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6. The question of ease of dispersal is crucial. Clearly, any movement of a soil mass from one continent to another or from a continent to an island could serve to disperse nematodes to a new area, and probably this has happened at times. However, certain genera are well distributed on some continents and are found rarely or not at all on others. The nematodes of Europe have been studied for years, whereas those of South America are hardly known. Nevertheless, the few collections from South America reveal a greater diversity in Leptonchoidea than is known from Europe after decades of intensive collecting.
7. These include North America, Europe, India, Australia, New Zealand, and some of Africa and the U.S.S.R. In South America, limited collecting has been done in Argentina, Brazil, Venezuela, Paraguay, and Chile. Islands studied include Puerto Rico, New Guinea, New Hebrides, Mauritius, and Japan.
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Sustained Tolerance to a Specific Effect of Ethanol on Posttetanic Potentiation in *Aplysia*

Abstract. *Perfusion with 0.8 molar ethanol in seawater specifically accelerates the rate of decay of posttetanic potentiation observed after repetitive electrical stimulation of an identified synapse in the abdominal ganglion of* Aplysia californica. *Repeated perfusion with seawater alternately with and without ethanol leads to a progressive diminution of this specific effect of ethanol, such that after the third application ethanol no longer has any effect on the rate constant of decay of posttetanic potentiation. This tolerance to the specific effects of ethanol persists for at least 11 hours after the last application of ethanol.*

Tolerance to alcohol refers to the diminution of its pharmacological efficacy with repeated exposures. In mammals, including humans, tolerance to ethanol is due in large part to adaptive changes in the nervous system rather than to an acceleration of ethanol metabolism (*1*, *2*). The nature of the adaptive change that produces tolerance is not known. In a previous study we used an identified synapse in *Aplysia californica* to demonstrate a specific neurophysiological effect of ethanol and other alcohols, namely an acceleration of the decay of posttetanic potentiation (PTP) (*3*) (Fig. 1). We now report that repetitive administration of ethanol over the course of a number of hours leads to the development of tolerance to this specific, neurophysiological effect.

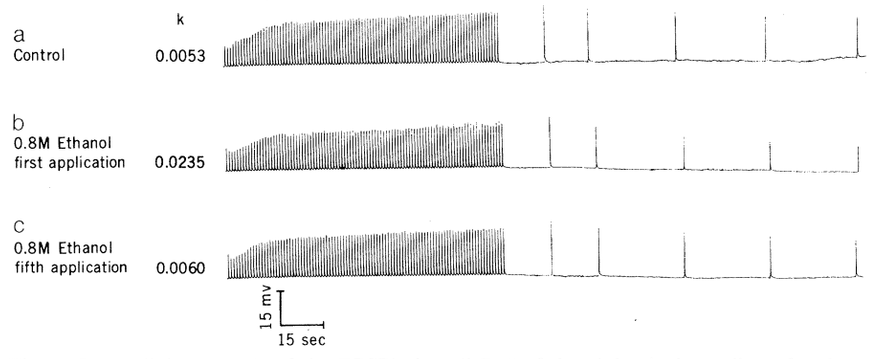

Fig. 1. Intracellular records of the EPSP's in cell R15 of the abdominal ganglion of *Aplysia californica* during stimulation of the right visceropleural connective by a train of 100 pulses at one pulse per second, followed by two test pulses at 15-second intervals and then test pulses every 30 seconds. Cell R15 was hyperpolarized to about −100 mv by injecting current through the second barrel of a double-barrel recording electrode. (a) Control preparation in fortified artificial seawater (*5*). (b) The stimulus pattern was given 30 minutes after starting the first application of artificial seawater containing 0.8*M* ethanol. The response during the train of stimuli is similar to that of the control preparation; however, the PTP observed after the train decays with a much faster time course. Beginning 10 minutes after this train the preparation was washed for 120 minutes before the second application of ethanol. (c) The stimulus pattern was given 30 minutes after starting the fifth application of 0.8*M* ethanol. The rate of decay of PTP here is similar to that in the control preparation. The calculated rate constant of PTP decay (*k*) is shown for each case.

Posttetanic potentiation is a presynaptic phenomenon which is produced after repetitive stimulation of an identified synapse in the abdominal ganglion of *A. californica* (*4*). Electrical stimulation of the right visceropleural connective gives rise to an excitatory postsynaptic potential (EPSP) that may be recorded with an intracellular electrode in cell R15 of the isolated abdominal ganglion by using standard electrophysiological methods (*4*, *5*). During and after repetitive stimulation of this synapse at a frequency of one pulse per second, a series of changes in the amplitude of the recorded EPSP is observed, as has been described in detail (*4*, *5*). After termination of a train of 100 stimuli, the test EPSP's elicited at 30-second intervals are of larger amplitude than the characteristic isolated EPSP of this synapse. This PTP decays with a single exponential time course toward the size of an isolated EPSP, so that after the train of 100 stimuli at one pulse per second, the EPSP amplitude returns to the control level within 20 minutes. When an identical experiment is conducted in the presence of 0.8*M* ethanol all responses during the train are, on the average, identical with those observed in the absence of ethanol. However, the rate constant of decay of PTP is strikingly accelerated (*3*) (Fig. 1). The effect of repeated administration of ethanol on this specific action (acceleration of the rate of decay of PTP) is the subject of this report.

In this investigation we alternated trains of stimuli in the presence and absence of 0.8*M* ethanol and studied the amplitude of all EPSP's elicited by each train as well as the rate constant of decay of PTP. In a typical experiment a train of 100 stimuli at one pulse per second was given and the rate of decay of PTP was determined by eliciting test EPSP's 15 and 30 seconds after the end of the train and then at 30-second intervals for 10 minutes. This stimulus pattern was repeated at 40-minute intervals, allowing about 30 minutes of rest between experiments. Solutions were changed at the beginning of the 30-minute rest period. Three consecutive control experiments with fortified artificial seawater (*5*) without ethanol were alternated with a single experiment with a solution of 0.8*M* ethanol in fortified artificial seawater. Continuous application of ethanol was not feasible since axonal conduction block often developed after prolonged perfusion with 0.8*M* ethanol.

