower frequency; (v) the SPL in the plateau region below the peak frequency is decreased as much as 10 to 15 dB. The SPL in the 0.25 to 3 kHz region remains unchanged.

The basilar membrane response curve 3/26/81 (Fig. 1) is replotted in Fig. 2. The SPL required for a basilar membrane vibration amplitude of 3×10^{-8} cm was obtained by linear extrapolation. For comparison, a neural tuning curve is shown; the threshold values for this curve are within the normal range for this cat in this frequency region (*14*). In the peak region, the low-frequency slope (86 dB per octave), the high-frequency slope (538 dB per octave), and the sharpness of resonance ($Q_{10\ dB} = 5.9$) are the same for the two curves. The principal difference is in the tip (peak) : tail (1 kHz) ratio. For the neural tuning curve, this ratio is about 44 dB, while for the basilar membrane tuning curve it is 30 dB. Tip-to-tail ratios exceeding 25 dB were observed in at least five other basilar membrane experiments. [The highest tip-to-tail ratio reported by Rhode (*4*) was 15 dB]. A second difference between our data and that of Liberman and Kiang (*15*) is that, for a given location on the basilar membrane, the frequency of the peak falls a factor of 1.4 to 1.9 below their frequency values.

These differences may be explained by the fact that the cochlea on which our measurement was made showed some high-frequency CM loss. Therefore, this basilar membrane response is not that of an entirely undamaged cochlea. Indeed, Liberman (*14, 16*) has shown that in noise-traumatized cats the tip-to-tail ratio of neural tuning curves decreases and the sensitivity in the region an octave below the peak frequency increases by 10 to 15 dB. Cody and Johnstone (*17*), recording from single auditory nerve fibers, showed that even a 1-minute exposure to a tone at 100 dB SPL results in a 30-dB loss in sensitivity in the peak region; also the peak frequency shifts down almost an octave.

The mechanical frequency response of the basilar membrane is susceptible to trauma, which affects the basilar membrane frequency response mainly in the peak region. This leads to the flat frequency response observed by most investigators, and quite often by us. However, when exceptional precautions are exercised to minimize cochlear damage, preparations are obtained in which the peak of the basilar membrane response is larger than previously seen. The basilar membrane frequency response under these conditions is closer in shape to neural tuning curves. In view of the above observations, the need for a second filter (*3*) must be reevaluated.

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References and Notes

1. G. von Bekesy, *J. Acoust. Soc. Am.* **21**, 233 (1947); B. M. Johnstone and A. J. Boyle, *Science* **158**, 89 (1967); W. S. Rhode, *J. Acoust. Soc. Am.* **49**, 1218 (1971).
2. J. P. Wilson and J. R. Johnstone, in *Symposium on Hearing Theory* (IPO, Eindhoven, Netherlands, 1972).
3. E. F. Evans and J. P. Wilson, *Science* **190**, 1218 (1975).
4. W. S. Rhode, *J. Acoust. Soc. Am.* **64**, 158 (1978).
5. D. O. Kim and C. E. Molnar, in *The Nervous System*, D. B. Tower, Ed. (Raven, New York, 1975).
6. J. P. Wilson and J. R. Johnstone, *J. Acoust. Soc. Am.* **57**, 705 (1975).
7. E. L. Lepage and B. M. Johnstone, *Hear. Res.* **2**, 183 (1980).
8. S. M. Khanna and D. G. B. Leonard, "Cochlear damage incurred during preparation for and measurement of basilar membrane vibrations," in preparation.
9. ———, "Basilar membrane vibrations measured in cat using a round-window approach," in preparation.
10. ———, in *Optics in Biomedical Sciences*, G. von Bally and K. Schindl, Eds. (Springer-Verlag, Berlin, in press); S. M. Khanna, in preparation.
11. W. G. Sokolich, *J. Acoust. Soc. Am.* **52** (Suppl. 1), S12 (Abstr.) (1977).
12. M. C. Liberman and D. G. Beil, *Acta Otolaryngol.* **88**, 161 (1979).
13. D. G. B. Leonard and S. M. Khanna, in preparation.
14. M. C. Liberman, personal communication.
15. ——— and N. Y. S. Kiang, *Acta Otolaryngol. Suppl.* **358**, 1 (1978).
16. M. C. Liberman, thesis, Harvard University (1976).
17. A. R. Cody and B. M. Johnstone, *Hear. Res.* **3**, 3 (1980).
18. M. C. Liberman, *J. Acoust. Soc. Am.* **63**, 442 (1978).
19. Supported by NIH grants 5 K04 NS 00292 and 5 R01 NS 03654.

