underpinnings of ethanol's behavioral effects. Our findings extend to chronically treated animals the evidence that hyperbaric exposure antagonizes the acute, membrane actions of ethanol which lead to intoxication. This indicates that hyperbaric exposure can be used in place of a more traditional, specific, receptor antagonist to investigate the cascade of events leading from the membrane to acute intoxication, tolerance, and physical dependence.

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Regeneration of Functional Synapses Between Individual Recognizable Neurons in the Lamprey Spinal Cord

Abstract. *In 4- to 5-year-old sea lamprey larvae that had recovered from complete transection of the spinal cord, pairs of giant interneurons on opposite sides of the scar were impaled with microelectrodes. In 4 of 30 pairs, stimulation of the caudal cell elicited a monosynaptic electrochemical excitatory postsynaptic potential in the rostral cell. Fifty percent of such pairs were synaptically linked in control lampreys without transections. These results show regeneration of functional synaptic connections between individual neurons in a vertebrate central nervous system.*

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Spinal cord injury in mammals results in abortive regeneration of axotomized neurons and permanent functional disability (*1*). Use of peripheral nerve grafts and bridges (*2*) and fetal tissue transplants (*3*) has produced morphological evidence of neurite growth for short distances (1 to 2 mm) in the mature central nervous system (CNS). However, a demonstration of the reestablishment of functional synaptic connections between known neuronal populations across the lesion site has been lacking.

A similarly limited distance of regeneration accompanies recovery of function in the spinally transected sea lamprey larva (*4*). Most fibers grow up to 5 mm across the scar (*5*), and their distal segments are electrically excitable (*6, 7*). We now report that giant interneurons (GI's; second-order, rostrally projecting sensory neurons) can reestablish functional synaptic contacts with other GI's across a healed spinal transection in sea lamprey larvae 4 to 5 years old.

Spinal cords from larvae that had recovered for more than 7 weeks after spinal transection and from control larvae without transections were studied in a transilluminated perfusion chamber (*4*). Pairs of GI's were impaled simultaneously with microelectrodes filled with 6 percent horseradish peroxidase (HRP) in 0.2*M* potassium acetate (*p*H 7.4) (Fig. 1A). The cells were stimulated intracellularly at frequencies of 0.2 to 33.3 Hz. After the responses were recorded, HRP was injected iontophoretically and the identities of the impaled cells were verified in whole mounts of the cord (*5*). In control animals, stimulation of the more caudal cell elicited a composite electrochemical excitatory postsynaptic potential (EPSP) in approximately 50 percent of GI pairs (Fig. 1B and Table 1). In adult lampreys 70 percent of such cell pairs are synaptically linked (*8*). The EPSP's followed stimulation at frequencies up to 33.3 Hz with a fixed latency and persisted in Ringer's solution with 20 m*M* Ca^{2+} (Fig. 1, C to F), which was added to eliminate polysynaptic activity (*9*). Thus the connections were monosynaptic.

In animals that had recovered from spinal transection, one GI located less than 8 mm rostral to the scar was impaled with one of two microelectrodes. Whole cord stimulation with a bipolar electrode positioned approximately 30 mm caudal to the scar evoked EPSP's of both variable and fixed latency in 17 of 20 cells studied (Fig. 2A). In Ringer's solution with 20 m*M* Ca^{2+} the variable-latency EPSP's disappeared (even after a tenfold increase in the stimulus voltage), while fixed-latency EPSP's persisted. This suggested that synapses had been formed on GI's directly by axons regenerating rostralward. To test this hypothesis, we impaled a second GI located contralateral to the first and caudal to the transection site. The responses to intracellular stimulation of this cell were recorded in the GI rostral to the scar. In 4 of 30 pairs examined, a fixed-latency, composite EPSP was observed that persisted at higher stimulation frequencies (3.3 Hz) and in the high Ca^{2+} Ringer's solution (Fig. 2B). The amplitude of the

Table 1. Electrophysiological properties of regenerated synapses. Chemical components were calculated by subtracting the pure electrical component, recorded during high-frequency stimulation when the chemical component is depressed, from the composite EPSP evoked at 0.2 Hz.

