have been "tagged" directly by the experience. Orientation, eye, forearm—key tags of the experience—have been retained straightforwardly. Results from the older cat show that by week 11, the reallocation is undetectable (*11*). This would be an upper limit of a possible critical period in response properties within the somatosensory cortex.

All these effects were present in the yoked animal but some to a lesser degree. This also applies to results in the visual and visual association cortices.

Stimulus control by the kitten, that is, one more association, apparently enhances the effect. The result in visual association also appears reasonable: the cells are primarily visual and secondarily somatic. Functional characteristics are thus somewhat between somatosensory and visual cortex but still reflect the experience in a simple one-to-one way; they mirror neurally the associative relationships (tags) that exist behaviorally in the training situation.

The most surprising results were obtained in the visual cortex. We expected that the visual cortex would be unaffected by the task because the total visual experiential time invested in it by the kittens was such a small fraction of their total visual experience (especially with regard to the unsafe stimulus). The presence of the cells with two different receptive fields was completely unexpected; after the fact, however, it is apparent that the transition from the unsafe to the safe stimulus carries important information for the animal and that this transition must be detectable, neurally represented. These cells do just that. The inversion phenomenon remains unexplained; this and other phenomena in the visual cortex may result, however, from an interaction between the training experience and those gained independently by the animal.

We have shown that notable plastic changes can be produced in normally reared kittens that have not been sensorially deprived. Because sensory deprivation was not involved, this experiment brings us closer to the means by which experience is recorded in the brain. Indeed, atrophy from disuse cannot explain the existence of cells with properties which are not normally present or the enlargement beyond normal limits of the cortical representation of the forearm. Similarity between these phenomena and those that take place in adult learning remains to be demonstrated.

However, and possibly more important than the abstract search for the engram, the results show that early learning produces plastic changes in the structure of the developing brain which then affect the way subsequent or concurrent experiences influence the animal. As we believe the changes to be permanent (*6*), it becomes imperative to determine if any parallels exist between these findings and early experience in human children.

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10. If the full history of the untrained forearm was known, much of the "randomness" might disappear.
11. In this brief report, we deliberately focus on the similarities between neural and behavioral events. Our working hypothesis is that the seeming dissociation in the older cat is only apparent in that the behavioral change is supported by a similar but less extensive neural modification.
12. Possible mechanisms for these effects could range from a simple modification in viewing preference generated by the safe and unsafe stimuli, to complex inhibitory interactions between neurons that are sharply tuned by the experience ("expert" cells) and the remainder of the local population.

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Membrane Glycolipids: Regional Synthesis and Axonal Transport in a Single Identified Neuron of *Aplysia californica*

Abstract. *Glycolipids moving along an identified axon of* Aplysia californica *were synthesized and incorporated into intracytoplasmic membranes solely in the perikaryon: direct injection of tritiated sugar into the axon revealed no local synthesis or exchange. There was no indication for transfer into axoplasm from glia. Insertion of glycolipids into nascent membranes occurs coordinately with insertion of protein components in the cell body.*

Transport of vesicles and other organelles along axons is a characteristic property of neurons. It is now well established that membrane proteins of these organelles are synthesized in the endoplasmic reticulum and Golgi apparatus of the cell body (*1*). However, the origin of lipids associated with transported organelles has not been established with certainty. Although it is widely thought that most lipid originates in the cell body (*2*), there is evidence that phospholipids and glycolipids can be synthesized and incorporated in axons and at synapses (*3–5*).

Regional localization of lipid synthesis is an intriguing problem: how neuronal membranes are formed and subsequently processed requires an understanding of the contribution of each cellular region to the elaboration of functional organelles. Although glycolipids constitute only a fraction of the total lipid in membranes, they have been investigated extensively because they are likely to be important in neuronal function and have been implicated in membrane specificity (*6*). In earlier studies, ^{3}H-labeled sugars were introduced into the vertebrate eye and labeled glycolipids were found in optic nerve tracts (*4, 5, 7*). Because of the complexity of the vertebrate visual system, it is not clear whether all of the glycolipid originated in retinal cell bodies, or if some was derived from local axonal or glial synthesis.

