way as mice in the second experiment except that none received larvae.

Among the groups in which two individuals were dosed with 50 or 150 larvae, the number of groups with dominant uninfected mice was not significantly different from the number for which chance alone would account (Table 1). Uninfected mice became dominant significantly more often in groups in which two individuals received a dose of 250 larvae (Table 1). The heaviest mouse in each group became dominant no more often than expected by chance [χ^2 (1) = 0.4171, $P > .05$]. Analysis of variance indicates that neither dosage nor dominance status influenced weight gain by individuals during the experiments [50 larvae $F(51) = 0.52, P > .67$; 150 larvae, $F(84) = 1.13$, $P > .32$; 250 larvae, $F(58) = 0.79$, $P > .51$]. None of the doses used in the second experiment resulted in dominant mice becoming subordinate significantly more often than occurred among the control groups (Table 2).

The dominance status of a male mouse is of prime importance in its gaining access to females (*4*). The same is true for other mammalian species (*5*). If parasites impair the development of dominance in males of other host species, this would support Freeland's (*6*) suggestion that female sexual characteristics (olfactory, visual, and so forth) evolved in part to induce competition by males so as to reveal their disease states. Males with high parasite loads are thus eliminated from the sexual arena, reducing the probability that a female will contract a pathogen capable of diminishing or eliminating her reproductive output. Also, healthier males may provide genes or gene complexes associated with resistance to pathogens, thereby increasing the probability that the offspring will survive and reproduce.

W. J. FREELAND
Department of Biological Sciences, Wright State University, Dayton, Ohio 45435, and *Department of Parasitology, University of Queensland, Brisbane, Queensland, 4067 Australia*

References and Notes

1. G. Hausfater and D. F. Watson, *Nature (London)* **262**, 688 (1976).
2. *Heligmosomoides polygyrus* were from a strain provided by Dr. Paul Weinstein, Department of Biology, University of Notre Dame. Worms were cultured by the methods of P. D. Van Zandt, *J. Elijah Mitchell Sci. Soc.* **77**, 300 (1961).
3. Mice do not establish dominance hierarchies. When male mice are grouped, one usually becomes dominant over the others. Dominance status among the nondominant mice is difficult or impossible to determine reliably. The criterion used most frequently and reliably to determine which individual is dominant is the relative absence of tail and rump wounds [F. H. Bronson, *Physiol. Behav.* **10**, 947 (1973); J. C. DeFries and G. E. McClearn, *Am. Nat.* **104**, 408 (1970); C. Desjardins, J. A. Maruniak, F. H. Bronson, *Science* **182**, 939 (1973); J. A. Lloyd, *Proc. Soc. Exp. Biol. Med.* **137**, 19 (1971); J. G. Vandenberg, *Anim. Behav.* **8**, 13 (1960); J. P. Scott, *Am. Zool.* **6**, 683 (1966)].
4. J. C. Defries and G. E. McClearn, in (*3*); J. M. Horn, *Behav. Genet.* **4**, 373 (1974).
5. R. C. Farentinos, *Anim. Behav.* **20**, 316 (1972); G. Hausfater, *Contrib. Primatol.* **7**, 1 (1975); T. Rowell, *Behav. Biol.* **11**, 131 (1974); H. K. Buechner, *IUCN (Int. Union Conserv. Nat. Nat. Resour. Publ.) New Ser.* **2** (No. 24), 853 (1974).
6. W. J. Freeland, *Biotropica* **8**, 12 (1976).
7. I thank C. Dobson for advice during the early phases of this study. Funded by NIMH grant R03 MH 32090.