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Ethanol in Low Doses Augments Calcium-Mediated Mechanisms Measured Intracellularly in Hippocampal Neurons

Abstract. *The electrophysiological effects of ethanol in low doses (5 to 20 millimoles per liter or 23 to 92 milligrams per 100 milliliters) were examined intracellularly in CA1 cells of rat hippocampus in vitro. Inhibitory and excitatory postsynaptic potentials were increased when ethanol was applied to the respective synaptic terminal regions. Postsynaptically, ethanol caused a moderate hyperpolarization with increased membrane conductance, even when synaptic transmission was blocked. Ethanol augmented the hyperpolarization that followed repetitive firing or that followed the eliciting of calcium spikes in the presence of tetrodotoxin, but not the rapid afterhyperpolarization in calcium-free medium. Ethanol appears to augment calcium-mediated mechanisms both pre- and postsynaptically.*

Ethanol has variable effects on central mammalian neuronal activity and transmitter release (*1*). Both increased and decreased rates of spontaneous firing of single units of rat hippocampus were observed with ethanol concentrations less than 80 mg/100 ml (*2*). In the hippocampus, extracellular measurements of evoked field potentials indicated that ethanol in low doses increased excitation (*3*) and inhibition (*3, 4*). We studied the cellular mechanisms of mildly intoxicating concentrations of ethanol using the mammalian hippocampal slice preparation (*5*), which is well suited for intracellular recordings.

Male Sprague-Dawley rats (250 to 350 g) were lightly anesthetized with ether, then decapitated. The hippocampal slices were prepared (*5*) and maintained on a mesh for recording at 32° to 35°C. Ethanol, dissolved in control medium, was perfused (four cells) or focally ejected onto the slice with a micropipette (66 cells). Standard intracellular electrophysiological recording techniques were used (*6*). Only cells with spikes greater than 75 mV were studied. The mean ± standard deviation (S.D.) for the resting potentials, when measured, was 55.4 ± 4.5 mV ($N = 18$).

After a characteristic delay of 0.5 to 3 minutes, ethanol caused a moderate hyperpolarization (Fig. 1A) of 0.5 to 8 mV (mean ± S.D., 2.7 ± 1.8 mV) in 78 percent of the cells and a moderate conductance increase (Fig. 1C) of 10 to 40 percent in 74 percent of the cells. All but one cell showed either or both of these effects. The reversal potential for the increased conductance, estimated from the intersection of the current-voltage curves (*7*) in control solution and after ethanol application was between −0.5 and −6.0 mV (mean ± S.D., −3.2 ± 2.6 mV) ($N = 5$) below the resting potential, suggesting increased Cl^- or K^+ conductance. When spontaneous spiking was present, a decreased frequency occurred in 10 of 12 cells (Fig. 1A). The amplitude of the injected depolarizing current pulse necessary to trigger a spike was usually increased after ethanol application, as expected from both the hyperpolariza-

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tion and conductance increase. These effects occurred in the presence of synaptic blockade with medium containing Mn^{2+} and a low concentration of Ca^{2+} (eight cells), no Ca^{2+} (five cells), or tetrodotoxin (TTX) (six cells), and did not depend on whether ethanol was applied onto distal apical dendrites or onto the soma. Potassium chloride electrodes were used to record from 11 cells to assess whether ethanol-induced hyperpolarization and conductance increase could be mediated by Cl^-. After 4 to 8 minutes of intracellular Cl^- injection (0.5 nA of hyperpolarizing current in three cells), small reversed inhibitory postsynaptic potentials (IPSP's) were seen (*8*), suggesting increased intracellular Cl^- concentration. However, since ethanol-induced hyperpolarization and conductance increase still occurred in these cells, the Cl^- ion apparently was not involved. These and all of the other described effects of ethanol were similar at all concentrations used (5 to 20 m*M*) and persisted for more than 15 minutes.