We have developed a novel approach for examining the synthesis and distribution of glycolipids in the different regions of a single, identified neuron. The origin of membrane constituents can be ascertained with assurance by using single neurons in the central nervous system of the marine mollusk, *Aplysia californica*. The identified giant neuron R2 of the abdominal ganglion is well suited for investigations of regional synthesis and axonal transport of membrane components because of its large size and long (2 to 4 cm) axon which runs unbranched in the right pleuro-abdominal connective. ^{3}H-Labeled sugars can be introduced by pressure injection directly into either the perikaryon (800 μm in diameter) (Fig. 1A$_1$) or axon (30 to 60 μm in diameter) (Fig. 1A$_2$). Since incorporated radioactivity is restricted to the injected neuron, we can evaluate the synthetic capability in the cell body or axon directly (*8, 9*). An additional advantage of using *Aplysia* is that glycolipids of glial cells and connective tissue which surround axons are labeled selectively by incubating connectives in the presence of ^{3}H-labeled sugars (*8–10*).

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^{3}H-Labeled glycolipids were extracted from membranes of both cell body and axon 15 hours after intrasomatic injection of *N*-[^{3}H]acetylgalactosamine into R2. Our criteria for identifying these substances as glycolipids include (i) solubility in chloroform-methanol, (ii) mobility on silicic acid thin-layer chromatography developed with organic solvents, and (iii) lack of mobility on thin-layer chromatographs developed with aqueous sodium borate which mobilizes water-soluble precursors and glycopeptides (*11, 12*). By these criteria, 25 percent of the total macromolecular radioactivity in membranes was in glycolipid, the rest was in glycoprotein. Qualitatively similar patterns were obtained from both the cell body and the axon when ^{3}H-labeled glycolipids from both regions of the neuron were compared by chromatography (Fig. 1, B and C). This similarlity suggested that the glycolipids originated in the cell body and were exported into the axon.

Axonal transport of the glycolipids can be demonstrated. We determined the time course of their appearance in the axon by analyzing the distribution of radioactivity between cell body and axon at various times after injection. ^{3}H-Labeled glycolipids appeared without delay, and the amount thereafter increased rapidly during the period the axon was examined (Fig. 2). At 15 hours, 30.1 ± 2.2 percent (± standard error) of the total labeled glycolipid of the neuronal membrane was in the axon. Once in the axon, the labeled glycolipids were rapidly translocated, and by 15 hours all of the glycolipid components had moved at least 2 cm along the axon away from the cell body. From the position of the distalmost radioactivity at 5, 10, 15, and 22 hours after intrasomatic injection, we estimate that the glycolipids are transported at approximately 50 mm per day at 15°C. Tritiated membrane glycoproteins (*9*) and serotonergic vesicles (*13*) that are actively transported in *Aplysia* axons also move at this rate, as do materials moving by rapid axonal transport in other cold-blooded animals (*14*).

As a further test for the idea that the glycolipids were moving by fast transport, we applied colchicine (10 m*M*), which blocks rapid axonal transport in *Aplysia* (*15*) and other animals (*16*). Transport of ^{3}H-labeled glycolipids was inhibited by more than 95 percent. Since colchicine does not inhibit the lateral diffusion of lipids in membranes (*17*), it is unlikely that this mechanism makes an important contribution to the quantitative movement of glycolipid from R2's cell body into the axon.

In order to determine whether the axon can incorporate or exchange precursors into lipids (*3-5*), we introduced *N*-[^{3}H]acetylgalactosamine directly into the axon of R2 by pressure injection (*10, 18*). No ^{3}H-labeled glycolipid was found. Although a small amount of radioactive material was extracted from the axon with chloroform and methanol, by our criteria it is not glycolipid: it migrated as a single component when examined by thin-layer chromatography with propanol and water (Fig. 1D), but moved to the front when the chromatogram was developed with aqueous sodium borate (*12, 19*).

An alternative source of the glycolipids in the axon of R2 may have been glial cells, since transfer of proteins from glial