12 February 1981; revised 8 May 1981

Epileptogenic Agents Enhance Transmission at an Identified Weak Electrical Synapse in *Aplysia*

Abstract. *To examine the possibility that alterations in the effectiveness of electrical synapses might participate in epileptogenesis, the effects of several convulsants on an identified weak electrical synapse in* Aplysia *were examined. Application of pentylenetetrazole, strychnine, or tetraethylammonium led to a dramatic increase in the size of the electrical postsynaptic potential mediated by the synapse; penicillin was considerably less effective. In a number of animals, the increased electrical synaptic effectiveness led to the abnormal conduction of spikes across the synapse. If convulsants have a similar action in mammalian cortex, enhanced transmission at weak electrical synapses may provide abnormal pathways for the flow of seizure activity and contribute in part to the synchronous firing of neurons characteristic of epileptic activity.*

Recent studies suggest that a surprising number of cells in the mammalian cerebral cortex are interconnected by weak electrical synapses (*1*, *2*). For example, MacVicar and Dudek (*3*) have recently found evidence for electrical coupling between hippocampal pyramidal cells by measurements of electrical coupling, observations of dye passage, and detection of gap junctions by freeze-fracture electron microscopy. In this instance and others, the electrical coupling is relatively weak in that action potentials in one cell do not lead to firing of the connected cell, but only to small postsynaptic potentials (PSP's). However, in

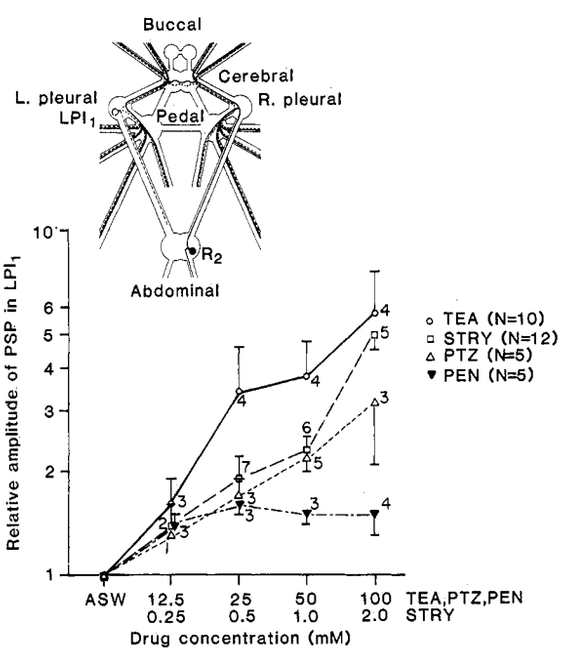

Fig. 1. Enhancement of electrical transmission at the R_2-LPl_1 synapse by convulsants. The PSP in the LPl_1 soma resulting from R_2 spikes provides a measure of synaptic effectiveness. Dose-response curves plotted on a log-log basis are shown for the four drugs. Abbreviations: *TEA*, tetraethylammonium; *STRY*, strychnine; *PTZ*, pentylenetetrazole; *PEN*, penicillin; *ASW*, artificial seawater; *N*, number of animals. Numerals on graph indicate number of observations; data are shown as mean ± standard error of the mean indicated in one direction. Drugs were applied in increasing concentration (Fig. 2A shows data from a typical trial). For comparison, the PSP's have been normalized by taking the initial control PSP in ASW as 1.0. (Inset) Schematic of the nervous system of *Aplysia* with the axonal trees of R_2 (solid line) and LPl_1 (dashed line) indicated (*5*, *15*). The cells are coupled by way of an electrical synapse in the cerebral ganglion. By virtue of the asymmetry in the axonal trees of the cells, a reciprocally functioning synapse appears to rectify: spikes in either cell produce 10-mV PSP's in the postsynaptic axon of the other cell in the cerebral ganglion. However, because the R_2 soma is much farther from the synapse than that of LPl_1, PSP's are recorded in the LPl_1 soma, while none are seen in the R_2 soma.

0036-8075/81/0724-0462$00.50/0

the hippocampus, which is a prime site for epileptogenesis, if this weak electrical coupling were to be enhanced by epileptogenetic factors, it might contribute to the synchronous firing characteristic of epileptic activity. To test this idea, we examined the effects of several convulsant agents on a weak electrical synapse in the nervous system of the marine mollusk *Aplysia californica*. We found that strychnine, pentylenetetrazole, and tetraethylammonium (*4*) substantially increased the effectiveness of the electrical synapse, while penicillin was only modestly effective. In some animals, the synapse was transformed from functioning relatively insignificantly to conducting spikes on a one-for-one basis.