In the absence of ethanol, PTP was observed to decay with an average rate constant of 0.0053 ± 0.0006 sec^{-1} (± the

standard error) (see Fig. 1 and closed circles in Fig. 2). During the first application of ethanol, the rate constant of decay of PTP increased in every preparation. The rate constant during the first application of ethanol was 0.0221 ± 0.0067 sec^{-1}. The difference from the control value is significant with $P < .02$ (two-tailed Mann-Whitney U test). During the second application of ethanol the effect on PTP decay was considerably reduced, so that the rate constant of PTP decay was now elevated by ethanol to an average of only 0.0113 ± 0.0032 sec^{-1} ($P \approx .1$ compared to control, by the two-tailed Mann-Whitney U test). The third, fourth, and fifth ethanol applications no longer had any significant effect on the rate constant of PTP decay (Fig. 2).

After the fifth application of ethanol, some of the preparations were perfused with fortified seawater up to an additional 18 hours and the effect of ethanol application was determined after 7 hours and again 11 hours later. After these prolonged intervals in the absence of ethanol, ethanol again was without effect in all cases (Fig. 2).

Our interpretation of the progressive and sustained failure of ethanol to affect PTP decay on repeated exposure is that the preparation becomes tolerant to the action of ethanol. An alternative possibility is that the isolated *Aplysia* ganglion shows progressive deterioration with prolonged maintenance in the perfusion chamber. This seems unlikely since the values remain normal throughout control experiments (Fig. 2). To test this possibility more directly, we determined the effect of an initial application of ethanol after maintaining preparations in the perfusion chamber with or without trains of stimulation for up to 24 hours. As was observed in the usual experiment, initial application of ethanol under these circumstances produced a marked acceleration of decay of PTP. Therefore, we conclude that it is the repeated exposure to ethanol with our experimental paradigm rather than prolonged maintenance in the perfusion chamber that accounts for the development of unresponsiveness to ethanol which we refer to as tolerance.

From observations of the effects of ethanol (*3*), other aliphatic alcohols (*3*), and temperature (*6*) on the rate of decay of PTP, we have inferred that the presynaptic nerve terminal that is responsible for PTP in this system decays back to its normal state through a process which is influenced by the fluidity of some membrane component of the terminal. The development of tolerance to the effect of ethanol demonstrated here and the development of tolerance to an effect of temperature on the rate constant of decay of PTP demonstrated previously (*6*) suggest that there are regulatory mechanisms that control the nature of this critical membrane component of the presynaptic nerve terminal. Evidence has been presented that in *Escherichia coli* adaptation to either changes in temperature (*7*) or aliphatic alcohols (*8*) is mediated by changes in the lipid composition of the plasma membrane. In this manner the fluidity of membrane components is kept fairly constant. A similar mechanism might be operative in the presynaptic nerve terminal, mediating tolerance to temperature or alcohol effects on the rate constant of decay of PTP. It should be pointed out, however, that this adaptive response, like the initial effect, is selective for the rate constant of decay of PTP and apparently does not involve other aspects of the neurotransmission process. Furthermore, in the tolerant state, the rate constant of decay of PTP remains normal in the absence of the treatment that induced tolerance. For example, development of tolerance to ethanol does not involve a detectable change in the rate constant of decay of PTP when tested in the absence of ethanol (Fig. 2). We have not, on the average, observed a hypersensitivity of the tolerant preparation in the absence of ethanol which might be analogous to a "withdrawal" phenomenon, although results with several preparations were suggestive of this. The mechanism for an adaptation that appears so highly specific remains to be determined.

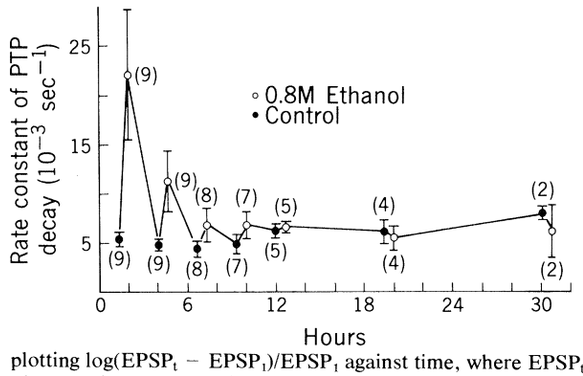

Fig. 2. Changes in the rate constant of PTP decay with repeated application of 0.8*M* ethanol. Identical stimulus patterns were given every 40 minutes. After three control experiments, 0.8*M* ethanol was introduced and a single experiment with ethanol was begun 30 minutes later. The preparation was then washed by perfusion with fortified artificial seawater and the procedure was repeated. The rate constant of PTP decay was determined by plotting $\log(EPSP_t - EPSP_1)/EPSP_1$ against time, where $EPSP_t$ is the amplitude of a test EPSP after a train and $EPSP_1$ is the size of the first EPSP of the train. Such plots give straight lines, the slope of which is the rate constant of PTP decay (*3*, *4*). Each data point represents the mean for the number of experiments which is shown in parentheses; error bars represent the standard error.

M. Elaine Traynor
Paul B. J. Woodson
Werner T. Schlapfer
Samuel H. Barondes
Departments of Neuroscience and *Psychiatry, University of California, San Diego, La Jolla 92093,* and *Veterans Administration Hospital, San Diego 92161*

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