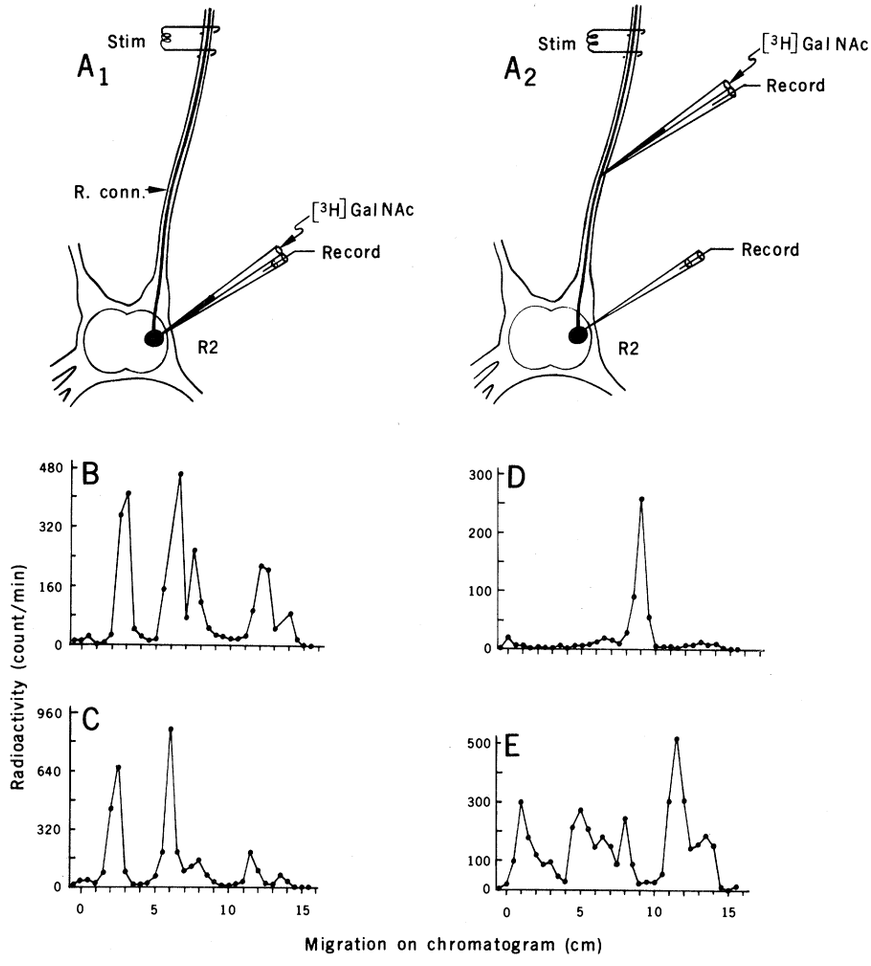

Fig. 1. Regional synthesis of glycolipids in *Aplysia* nervous tissue. (A) Diagrams of the dorsal surface of the abdominal ganglion showing the technique of pressure injection into (A_1) the cell body and (A_2) the axon of the giant neuron, R2. For intrasomatic injection of concentrated *N*-[^{3}H]acetylgalactosamine ([^{3}*H*]*GalNAc*) (New England Nuclear; 25 Ci/mmole) the central nervous system was removed from the animal and placed in artificial seawater in a chamber designed for recording and stimulating nerves (*8*); R2 is shown penetrated by a double-barreled micropipette, one barrel for recording and the other for pressure injection of between 1.0 to 90 pmole of the sugar. After injection, nervous tissue was maintained in artificial seawater at 15°C. The nervous system was then rapidly frozen with solid CO_2 on a brass block. The right connective (*R. conn.*) containing the major axon of R2 was cut from the ganglion containing the cell body. Crude, washed membrane fractions were obtained from the two regions by centrifugation at 105,000*g* (*8*). (B to E) Chromatography on polysilicic acid glass fiber plates (*25*) (Gelman Instrument; ITLC, type SA) of ^{3}H-labeled glycolipids from washed membranes. Chromatograms were developed with propanol and water (80:20 by volume) and were cut into 0.5-cm pieces for liquid scintillation counting (*8*). (B) ^{3}H-Labeled glycolipids from R2's cell body 15 hours after intrasomatic injection. Identical chromatographic patterns were obtained with membranes prepared from single R2 cell bodies dissected out of the ganglion. (C) ^{3}H-Labeled glycolipids from axonal membranes of the same neuron whose cell body pattern is shown in (B). (D) Chloroform-methanol extractable radioactivity from axonal membranes of an R2, 15 hours after intraaxonal injection with 10 pmole of *N*-[^{3}H]acetylgalactosamine. (E) ^{3}H-Labeled glycolipids synthesized during incubation of nervous tissue at 15°C in artificial seawater containing 4 μM *N*-[^{3}H]acetylgalactosamine. After 20 hours, the right connective was cut from the ganglia and glycolipids were extracted from the washed membrane fraction.

cells to axoplasm has been demonstrated in squid (*20*). In *Aplysia*, glial cells and the connective tissue that surround the axons can be labeled by incubating the connective in the presence of ^{3}H-labeled sugars (*9, 10*). The chromatographic pattern of labeled glycolipids extracted from the right pleuro-abdominal connective after incubation with *N*-[^{3}H]acetylgalactosamine (Fig. 1E) was different from that obtained from the right connective extracted after injection of R2's cell body (compare Fig. 1, B and E). To see if glycolipids from glia can be transferred into the axon, we used a procedure that extruded about 70 percent of R2's axoplasm (*21*). When axoplasm was extruded from a connective which had been incubated in *N*-[^{3}H]acetylgalactosamine, only 9.2 ± 1.5 percent ($N = 6$) of the ^{3}H-labeled glycolipid was squeezed out of the tissue. Since it is difficult to avoid rupture of labeled supporting cells in the sheath of the connective during this procedure, the low value of 9 percent is an overestimate. Thus, transfer of glycolipid between glial cells and axons is unlikely to be a source of glycolipid for organelles in the axon.