Group	Number of animals	Number of pairs connected	Conduction velocity (m/sec)	Amplitude of electrical component (mV)	Amplitude of chemical component (mV)
Control GI-GI pairs	13	12 of 23 (52 percent)	1.68 ± 0.21	1.46 ± 0.21	0.66 ± 0.11
GI-GI pairs separated by transection	16	4 of 30 (13 percent) [5 of 31 (16 percent)]*	0.74 ± 0.16†	0.85 ± 0.12‡	2.42 ± 0.58§
GI-GI pairs caudal to transection	9	5 of 15 (30 percent)	1.16 ± 0.23	1.86 ± 0.31	3.00 ± 1.77‖

* In one experimental pair, a fixed-latency EPSP was observed but the procedure in high Ca^{2+} Ringer's solution could not be completed. † Significantly different from corresponding control value at $P < 0.05$ (two-tailed Student's t-test with Bonferroni correction). ‡ $P < 0.01$. § $P < 0.005$. ‖ Welch procedure used for t-test.

early (electrical) component remained constant through all maneuvers, while the amplitude of the late (chemical) component fluctuated during repetitive stimulation and, in some cases, was increased in high Ca^{2+} Ringer's (Fig. 2B4). Five of 15 pairs of GI's in which both cells were located caudal to the site of transection (and therefore had both been axotomized) were also connected by fixed-latency, composite EPSP's.

The characteristics of the observed EPSP's from the three types of GI pairs (control, across a scar, and below a scar) are compared in Table 1. The calculated conduction velocity between pairs of cells located caudal to a scar was not significantly different from that in control animals, and these values agreed with previous measurements (*7*). Therefore the conduction velocity of the proximal portion of a transected axon is not greatly different from that in a control axon. However, between cells on opposite sides of a scar, conduction velocities were two to three times lower (0.74 ± 0.16 versus 1.68 ± 0.21 m/sec; $P < 0.01$). From these data we estimate the average conduction velocity of the regenerated segments beyond the transection site to be approximately 0.33 m/sec. This is consistent with morphological findings that neurites taper as they grow into and beyond the scar (*4*) and also with electrophysiological measurements from antidromic activation of GI's (*7*). The amplitude of the chemical EPSP was greater in GI's of spinally transected animals, both for cell pairs across and below a healed scar. In all control GI pairs the electrical component was larger than the chemical component. After recovery from spinal transection the situation was reversed, so that the EPSP's now resembled those of normal adult sea

