response for trichloroethylene (*12*). Thus for a more thorough test of a chemical, several liver preparations from different species and with various inducers should be tried. In the case of the Fyrol FR2, a weak mutagen in the test system, the mutagenicity was missed in the original study (*1*) because only the PCB-induced rat liver homogenate was used.

Tris-BP has been shown to be a mutagen in several test systems (*1*, *2*), to be a potent animal carcinogen (*3*), to be absorbed from fabric through human skin (*5*), and to cause testicular atrophy and sterility in animals (*4*). Fyrol FR2, a chlorinated chemical closely related to tris-BP in structure, is shown to be a mutagen in this report and thus is likely to be a carcinogen (*17*). Fyrol FR2 has also been found to be positive in a test for sister chromatid exchange, but its activity in this test is weak compared to that of tris-BP (*19*). Most nonpolar chemicals, including tris-BP and five other phosphotriesters, are absorbed through human skin (*20*); Fyrol FR2 is also likely to be absorbed. The first cancer test in experimental animals is in progress at the National Cancer Institute; the results will not be known for several years. Considering that alternatives to the use of dangerous flame-retardant additives are available (*2*), we recommend that these alternatives be used.

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8. Fyrol-FR2 (Stauffer, 99 percent pure) was obtained from D. J. Lisk of Cornell University, and commercial grade Fyrol-FR2 (96 percent pure) was supplied by Stauffer Chemical Company. The 1,3-dichloro-2-propanol was obtained from Aldrich Chemical Company; 1,3-dichloro-2-propanone from Eastman Company; and Antiblaze 19 (a mixture of cyclic phosphate esters) from Mobil.
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Spine Stems on Tectal Interneurons in Jewel Fish Are Shortened by Social Stimulation

Abstract. *Spined pyriform interneurons in community-reared jewel fish have more dendritic branches and spines in the deep tectal layers than those in isolates reared without visual-tactile contact with conspecifics. Furthermore, in the same dendritic loci in which the community-reared fish had more spines, the spine stems were shorter. The findings suggest that social stimulation induces localized formation of spines, which swell with synaptic activation. Shortening of the spine stem through elongated swelling of the spine head is likely to alter synaptic effectiveness through changes in electrotonic conductance.*

For years, the formation of new synaptic contacts or changes in conductivity of existing contacts has been hypothesized to be the basis of learning. In studies of mammals, social deprivation during rearing has been reported to reduce the receptive surface of neurons in relevant brain areas (*1*). Changes in synaptic density and terminal size, along with postsynaptic thickening, have also been reported (*2*). In a recent Golgi study, we found that cichlid fish deprived of seeing their own species have fewer dendritic spines and branches on specific tectal interneurons than do fish reared in community tanks (*3*). We also noticed that spines on the interneurons of socially deprived fish had long slender stems. In this report, the spine stem is defined as the thin stalk between the dendrite and the larger bulbous portion of the spine. Although spines have been reported to change shape somewhat with visual deprivation (*4*), to swell considerably during electrical stimulation (*5*), and possibly to have unusually long, thin stems in fetal and retarded humans (*6*), to our knowledge there are no reported data on the effects of restricted experience on stem length. We now report, from further study of the same material, that visual stimulation in the appropriate social context shortens spine stems, particularly in dendritic loci where spine density is also affected.

Jewel fish (*Hemichromis bimaculatus*, Gill 1862) obtained from the same spawn were reared in total isolation for 73 days and then assigned to isolate and control groups. Both groups experienced similar amounts of olfactory and auditory stimulation from nearby jewel fish and relatively equivalent amounts of background visual pattern stimulation from conspicuously colored graphics and other moving fish. Isolates and controls differed in that isolates could not associate these perceptual inputs with the configuration and behavioral repertoire of their own species. In place of jewel fish, the isolates were reared with blind cave characins (*Anoptichthys jordani*). They observed these eyeless unpigmented fish through the windows and permeable screens of their adjacent compartments, which were suspended in a flat 73-liter aquarium. The fish in the control group, on the other hand, were released in community tanks where they were in contact with conspecifics with which they frequently engaged in vicious territorial fighting.