Both excitatory postsynaptic potentials (EPSP's) and IPSP's were increased as a result of exposure to ethanol by perfusion in four of four cells (Fig. 1B). Selective application of ethanol to the apical dendrites of the stratum radiatum, which contains the excitatory terminal endings, increased the intracellularly measured EPSP amplitudes (Fig. 1B) in six of eight cells (by > 50 percent), whereas the amplitudes of the orthodromically elicited IPSP increased in only one of eight cells. Some initially subthreshold EPSP's become sufficiently large to trigger a spike in the presence of ethanol (Fig. 1B). This relatively large increase in EPSP could not be explained by the small hyperpolarization mentioned above. When ethanol was focally ejected onto the somatic area of the recorded neuron—the presumed location of inhibitory synapses—the orthodromic IPSP from stratum radiatum stimulation was increased in 16 of 19 cells (Fig. 1B), but the EPSP was also increased in only 3 of the 19 cells. The IPSP's increased in size, even though most cells were hyperpolarized in the presence of ethanol. These data suggest that ethanol might augment both excitatory and inhibitory neurotransmitter release at the nerve terminal location, as has been shown at the vertebrate neuromuscular junction (*9*). However, ethanol could augment the postsynaptic response to the putative transmitter, since it caused increased sensitivity to acetylcholine applied by iontophoresis at the vertebrate neuromuscular junction (*10*). Nestoros (*11*) showed in vivo that ethanol specifically augments the inhibition of spontaneous cortical neuronal spiking mediated by γ-aminobutyric acid (GABA). However, we were unable, in ten cells, to demonstrate any ethanol-mediated augmentation of CA1 pyramidal cell responses to pressure-ejected GABA (10^{-2} to 10^{-4}*M*). These GABA responses (*12*) are presumably mediated by increased Cl^- conductance.

Because the postsynaptic effect seemed not to be mediated by increased Cl^- conductance, we investigated ethanol effects on a known postsynaptic K^+ conductance mechanism. A long-lasting afterhyperpolarization (AHP) due to increased K^+ conductance, probably activated by Ca^{2+} influx, has been demonstrated after repetitive firing in CA1 cells (Fig. 2A) (*13–16*). With drop application of ethanol to the soma, this AHP increased in six of six cells (Fig. 2B). In order to unmask Ca^{2+} spikes, we applied TTX, which blocks Na^+ spikes. Broader, shorter Ca^{2+} spikes elicited by much greater depolarization (*13, 17*) were followed by an AHP (*13*) (Fig. 2A). Ethanol increased this AHP (Fig. 2, A and C), but did not affect or even slightly decreased (< 10 percent) the depolarization-induced Ca^{2+} spike (Fig. 2A) in six of six cells. In order to diminish Ca^{2+} conductance, a solution containing Mn^{2+} and low Ca^{2+} was used. The diminished AHP (*13*) was minimally increased by ethanol in five of seven cells. To prevent completely inward Ca^{2+} current and to unmask an AHP that was presumably not Ca^{2+}-dependent (*18*), we bathed slices in Ca^{2+}-free solution for at least 1