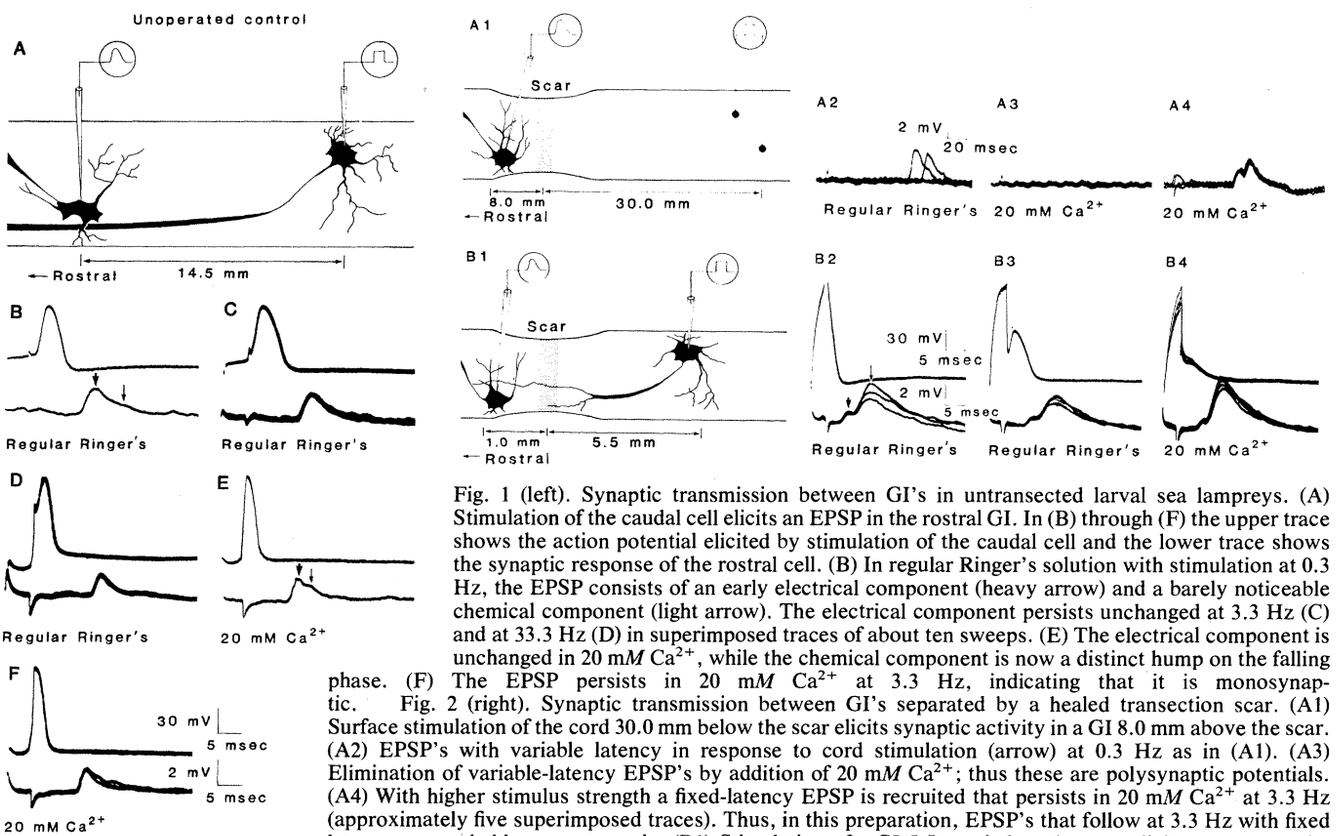

Fig. 1 (left). Synaptic transmission between GI's in untransected larval sea lampreys. (A) Stimulation of the caudal cell elicits an EPSP in the rostral GI. In (B) through (F) the upper trace shows the action potential elicited by stimulation of the caudal cell and the lower trace shows the synaptic response of the rostral cell. (B) In regular Ringer's solution with stimulation at 0.3 Hz, the EPSP consists of an early electrical component (heavy arrow) and a barely noticeable chemical component (light arrow). The electrical component persists unchanged at 3.3 Hz (C) and at 33.3 Hz (D) in superimposed traces of about ten sweeps. (E) The electrical component is unchanged in 20 m*M* Ca^{2+}, while the chemical component is now a distinct hump on the falling phase. (F) The EPSP persists in 20 m*M* Ca^{2+} at 3.3 Hz, indicating that it is monosynaptic. Fig. 2 (right). Synaptic transmission between GI's separated by a healed transection scar. (A1) Surface stimulation of the cord 30.0 mm below the scar elicits synaptic activity in a GI 8.0 mm above the scar. (A2) EPSP's with variable latency in response to cord stimulation (arrow) at 0.3 Hz as in (A1). (A3) Elimination of variable-latency EPSP's by addition of 20 m*M* Ca^{2+}; thus these are polysynaptic potentials. (A4) With higher stimulus strength a fixed-latency EPSP is recruited that persists in 20 m*M* Ca^{2+} at 3.3 Hz (approximately five superimposed traces). Thus, in this preparation, EPSP's that follow at 3.3 Hz with fixed latency are probably monosynaptic. (B1) Stimulation of a GI 5.5 mm below the scar elicits monosynaptic, composite EPSP's in another GI 1.0 mm above the scar. (B2) The electrical component (heavy arrow) has a constant amplitude and latency, while the chemical component (light arrow) has a variable amplitude. (B3) Constancy of the electrical component at 3.3 Hz while the chemical component decays in amplitude with repetitive stimulation. (B4) Persistence of the EPSP in 20 m*M* Ca^{2+} even at 3.3 Hz. Therefore the connection is monosynaptic. Note the increase in the amplitude of the chemical component in high Ca^{2+} Ringer's solution compared with regular Ringer's. The action potential in the presynaptic GI deteriorated during the experiment.

lampreys, in which the chemical component is also usually larger than the electrical component.