Four adult females from each group, selected on the basis of size and weight, were killed in pairs at 402, 508, and 529 days (*7*). Whole brains were excised and

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placed in rapid Golgi fixative (0.2 percent OsO_4 and 2.7 percent $K_2Cr_2O_7$) for 3 to 6 days followed by 0.5 percent $AgNO_3$ for 24 to 48 hours. After the optic tectum was cut in 120-μm coronal sections and the tissue was washed and cleared (*3*), each section was mounted separately and coded with a random number. Four months after quantifying the spine density and dendritic bifurcations on a common, but infrequently stained, pyriform interneuron (for each group, $N = 9$), we reexamined the material to determine how the lengths of spine stems might vary systematically with spine density and dendritic location. Because of darkening of some tissue with age, only seven interneurons from three isolate fish and eight from three control fish were measured. We used a magnification of 1000 (oil) and a linear reticle scale with 0.51-μm increments. Beginning with the apical dendrite ascending from the perikaryon and moving to the primary, secondary, and tertiary dendritic branches radiating from the apical dendrite, we measured all spines conforming to the following criteria: those which (i) lay perpendicular to the plane of vision in clear unobstructed focus, (ii) were between 1.91 and 3.44 μm in overall length, (iii) exhibited no bifurcations, and (iv) had stem lengths of at least 0.25 μm. Moreover, measurements of stem length did not include bulbous stem varicosities. Two surveys, each made by an

Table 1. Comparison of mean spine-stem length between control and isolate interneurons. Stem lengths are grouped according to the overall spine length and the distance from perikaryon. Data were examined by cross-sectional time-series multiple regression adjusted to compare the stem lengths as a function of the distance of their stratum from the perikaryon. Asterisks indicate the significance level of comparisons between control and isolate interneurons: $*P < .05$; $**P < .01$; $***P < .005$; $****P < .0005$. Daggers indicate comparisons of controls at different strata: $\dagger P < .05$; $\dagger\dagger P < .01$.

Distance of stratum from perikaryon (μm)	Overall spine length (μm)							
	1.91 to 2.42		2.43 to 2.93		2.94 to 3.44		All lengths	
	Controls ($N = 32$)	Isolates ($N = 20$)	Controls ($N = 89$)	Isolates ($N = 63$)	Controls ($N = 33$)	Isolates ($N = 33$)	Controls ($N = 154$)	Isolates ($N = 116$)
0 to 64.80	0.83	1.02	1.09	1.36***	1.34	1.83*	1.08	1.45****
64.81 to 162.0	1.11††	0.91	1.21	1.19	1.43	1.60	1.24†	1.24
All strata	0.98	1.00	1.12	1.24	1.38	1.79*	1.14	1.33**