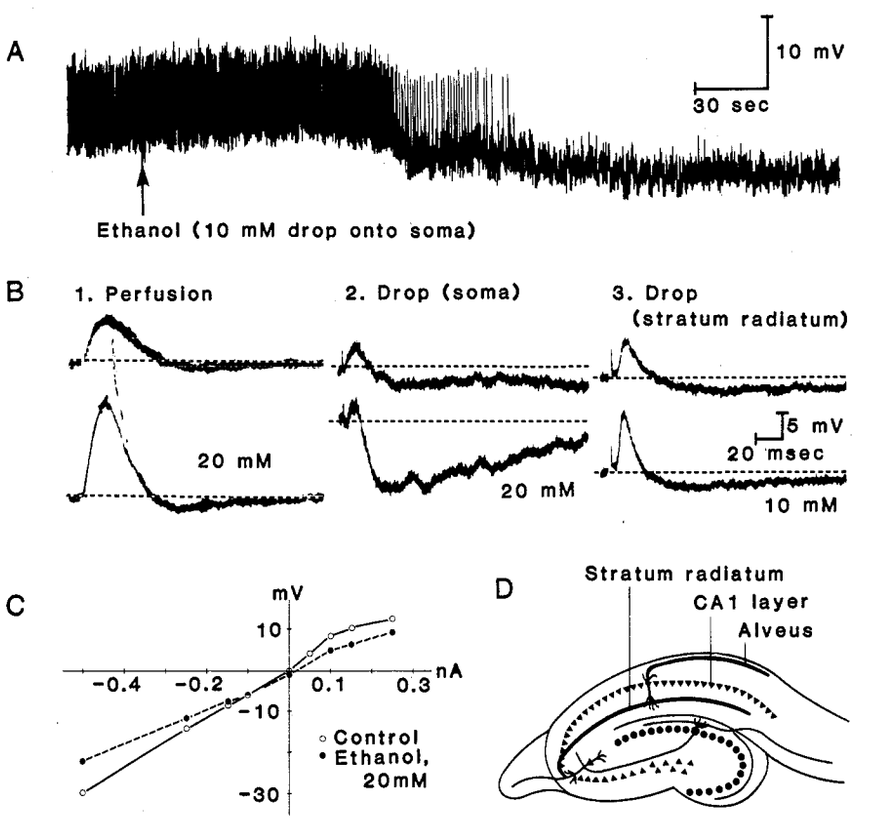

Fig. 1. Effects of low doses of ethanol measured intracellularly in CA1 cells of a hippocampal slice preparation. (A) Spontaneous activity and resting membrane potential obtained with a potassium acetate electrode were monitored on a d-c chart recorder (Gould). Approximately 1.5 minutes after focal application of a drop containing 10 m*M* ethanol onto the somatic region of the CA1 cell, the cell was hyperpolarized; this was followed by cessation of the spontaneous spiking. Spike heights were attenuated by the chart recorder. (B) An EPSP-IPSP sequence elicited by stratum radiatum stimulation. (1) Bath perfusion of 20 m*M* ethanol caused an increased EPSP and IPSP. The stimulation for both records was 18 μA. The EPSP sometimes reached threshold causing the neuron to fire (lower trace). (2) Drop application of ethanol (20 m*M*) onto the somatic area caused a greatly enhanced IPSP 5 minutes after ethanol application. (3) Ethanol (10 m*M*) focally applied to the stratum radiatum increased the EPSP 2 minutes after ethanol application. Stimulation currents for (2) and (3) were 22 and 100 μA, respectively. (C) Injected current plotted against the peak voltage response. Cell input resistance (R_{in}) was decreased after ethanol exposure; R_{in} was measured in all cells with 100-msec constant-current pulses injected by means of an active bridge amplifier. With no injected current, the ethanol plot crosses the *y*-axis at −1 mV, reflecting the degree of resting membrane hyperpolarization seen 5 minutes after ethanol exposure in this cell. Control R_{in} was 60 megohms, reduced with ethanol perfusion to 44 megohms. (D) Diagram of the hippocampal slice preparation. The stratum radiatum contains excitatory afferents to the apical dendrites of CA1 cells.

hour. This nondependent AHP (unlike the longer Ca^{2+}-dependent AHP) is sensitive to tetraethylammonium chloride (*16*) and is resistant to Mg^{2+} (*15*). Ethanol had no effect on this AHP in five of five cells.

The simplest explanation of the above data is that ethanol augments Ca^{2+}-mediated pre- and postsynaptic mechanisms, possibly by increasing intracellular Ca^{2+} concentration or effectiveness. Augmented nerve-evoked presynaptic transmitter release is associated with increased inward Ca^{2+} current (*19*). However, the same effect could occur with increased intracellular Ca^{2+} activity from intracellular sources such as mitochrondria (*20*). At low doses, ethanol increases Ca^{2+} accumulation in murine brain synaptosomes (*21*). In the presence of EGTA without Ca^{2+}, spontaneous, nonspike-induced transmitter release was increased at the neuromuscular junction by ethanol (*22*). Since we did not observe increased spontaneous postsynaptic potentials after application of ethanol, with or without synaptic blockade, the posysynaptic effects appear to be independent of presynaptic transmitter release. Enhanced postsynaptic neurotransmitter sensitivity seems unlikely in view of the lack of ethanol-GABA interaction. If the postsynaptic ethanol effects occurred in the presynaptic terminal, the hyperpolarization would be expected to increase, and the conductance increase should decrease the nerve-evoked transmitter release.