The electrical synapse we examined connects two cholinergic giant neurons: R_2, whose soma is in the right abdominal ganglion, and LPl_1, whose soma is in the left pleural ganglion (Fig. 1, inset). These neurons are homologous, forming a left-right pair, differing only in the asymmetrical location of their cell bodies and the resulting difference in their axonal trees. As a result, even though the synapse itself does not rectify, a spike in LPl_1 has no measurable effect in the soma of R_2, while a spike in R_2 produces a small PSP of less than 1 mV in the cell body of LPl_1 because the electrical synapse is much closer to the soma of LPl_1 than to that of R_2 (*5*, *6*).

To measure the strength of the synapse, we recorded the depolarizing amplitude of the PSP in LPl_1. Normally, the PSP is diphasic, resulting from low-pass filtering of the presynaptic spike, and has a brief depolarizing phase followed by a longer lasting hyperpolarization (Fig. 2A, ASW). With each of the four convulsants we tried (*7*), the depolarizing component of the PSP was enhanced (*8*) and the hyperpolarizing component eliminated. Except when penicillin was used, the amplitude of the depolarizing phase of the PSP grew progressively with increasing drug concentration (Fig. 1). The drug concentrations ranged from the threshold concentration for achieving a clear effect to eight times that concentration, above which the drugs began to produce conduction blockade. Tetraethylammonium application at the maximum concentration used led to about a sixfold increase in PSP size, strychnine to a fivefold increase, pentylenetetrazole to a threefold increase, and penicillin to at most a 1.5-fold increase.

To obtain insights into the mechanisms of the synaptic enhancement, we placed an electrode in the R_2 axon close to the electrical synapse (in the cerebral ganglion) to monitor the presynaptic action potential. By this means, we found that the different agents acted through distinctly different mechanisms. Strychnine does not affect the resting input resistance of the cells (measured with small hyperpolarizing current pulses), but rather enhances synaptic transmission by broadening the presynaptic action potential (*9*) and blocking the hyperpolarizing afterpotential of the spike (Fig. 2A). Because of the low-pass filter properties of the postsynaptic axon, a broader (thus lower frequency) presynaptic spike produces a larger postsynaptic depolarization. In addition, the PSP decays more slowly (thus lasting longer and reaching a greater average amplitude) when it is not repolarized by the hyperpolarizing afterpotential of the presynaptic spike.

Tetraethylammonium resembles strychnine in acting presynaptically. But rather than primarily affecting the presynaptic axonal spike (which broadens at most twofold), tetraethylammonium application leads to the firing of somatic spikes that may be increased 50-fold in

Table 1. Frequency with which spike conduction across the R_2-LPl_1 synapse was elicited by convulsant application. Each value is the fraction of animals (N is the number of animals used) in which the convulsant led to more than 10 percent or more than 90 percent spike following. Spike following was measured by recording about 20 spikes in each cell and observing the percent that led to firing of the follower cell. The maximum drug concentration indicated in Fig. 2A was used unless a lower concentration was maximally effective. Usually, spikes conducted across the synapse from LPl_1 to R_2 more frequently than from R_2 to LPl_1.

Drug	N	Fraction showing conduction			
		$R_2 \rightarrow LPl_1$		$LPl_1 \rightarrow R_2$	
		> 10 percent	> 90 percent	> 10 percent	> 90 percent
Tetraethylammonium	10	0.5	0.2	0.8	0.4
Strychnine	12	0.25	0.17	0.5	0.25
Pentylenetetrazole	5	0.4	0	0.4	0.2
Penicillin	5	0	0	0	0