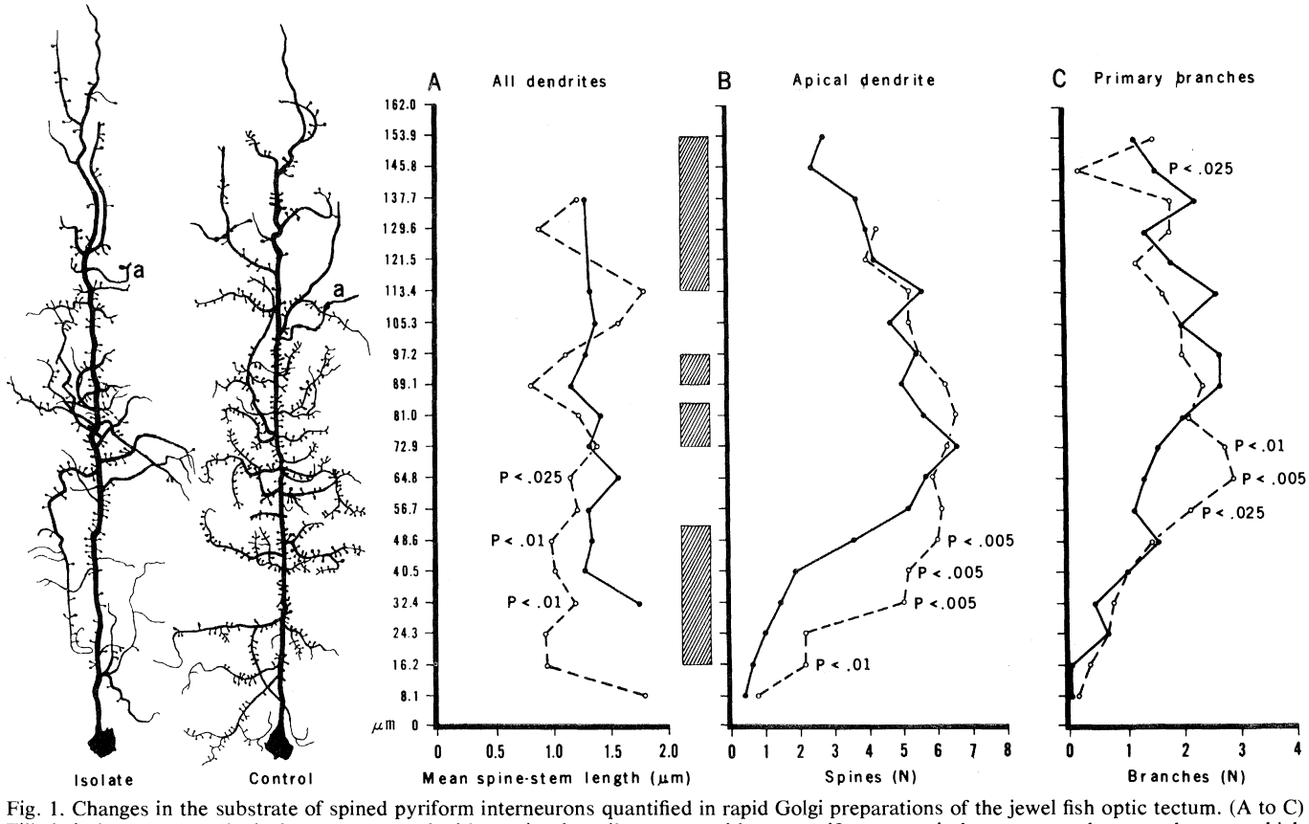

Fig. 1. Changes in the substrate of spined pyriform interneurons quantified in rapid Golgi preparations of the jewel fish optic tectum. (A to C) Filled circles represent the isolate group reared without visual-tactile contact with conspecifics; open circles represent the control group, which was reared in a community tank. Examples of isolate and control interneurons appear in scale at the left of the ordinate. Their axons are labeled by the letter *a*. Cross-hatched areas represent plexiform layers in the following strata: top, inner plexiform layer of the stratum griseum centrale; middle, lower half of stratum griseum centrale; bottom, stratum album centrale. Significant differences between groups per 8.1-μm stratum were determined by one-tailed *t*-tests of average spine-stem lengths on all dendrites of each neuron (A) and spine and primary bifurcation counts on the apical dendrite of each neuron (B and C). Multiple-regression trend analyses indicated that the groups differ in the following trend components throughout the lengths of the interneurons: (A) linear component per 0.51-μm stratum ($P < .01$), (B) linear and curvilinear components per 8.1-μm stratum ($P < .0005$ and $P < .005$, respectively), and (C) curvilinear component per 8.1-μm stratum ($P < .025$). The illustrated averaged data only approximate these trend components.

independent observer, measured 270 and 290 spines, respectively. Of these, 159 spines appeared in both surveys. Measurements of the stem length of 102 of these spines were virtually the same ($\pm$ 0.12 μm). The measurements of those 102 matched spines were then averaged and treated by cross-sectional time-series multiple regression (*8*) adjusted to compare the stem lengths as a function of their stratum distance from the perikaryon. We compared the stem lengths both between and within groups in the deeper tectal strata (0 to 64.8 μm from the perikaryon) and in the higher strata (64.81 to 162.0 μm) (Fig. 1A). We selected these strata for study because the controls exhibit a greater density of spines and primary bifurcations along the apical dendrite in the deep tectal strata (Fig. 1, B and C).

As in our initial study, major differences between groups appear in the deep tectal strata. Spine stems on control-group interneurons in the deep tectal strata are considerably shorter ($\bar{X}$ = 1.11 μm) than those on isolate-group interneurons ($\bar{X}$ = 1.65 μm) [F (1, 98) = 19.93, $P < .0005$]. The amount of unique variance (semipartial r^2) explained by the rearing conditions was 17 percent. Spine stems in the deeper tectal strata of the controls are also significantly shorter than those in the higher strata [F (1, 98) = 7.24, $P < .01$].

We also analyzed the complete sample of 270 spines measured in our first survey. The results are virtually the same as our combined survey data. With this larger sample of spines, stratum comparisons could be made on the basis of overall spine length (Table 1). The greatest differences in stem lengths between groups appear in the stratum album centrale, a structure receiving numerous intrinsic projections and centripetal projections from a variety of sensory modalities (Figs. 1A and 2). These include optic and somatosensory afferents (*3, 9*).