The ethanol-induced postsynaptic hyperpolarization and conductance increase, which were independent of intracellular Cl^- injections, probably resulted from increased K^+ conductance (*23*). These effects were independent of extracellular Ca^{2+} since they occurred in Ca^{2+}-free medium.

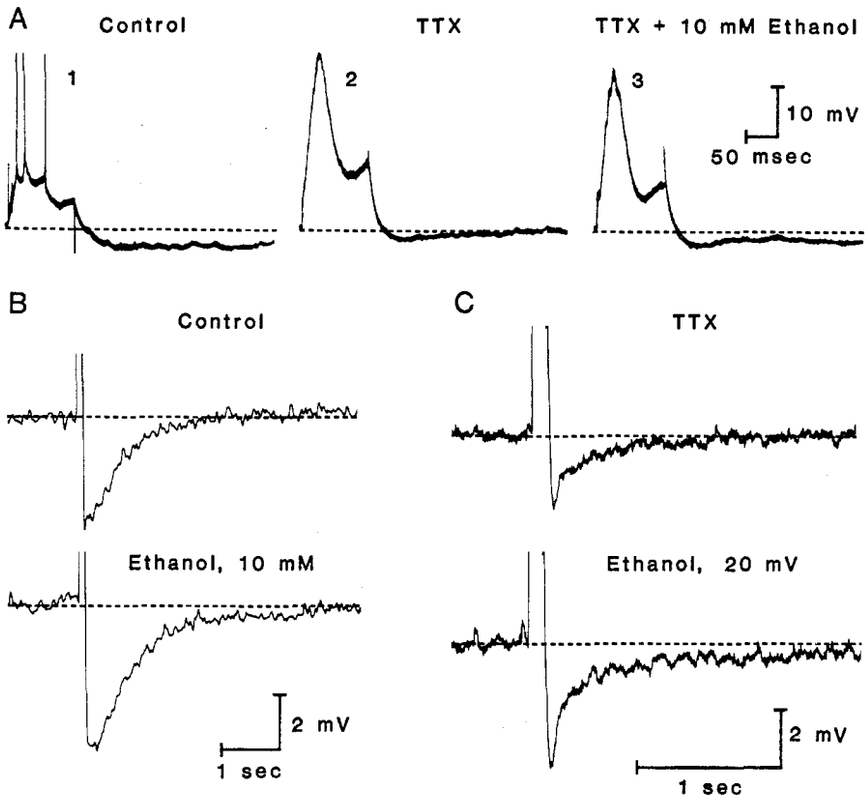

Fig. 2. Augmentation of AHP's by ethanol. (A) (1) Postrepetitive firing AHP in control medium with a 0.35-nA, 100-msec constant-current pulse. Spikes were retouched and full heights (not shown) were 80 mV. (2) TTX ($2 \times 10^{-5} M$) was applied in large drops to the stratum radiatum, pryamidale, and oriens, blocking fast Na^+ spikes. A higher injected depolarizing current (3.5 nA) elicited a Ca^{2+} spike (*12, 13*) followed by an AHP that was probably mediated by increased K^+ conductance (*12*). (3) Three minutes after drop application of 10 m*M* ethanol to the soma, the AHP (at 3.5 nA) was increased, but the Ca^{2+} spike was slightly decreased. Bridge balance was not corrected after ethanol application. (B) Increases in depth and length of AHP after drop application of 10 m*M* ethanol to the soma in control solution. The current pulse for control and ethanol measurements was 0.75 nA, and each pulse caused four spikes. A chart recorder was used for (B) and (C), and depolarizing responses to 100-msec current pulses were cut off because of high gain. (C) In the presence of TTX, the AHP following the Ca^{2+} spike increased in depth and was markedly prolonged after drop application of 20 m*M* ethanol to the soma. A 1.0-nA constant-current depolarizing pulse was used.