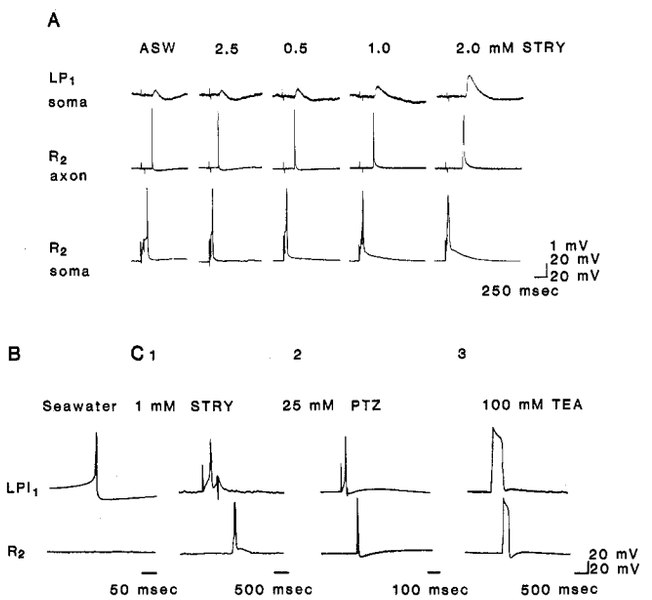

Fig. 2. (A) Strychnine (*STRY*) enhancement of electrical transmission. Electrodes were placed in the R_2 soma, the R_2 axon in the synaptic region in the cerebral ganglion, and the LPl_1 soma. Spikes were triggered in the R_2 cell body by short depolarizing current pulses. The spike in the R_2 axon follows the R_2 soma spike with a constant but considerable delay due to conduction time. In addition, strychnine reduces conduction velocity, leading to greater delays with higher drug concentrations. The immediately presynaptic axon spike shown leads to a PSP in the LPl_1 axon (not recorded) that spreads passively to the LPl_1 soma, producing the recorded PSP. With increasing strychnine concentration, the presynaptic spike broadens, resulting in an ~ fivefold increase in the depolarizing amplitude of the PSP in LPl_1. (B) Under normal conditions, the firing of LPl_1 (a spontaneous spike is shown) has no measurable effect in the R_2 soma [the time required for conduction from LPl_1 to R_2 is about 70 msec (*5*)]. (C) In some cases PSP enhancement leads to spike conduction through the R_2-LPl_1 electrical synapse (in the case shown the direction is from LPl_1 to R_2). Here, spikes in LPl_1 are triggered by short intracellular depolarizing current pulses. For each drug, we show one example from a series of spikes evoked regularly in LPl_1 where conduction from LPl_1 to R_2 occurred repeatedly. The same demonstration could be made for conduction from R_2 to LPl_1, but conduction across the synapse in this direction occurred less frequently (see Table 1). Examples shown are from five animals.

duration. As a result, a single somatic spike triggers multiple axonal spikes at an abnormally high frequency, which leads to effective temporal summation of the resulting axonal PSP's and to enhancement of the now compound PSP recorded in the LPl_1 soma (*10*).

In contrast, pentylenetetrazole mainly affects postsynaptic properties. With its application, R_2 and LPl_1 are transformed from silent cells to bursting pacemakers (*11*). The induced pacemaker potential leads to parallel cyclic depolarizations and increases in input resistance, both maximal in each cycle at about the instant when burst firing begins. As a result, presynaptic spikes coincident with these maxima in input resistance produce PSP's of enhanced effectiveness. Measurements of d-c coupling across the synapse suggest that the convulsants act on the membranes of the cells and do not appreciably affect the properties of the gap junctions that make up the actual connection (*10*).

If enhanced electrical PSP's should result in spike conduction across the synapse, they would have special clinical significance. To explore this possibility, we looked for spikes conducting from one cell to the other cell. We have described the R_2-LPl_1 synapse as weak because single spikes in one cell never conduct across the synapse to the other cell under normal conditions in adult animals (Fig. 2B) (*5, 6*).