Pearson product-moment correlations based on the initial survey of spines provided group comparisons between the density of spines per 8.1-μm unit length of each dendrite and the length of spine stems within the same locus. The spines on the apical dendrite were treated separately from those on primary through tertiary branches. For the control-group interneurons, the number of spines and spine-stem length on the apical dendrite were negatively correlated (r = $-.307$, d.f. = 56, $P < .02$); the negative correlation for the primary through tertiary branches was not significant. In contrast, for the isolate-group interneurons, low positive correlations between number of spines and stem length are significantly different from the control correlations ($P < .005$). If proximal spines develop first and synaptic activation shortens spine stems through elongated swelling of spine heads, we could expect that older spines on proximal portions of primary and secondary dendrites should have shorter stems than distal and presumably younger ones near the dendritic tips. This hypothesis is based on the likelihood of increasing presynaptic contacts and activation with age or experience (or both). Comparing the position order of each spine on the dendrite with its stem length, we found a significant positive correlation for only the control-group interneurons (r = .369, d.f. = 54, $P < .005$, one-tailed test). The isolates, on the other hand, show no systematic lengthening of the spine stems as one progresses out the dendrite. Other researchers have observed shorter stems on the proximal regions of pyramidal dendrites in nondeprived mice and humans, but have provided no information on the effects of isolation in deprived subjects (*6, 10*).

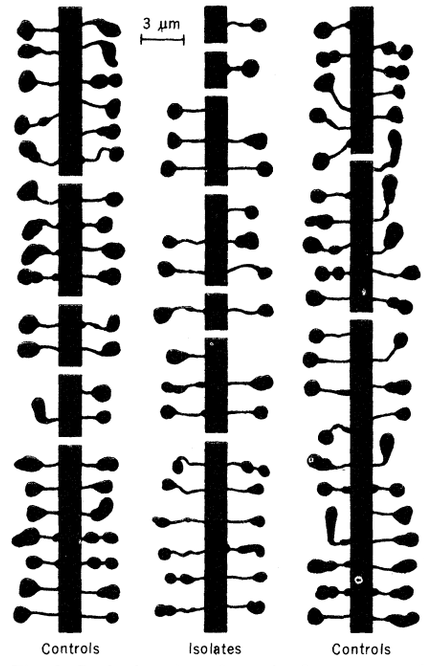

Fig. 2. Scale drawings from the first survey of all spines quantified on apical and primary through tertiary dendritic branches in the stratum album centrale. Each bar segment represents the dendritic base of one interneuron. Spine-stem alignment is relatively perpendicular to the bar for comparison of overall length, stem curvature, and stem varicosities. Note the greater number of spines with elongated varicose heads and concomitantly shorter stems on control-group interneurons.

In seeking generality with mammalian studies of the effects of visual deprivation, we measured the lengths of spine stems on pyramids in mouse visual cortex shown in photomicrographs published by Valverde. Spine stems were shortest on the mouse exposed longest to light (*11*). Further support for the hypothesis that synaptic activation elicits spine-head swelling with concomitant shortening of the stem comes from research with tetanizing stimulation of mammals (*12*). Posttetanic potentiation lasting for weeks (*13*) has also been found in the same neural circuits that exhibit spine swelling lasting as long as 23 hours (*14*). Long-term monosynaptic potentiation and spine swelling may be functionally related. For some time, researchers have suggested that long, thin spine stems would offer greater ohmic resistance than shorter or wider stems (*15*). Increasing the electrotonic conductance of the spine through permanent alteration of the stem has been proposed as one mechanism for changing the influence the synapse exerts on the neuron's behavior (*16*).

Reviewing group differences in spine-stem length in relation to the receptive surface of specific tectal interneurons in jewel fish, we conclude that visual stimulation in the appropriate context, as obtained by social interactions with conspecifics, can augment the growth of dendritic branches and the formation of spines and can change neuronal postsynaptic morphology. Long-term neural plasticity may involve two stages of morphological change, one immediate and one eventual: (i) Change in the immediate substrate could be mediated by rapid spine-head swelling with stem shortening during synaptic activation (which seems likely to decrease electrotonic resistance and the time constant) and thus alter synaptic effectiveness. (ii) Changes in the shape of the receptive surface, especially at specific loci, could be contingent on local synaptic activity resulting from specific environmental stimulation working in concert with the neuron's genetic program. The resultant increased connectivity could provide the resource for future synaptic modification.