Ethanol did not affect the—presumably Ca^{2+}-independent—brief AHP seen in Ca^{2+}-free medium, but did augment the long-lasting AHP, which is most likely Ca^{2+}-mediated, according to experiments in central vertebrate neurons (*18, 24*) including hippocampal pyramidal cells (*13–15, 25*). Because the Ca^{2+} spikes in TTX solution were not increased by ethanol, we suggest that ethanol augmented the Ca^{2+}-mediated AHP by increasing intracellular Ca^{2+} activity or effectiveness. Increased intracellular Ca^{2+} is an effective inhibitor of inward Ca^{2+} current (*26*). Furthermore, in voltage-clamped dorid neurons (*27*), intraneuronal free Ca^{2+} and not inward Ca^{2+} current is related to the activation of the Ca^{2+}-dependent K^+ conductance. The increased IPSP's seen with ethanol could have resulted in part from augmented postsynaptic Ca^{2+}-induced K^+ conductance (*28*). One recently suggested hypothesis is that anesthetics (and ethanol) may cause Ca^{2+}-dependent permeability systems (such as K^+) to be more sensitive to intracellular ionized Ca^{2+}, without necessarily raising the Ca^{2+} concentration (*29*). Although all of the observed effects of ethanol are mediated by Ca^{2+}, confirmation of our hypothesis awaits intracellular measurements of changes in Ca^{2+} activity after application of ethanol.

The delay of 1.5 to 3 minutes until onset of the effects of ethanol suggests an indirect, time-dependent process that includes diffusion across membranes. The combination of the ethanol-induced excitation (increased EPSP's) and inhibition (increased IPSP's, postsynaptic hyperpolarization, conductance increase, and augmented AHP's) might help to explain the mixed excitatory and inhibitory effects of low-dose ethanol on the central nervous system, particularly in the local circuits of the hippocampus.

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References and Notes

1. H. Kalant, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **24**, 1930 (1975); B. Tabakoff, E. Noble, K. Warren, in *Nutrition and the Brain*, R. J. Wurtman and J. J. Wurtman, Eds. (Raven, New York, 1979), vol. 4, p. 159; H. Kalant, in *Advances in Pharmacology and Therapeutics*, G. Olive, Ed. (Pergamon, New York, 1979), vol. 8, p. 199.