In striking contrast to the normal situation, we found that pentylenetetrazole, strychnine, and tetraethylammonium not only enhanced the electrical PSP but in some animals did so sufficiently to result in spike conduction from cell to cell. Figure 2C shows single examples from a series of spikes evoked in each cell, once every 3 seconds or more slowly, where spikes in $LP1_1$ led repeatedly to postsynaptic spikes in R_2. Stimulation of R_2 also led to spikes in $LP1_1$, but conduction was less frequent in this direction (see Table 1). With the convulsant concentrations used, tetraethylammonium (*4*) was the most consistently effective, leading to spike conduction across the synapse (from $LP1_1$ to R_2) in 80 percent of animals (*12*). Strychnine (50 percent) and pentylenetetrazole (40 percent) were less effective. In some cases with each of these three convulsants more than 90 percent of the spikes evoked in one cell conducted to the other (Table 1). Penicillin, on the other hand, never induced spike following ($N = 5$). Spike conduction appears to be the direct result of PSP enhancement, since at the maximum concentrations of the convulsants used, their enhancement of PSP amplitude was roughly correlated with their effectiveness in producing spike conduction through the synapse (Fig. 1 and Table 1); essentially the same hierarchy of effectiveness—100 m*M* tetraethylammonium > 2 m*M* strychnine > 100 m*M* pentylenetetrazole > 100 m*M* penicillin—holds for both PSP enhancement and frequency of drug-induced spike following.

Intra-axonal recordings suggest that the variation in convulsant-induced spike conduction may be the result of differences in the baseline strength of the synapse from animal to animal. In the absence of drugs, the axonal PSP recorded in either axon in the synaptic region in the cerebral ganglion ranges from 5 to 17 mV (*10*). With a threshold of about 22 mV for spike initiation in the axons (*5*), an increase in initially large axonal PSP's would lead to suprathreshold depolarizations, while initially smaller PSP's would still remain subthreshold.

The finding that epileptogenic agents, in addition to their recognized effects on chemical synapses (*13*), enhance electrical synaptic transmission may have clinical significance. Epilepsy involves synchronous firing of neurons and propagation of electrical activity along pathways that are not normally used (*14*). In particular, the synchronizing ability of electrical synapses (*1*), enhanced by epileptogenic factors, might contribute to the synchronous activity that typifies epileptic cortex. In addition, spike conduction by way of normally nonfunctional electrical connections might determine new, abnormal pathways for the flow of seizure activity. Thus characteristic sites of initiation and direction of spread of seizures may be determined in part by variations in the effectiveness of electrical connections from region to region and from animal to animal. Indeed, electrical synaptic organization could provide an anatomic or genetic basis for varying predispositions to epileptogenesis.

STEPHEN G. RAYPORT
ERIC R. KANDEL
Center for Neurobiology and Behavior, Departments of Physiology and *Psychiatry, Columbia University College of Physicians and Surgeons,* and *New York State Psychiatric Institute, New York 10032*