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Regeneration of Symmetrical Hindlimbs in Larval Salamanders

Abstract. *The complete circle rule of the polar coordinate model of pattern regulation was tested for regenerating hindlimbs of* Ambystoma *larvae. The hindlimbs were made symmetrical in the circumference of either the thigh or the shank, and their ability to regenerate from both levels was observed. Thighs composed of two anterior halves failed to regenerate, whereas thighs composed of two posterior halves often regenerated distally complete, correspondingly symmetrical limbs; shanks composed of either two anterior or two posterior halves regenerated distally complete, correspondingly symmetrical limbs. These results are in contrast to what is predicted by the complete circle rule and suggest a modification of this rule.*

French *et al.* (*1*) have proposed a polar coordinate model to account for epimorphic pattern regulation in insect imaginal disks as well as regenerating insect and amphibian appendages. The cells of these structures are hypothesized to respond to two components of positional information, one component corresponding to position around the circumference of the limb, the other to position along a radius of that circle. In amphibian limbs, the circumferential component is represented by a circular sequence of positional values, and the proximal-distal axis of the limb is represented by the sequence of values along the radius of the circular sequence. According to the model, regeneration of amphibian limbs is governed by two rules for cellular interactions. The shortest intercalation rule states that missing values in either the radial or circular sequences are filled in along the shortest route by intercalary regeneration. The complete circle rule states that distal transformation of blastema cells will take place only if these cells are derived from a limb stump possessing a complete circular sequence of positional values. A complete circular sequence is present after simple amputation or can be generated by shortest intercalation when normally nonadjacent positional values of the circular sequence are apposed.

Bryant (*2*) has shown that adult *Notophthalmus viridescens* upper arms made symmetrical in the circular sequence by grafting together either two anterior or two posterior halves fail to regenerate after amputation; this result provides evidence for the necessity of a complete circular sequence of positional values for distal transformation. I now report the results of similar experiments in which upper and lower hindlimbs of *Ambystoma* larvae were made symmetrical with respect to their anterior and posterior halves (hereafter called double anterior and double posterior limbs). Both double anterior and double posterior lower hindlimbs regenerate correspondingly symmetrical limbs. Double anterior upper hindlimbs fail to regenerate, but double posterior upper hindlimbs regenerate symmetrical limbs. The production of symmetrical regenerates by symmetrical hindlimbs is a result opposite that predicted by the complete circle rule of the polar coordinate model.

Hindlimbs of *Ambystoma mexicanum* or *Ambystoma tigrinum* (length from snout to tail, 60 to 80 mm) were made symmetrical in either anterior or posterior regions of the thigh or shank (Fig. 1). Controls consisted of longitudinally halving the thighs and shanks and putting the halves back together in the normal orientation. Grafts become revascularized within 3 to 5 days after the operation and, after an initial period of edema lasting about 1 week, became well integrated with the host tissues. After a healing period of 10 to 32 days, amputation was performed through the middle of the grafted region (mid-thigh or distal shank).

The limb skeletons were examined by methylene blue staining after a 25- to 50-day period of regeneration (Tables 1 and 2). No difference in the results was noted between the two species. All control limbs regenerated normally (Fig. 2A). Some of the symmetrical thighs and shanks also regenerated normally. No sign of the grafted half was observed in the latter cases at the end of the experiment, which suggests that it had resorbed. It is likely that the host stump tissue in these cases formed an oblique wound surface, which would encompass the normal stump circumference and thus allow normal regeneration to occur. This hypothesis is strengthened by two observations: (i) In all remaining cases the graft was still visible on the limb stump at the end of the experiment. (ii) I have often observed this mode of normal regeneration in half-upper or lower hindlimbs of axolotls of the size used in the present experiments. The half-hindlimb rounds off and regresses somewhat, and a blastema forms from the whole stump circumference at a 45° angle to the longitudinal axis of the limb. The blastema subsequently straightens by faster growth on one side.

For the symmetrical thighs, the most significant finding was a nonequivalence in the regenerative ability of double anterior and double posterior stumps. Although normal-looking blastemas often formed on double anterior thigh stumps and advanced as far as the late bud stage, they were able to redifferentiate only a conical cap of cartilage continuous with the tip of the femur or a short spike of cartilage that articulated with the femur (Fig. 2B). Several double anterior limbs

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