2. L. A. Grupp, *Psychopharmacologia* **70**, 95 (1980).
3. S. A. Newlin, J. Mancillas-Trevino, F. E. Bloom, *Brain Res.* **209**, 113 (1981).
4. D. Durand, W. A. Corrigall, P. Kujtan, P. L. Carlen, *Can. J. Physiol. Pharmacol.* **59**, 979 (1981).
5. C. Yamamoto and H. McIlwain, *J. Neurochem.* **13**, 1333 (1966); P. A. Schwartzkroin, *Brain Res.* **85**, 423 (1975). Slices were continuously perfused by control medium composed of (in m*M*) NaCl, 124; KCl, 3.5; KH_2PO_4, 1.25; Mg_2SO_4, 2; $CaCl_2$, 2; $NaHCO_3$, 26; and dextrose, 10. When synaptic blockade was required, a solution containing a small amount of Ca^{2+} (0.5 m*M*) and 2 to 4 m*M* manganese chloride was used with KH_2PO_4 removed to avoid precipitation. In this solution, EPSP's and IPSP's could not be elicited after 20 minutes of perfusion. Ca^{2+}-free solution was also prepared as follows (in m*M*): NaCl, 130; KCl, 6.2; MgCl, 1.3; MnCl, 2.4; tris, 25; and dextrose, 10. The *p*H of all solutions was between 7.35 and 7.40.
6. Microelectrodes (60 to 130 megohms), filled with 3*M* potassium acetate, 2*M* $KMeSO_4$, or 3*M* KCl, were used for intracellular recordings. Data were stored on tape and plotted on a d-c chart recorder or photographed from the oscilloscope. The stratum radiatum was stimulated by a monopolar tungsten electrode at 0.2 Hz (0.1 msec, 10- to 120-μA constant-current pulses) to evoke orthodromic EPSP's, spikes, and IPSP's. Antidromic spikes and IPSP's were similarly evoked from alveus stimulation (Fig. 1D). To block Na^+ action potentials and to bring out Ca^{2+} spikes, we applied TTX (2×10^{-5}*M*) in large drops to the strata radiatum, pyramidale, and oriens.
7. R. Werman, *Comp. Biochem. Physiol.* **18**, 745 (1966); B. L. Ginsborg, *Pharmacol. Rev.* **18**, 289 (1967).
8. B. E. Alger and R. A. Nicoll, *Brain Res.* **200**, 195 (1980).
9. P. W. Gage, *J. Pharmacol. Exp. Ther.* **150**, 236 (1965); P. L. Carlen and W. A. Corrigall, *Neurosci. Lett.* **17**, 95 (1980).
10. P. W. Gage, R. N. McBurney, G. T. Schneider, *J. Physiol. (London)* **244**, 409 (1975).
11. J. N. Nestoros, *Science* **209**, 708 (1980).
12. CA1 cell response to pressure-ejected GABA consisted of a hyperpolarization with somatic application, sometimes preceded by a depolarization, particularly with higher doses and dendritic application. Both membrane potential changes were accompanied by a conductance increase. The threshold dose was 10^{-3}*M*. These responses, which occurred within seconds after the pressure ejection (unlike the 0.5- to 3-minute delay after ethanol application) have been reported by others [B. E. Alger and R. A. Nicoll, *Nature (London)* **281**, 315 (1979); P. Andersen, R. Dingledine, L. Gjerstad, I. A. Langmoen, A. Hosfeldt-Laursen, *J. Physiol. (London)* **305**, 279 (1980); M. Segal, *ibid.* **303**, 423 (1980)]. Ethanol did not augment depolarization induced by focally applied 1*M* glutamate in two cells.
13. J. R. Hotson and D. A. Prince, *J. Neurophysiol.* **43**, 409 (1980).
14. B. E. Alger and R. A. Nicoll, *Science* **210**, 1122 (1980).
15. B. Gustafsson and H. Wigstrom, *Brain Res.* **206**, 462 (1981).
16. P. A. Schwartzkroin and D. A. Prince, *ibid.* **185**, 169 (1980).
17. P. A. Schwartzkroin and M. Slawsky, *ibid.* **135**, 157 (1977).
18. E. F. Barrett and J. N. Barrett, *J. Physiol. (London)* **255**, 737 (1976).
19. E. P. Rubin, *Calcium and the Secretory Process* (Plenum, New York, 1974); R. Llinas, in *Neuroscience Study Program*, R. P. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1979), vol. 4, p. 555.
20. M. E. Sandoval, *Brain Res.* **181**, 347 (1980).
21. E. K. Michaelis and S. L. Myers, *Biochem. Pharmacol.* **28**, 2081 (1979); M. B. Friedman, C. K. Erickson, S. W. Leslie, *ibid.* **29**, 1903 (1980).
22. D. M. J. Quastel, J. T. Hackett, J. D. Cooke, *Science* **172**, 1034 (1971).
23. K. Krnjevic and A. Lisiewicz, *J. Physiol. (London)* **225**, 363 (1972); K. Krnjevic, E. Puil, R. Werman, *Can. J. Physiol. Pharmacol.* **54**, 73 (1976); *J. Physiol. (London)* **275**, 225 (1978).
24. K. Krnjevic, E. Puil, R. Werman, *J. Physiol. (London)* **275**, 199 (1978).
25. P. A. Schwartzkroin and C. E. Stafstrom, *Science* **210**, 1125 (1980).
26. S. Hagiwara and L. Byerly, *Annu. Rev. Neurosci.* **3**, 69 (1981).
27. R. Eckert and D. Tillotson, *Science* **200**, 437 (1978).
28. R. H. Thalmann and G. F. Ayala, *Soc. Neurosci.* **6**, 200 (1980); R. A. Nicoll and B. E. Alger, *Science* **212**, 957 (1981).
29. P. F. Baker and A. H. V. Schapira, *Nature (London)* **284**, 168 (1980).
30. Supported by National Institute of Health grant RO1 NS16660-01 and Medical Research Council of Canada grant Ma 6019. We thank J. F. MacDonald and J. M. Wojtowicz for discussions, A. Padjen for reading the manuscript, and L. A. Staines for typing.
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Iodine-125–Labeled Triiodothyronine in Rat Brain: Evidence for Localization in Discrete Neural Systems