References and Notes

1. M. V. L. Bennett, in *Handbook of Physiology*, section 1, *The Nervous System*, vol. 1, *Cellular Biology of Neurons*, E. R. Kandel, Ed. (American Physiological Society, Bethesda, Md., 1977), p. 357.
2. K. Mollgard and M. Moller, *Adv. Neurol.* **12**, 79 (1975); J. J. Sloper and T. P. S. Powell, *Proc. R. Soc. London Ser. B* **203**, 39 (1978); C. Sotelo and H. Korn, *Int. Rev. Cytol.* **55**, 67 (1978); M. J. Gutnick and D. A. Prince, *Science* **211**, 67 (1981).
3. B. A. MacVicar and F. E. Dudek, *Brain Res.* **196**, 494 (1980); *Soc. Neurosci. Abstr.* **6**, 407 (1980); *Science*, in press; personal communication.
4. Although tetraethylammonium is not usually thought of as a convulsant, we consider it as such because in a number of instances its action leads to the unmasking of Ca^{2+} currents which have been implicated in burst firing in hippocampal neurons [R. K. S. Wong and D. A. Prince, *Brain Res.* **159**, 385 (1978)], and its application leads to paroxysmal depolarization shiftlike events in these cells [P. A. Schwartzkroin and T. A. Pedley, *Epilepsia* **20**, 267 (1979)].
5. S. G. Rayport and E. R. Kandel, *J. Neurophysiol.* **44**, 555 (1980).
6. G. M. Hughes and L. Tauc, *J. Physiol. (London)* **197**, 511 (1968); J.-M. Meunier and L. Tauc, *Arch. Ital. Biol.* **111**, 305 (1973).
7. To test the properties of the synapse, we impaled both cells with low-resistance double electrodes in the isolated nervous system. The sheath over the cells and that over the cerebral ganglion overlying the synapse were cut, in the former case to ease penetration of the cells with the low-resistance electrodes and in the latter case to reduce diffusional barriers and permit axonal recordings. The chamber volume was 3 ml, and at least ten chamber volumes (30 ml) and a 10-minute wait were allowed before data on drug effects were taken. Drugs were dissolved just before use in artificial seawater (ASW; 460 m*M* NaCl, 10 m*M* KCl, 11 m*M* $CaCl_2$, 55 m*M* $MgCl_2$, 10 m*M* $NaHCO_3$, and 10 m*M* tris buffer, *p*H 7.6) at the maximum or twice the maximum concentration to be used and were then diluted. Strychnine as strychnine sulfate (Sigma) was dissolved in distilled water, to which the seawater salts were then added as a concentrated solution; strychnine concentrations used here reflect the concentration of strychnine itself and are thus twice the concentration of strychnine sulfate (which contains two strychnine molecules) dissolved. Pentylenetetrazole (Sigma) and tetraethylammonium (Eastman) were added to the ASW directly (as they are readily soluble), producing slightly hyperosmolar solutions. Penicillin G Sodium (Sigma) was dissolved in ASW, substituting for NaCl.
8. Enhancement of electrical synaptic transmission by tetraethylammonium was first observed in mollusks by M. S. Berry and V. W. Pentreath [*J. Exp. Biol.* **62**, 797 (1975)] and has also been seen in vertebrates [A. I. Shapovalov and B. I. Shiriaev, *Exp. Brain Res.* **33**, 299 (1978)]. E. Labeyrie and H. Korn [*C. R. Acad. Sci. Ser. D* **282**, 1617 (1976)] reported an increase in amplitude of the electrical component of eighth nerve input onto the Mauthner cell with pentylenetetrazole application.
9. Following a mechanism for modulating electrotonic transmission proposed by M. V. L. Bennett [*Ann. N.Y. Acad. Sci.* **137**, 509 (1966)].
10. S. G. Rayport, in *Physiology and Pharmacology of Epileptogenic Phenomena*, M. R. Klee, Ed. (Raven, New York, in press); in preparation.
11. R. J. David, W. A. Wilson, A. V. Escueta, *Brain Res.* **67**, 549 (1974).
12. We recognized spike conduction across the synapse when at least 10 percent of the spikes recorded (at least two of a series of 20) in one cell led to firing of the other cell. The possibility of coincident firing was ruled out by the observation that the postsynaptic spikes had the characteristics of antidromic action potentials and followed at fixed latency.
13. G. F. Ayala, M. Dichter, R. J. Gumnit, H. Matsumoto, W. A. Spencer, *Brain Res.* **52**, 1 (1973); P. A. Schwartzkroin and A. R. Wyler, *Ann. Neurol.* **7**, 95 (1980); D. Johnston and T. H. Brown, *Science* **211**, 294 (1981).
14. H. H. Jasper, A. A. Ward, A. Pope, *Basic Mechanisms of the Epilepsies* (Little, Brown, Boston, 1969); M. Rayport, in *Neurophysiology Studied in Man*, G. G. Somjen, Ed. (Excerpta Medica, Amsterdam, 1972), p. 100.
15. G. M. Hughes, in *Neurobiology of Invertebrates*, J. Salanki, Ed. (Plenum, New York, 1968), p. 423.
16. We are grateful to B. A. MacVicar and F. E. Dudek for sharing their unpublished results with us and to M. V. L. Bennett, V. F. Castellucci, T. J. Carew, E. Shapiro, and K. Weiss for their critical reading of a previous draft of this report. Supported by a grant from the Klingenstein Foundation and by National Institutes of Health medical scientist training grant GM-07367 (to S.G.R).

9 February 1981