The reason for the increase in chemical EPSP amplitude in the regenerated synapses is not yet known. One possibility is that GI's which had been disconnected from some of their synaptic input developed denervation supersensitivity. Consistent with this is the finding that, when both GI's were below the transection, the chemical EPSP was also increased (although because of the small sample size this was not statistically verifiable), while the electrical component was not. Two other possibilities, that regenerating connections are formed closer to the cell body or include more points of synaptic contact, are both less likely because the electrical components in the regenerated synapses were reduced.

The unequivocal demonstration of functional synapse formation by regenerating neurites ideally should involve simultaneous impalement of both the pre- and postsynaptic cells because (i) it is possible that synaptic activity evoked by extracellular stimulation might result from antidromic activation of axon collaterals of cells on the same side of the lesion as the target neuron and (ii) regenerated axons might release substances diffusely into the extracellular environment and activate neurons nonsynaptically. Such direct evidence has been obtained in invertebrate ganglia (*10*), but in the vertebrate CNS synaptic regeneration has been suggested by less direct methods. Morphological evidence has been found for the formation of synapses by regenerating fibers in the spinal cords of lampreys (*11*), bony fish (*12*), and amphibians (*13*). A combination of anatomical, behavioral, and evoked-potential studies has suggested regeneration of retinotectal synaptic connections in fish and amphibians (*14*). Stimulation of regenerated Mauthner axons has evoked compound action potentials in ventral roots of *Xenopus* tadpoles (*15*). Finally, excitation of muscle nerves in the forearms of frogs that had recovered from lesions of the second dorsal root elicited short-latency EPSP's in ipsilateral motor neurons (*16*). The present study shows that regenerated [as opposed to collaterally sprouted (*17*)] axons of vertebrate CNS neurons form functional synaptic connections. This suggests that similar connections may well be formed by regenerating axons from mammalian grafts and bridges (*2, 3*), where the distance of axon growth is similar to that in the lamprey spinal cord.

We previously showed that the regenerating neurites of GI's and other neurons of the lamprey CNS grow selectively in the direction of their original paths (*4, 5, 18*). Whether these axons show similar specificity in their choice of postsynaptic targets remains to be determined.

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Huntington's Disease: Two Families with Differing Clinical Features Show Linkage to the G8 Probe

Abstract. *To test the hypothesis that interfamily variability in Huntington's Disease (HD) is due to mutation at different loci, linkage analysis was undertaken in two large HD kindreds that differed in ethnicity, age-at-onset, and neurologic and psychiatric features. Both families showed linkage of the HD locus to the G8 probe. Several recombinants were documented in each family, and the best estimate of the recombination fraction for the two families was 6 percent with a 95 percent confidence interval of 0 to 12 percent. Although the data support the existence of a single HD locus, use of the G8 probe for presymptomatic testing in these kindreds would have resulted in a 12 percent error rate in genotype assignment at the HD locus.*

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Huntington's disease (HD) (*1*) is an inherited neuropsychiatric disorder, characterized by gradually worsening chorea, impairment of voluntary movements, dementia, and a variety of emotional symptoms, particularly severe depression (*2, 3*). The symptoms progress without remission until patients are physically incapacitated and severely impaired in cognition. Death occurs 15 years after onset (on the average) usually from subdural hematoma due to head trauma or from suffocation due to aspiration of food. The disease is inherited as an autosomal dominant trait, with onset of symptoms usually delayed until middle adult life. The paternal transmission effect is well documented in HD, but its cause is not understood. Affected children of affected fathers tend to have a significantly earlier onset than the affected children of an affected mother (*4*).

The disease is severe and the offspring are at high risk (50 percent) for eventually having symptoms. However, because of the delayed onset, even offspring of