Abstract. *Autoradiograms prepared from adult rat brains demonstrate that nerve cells and neuropil in different brain regions selectively concentrate and retain intravenously administered triiodothyronine, by mechanisms susceptible to saturation with excess triiodothyronine. A neuroregulatory role for thyroid hormones, strongly supported by the observations, may account for their marked effects on behavior and the activity of the autonomic nervous system.*

Marked changes in nervous system functions which develop in hypothyroid and hyperthyroid individuals are generally attributed to hormone-mediated events originating outside the nervous system (*1*). Recently, however, new observations have again raised the possibility that thyroxine (T_4) and its metabolites may function directly within the mature brain. These observations demonstrate that intravenously administered labeled T_4 slowly enters selected regions of rat brain by a saturable mechanism and becomes progressively more concentrated in the nerve terminal fraction. There it gives rise, through monodeiodination, to triiodothyronine (T_3), a metabolite with severalfold greater activity than its fully iodinated precursor. When T_4 supplies are low, as in hypothyroidism, the fractional conversion of T_4 to T_3 is unchanged or even reduced in most T_3-forming tissues, whereas it increases markedly in the brain. Intracerebral labeled T_3 (T_3*), whether generated in situ or taken up as such from the systematic circulation, remains concentrated in an osmotically sensitive compartment of the nerve terminal fraction long after the injected T_3* has largely disappeared from the circulation (*2*).

The foregoing evidence was obtained exclusively from biochemical studies requiring disruption of cytoarchitecture during tissue homogenization and extraction. To avoid the potential artifacts inherent in these methods, we reexamined the problem of the T_3 distribution in brain by using autoradiography. Earlier efforts to do so had been hampered by the low specific activity of labeled thyroid hormone preparations then available (in the range of 80 μCi/μg). In spite of this limitation, a number of different studies have provided preliminary evidence in support of a differential regional and subcellular distribution of iodothyronines in the brains of guinea pigs, rats, and *Rana pipiens* tadpoles (*3*).

In our experiments in which we used high specific activity T_3*, we sought first to identify the histologic correlates of previously observed gross anatomical differences in the regional distribution of thyroid hormones. For this purpose, two 150-g male Sprague-Dawley rats (Zivic-Miller Laboratories), surgically thyroidectomized 1 week earlier (*4*), received 1 mCi of ^{125}I-outer (phenolic) ring–labeled T_3 (specific activity, ~ 3000 μCi/μg; from P. Shadden, Abbott Laboratories) given under light ether anesthesia into the jugular vein. Rats were decapitated 3 hours after the injection, and the brains were processed for thaw-mount autoradiography (*5*). Parallel biochemical analyses confirmed earlier findings that radioactivity in brain homogenates at both 3- and 10-hour intervals after intravenous T_3* administration was more than 80 percent due to T_3 itself, whereas iodide never accounted for more than 8 percent.

To visualize labeling patterns at low magnification without the use of tinctorial stains, we left the brain sections exposed to the photographic emulsion for 7 months. The autoradiograms revealed clear-cut differences in the distribution of T_3* and its labeled metabolites in different brain regions and emphasized the selectivity of neuronal cell and neuropil labeling within regions (Fig. 1). For example, in the cerebellum (Fig. 1d), strong labeling of Purkinje cells contrasted with lesser labeling over basket cells. On the other hand the intensity in neuropil was different in different regions of the cortex and caudate, whereas in general neuropil labeling contrasted with the lower silver-grain density in white matter of the corpus callosum (Fig. 1, a to c). It therefore seems evident that gross dissections can provide only a hint

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