Experiments with anisomycin provided further evidence that the ^{3}H-labeled glycolipid in the axon originates in the cell body. Brief exposure to this inhibitor of protein synthesis has been shown to block export of newly synthesized membrane glycoprotein from the cell body into the axon (*22*). Treatment of the isolated nervous system with anisomycin starting 4 hours before intrasomatic injection of *N*-[^{3}H]acetylgalactosamine reduced incorporation of the sugar into glycolipids by only 44 ± 3.9 percent ($N = 3$), but decreased by more than 90 percent the appearance of ^{3}H-labeled glycolipid in the axon of R2. The labeled glycolipids formed in the cell body under these conditions were similar to those made in untreated cells.

Unlike colchicine, anisomycin has no effect on translocation of materials once they reach the axon (*22*). Much longer periods of exposure to the drug were necessary to reduce the appearance of existing storage granules in the axon of a serotonergic neuron of *Aplysia* (*13*). These differential effects can be understood if it is assumed (i) that anisomycin does not interfere with the export process, (ii) that membrane components must be incorporated into completed organelles before they can be exported, and (iii) that completion of organelles requires continued protein synthesis (*22*). Similar differential inhibition of lipids has been observed in the vertebrate optic

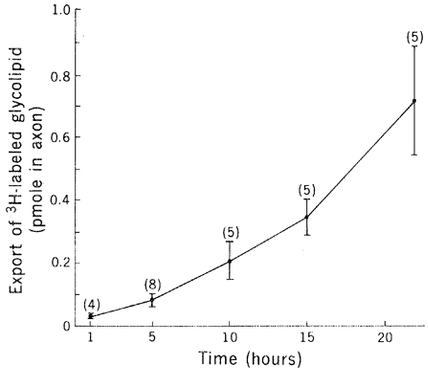

Fig. 2. Time course of appearance of ^{3}H-labeled glycolipids in R2's axon after intrasomatic injection of *N*-[^{3}H]acetylgalactosamine. After injection, the isolated nervous system was maintained at 15°C in artificial seawater. To measure export of labeled glycolipid into the axon, the right connective was cut from the ganglion; both ganglion and nerve were fractionated and membranes extracted separately as described in the legend to Fig. 1. Each point represents the mean and standard error of four to eight individual neurons. Under our conditions of counting, 10,500 count/min represents 1 pmole.

system in the presence of cycloheximide (*2*). Since labeled membrane constituents are present in anisomycin-treated cells, and since existing organelles are exported under these conditions but do not contain the labeled components, it is likely that exchange of glycolipid into completed organelles is negligible.

The logistics of organelle distribution are most obvious in nerve cells where vesicles and other components made in the cell body must travel to distant axon terminals. Studies in *Aplysia* neurons in which membranes in the cell body contain ^{3}H-labeled glycoprotein or ^{3}H-labeled glycolipid indicate that these organelles reach their destination by fast axonal transport (*23*). Formation of organelles involves not only the synthesis of proteins, but also their sequential and directional assembly. Synthesis and assembly are processes that occur in the endoplasmic reticulum and Golgi apparatus (*24*). Our experiments show that glycolipids are also only synthesized in the cell body and do not appear to exchange with glycolipids in completed organelles. Since lipid is an essential constituent of membrane, our results imply that insertion of glycolipid into nascent membrane is programmed coordinately with insertion of protein.

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Factors in Summer Ozone Production in the San Francisco Air Basin

In the recent report by Sandberg *et al.* (*1*) it is suggested that biogenic emissions are responsible for the high ozone levels observed in the San Francisco air basin.

First, for biogenic emissions to increase with previous winter rain, one would need to establish that summer vegetation is directly dependent on that rain. Miller and Cooper (*2*) find that tree-ring width is not correlated with previous winter weather. Furthermore, it does not necessarily follow that heavy winter rains will produce more summer vegetation since soil will reach saturation and any further rainfall will run off. One should therefore perform a detailed study of the types of vegetation in an area, their dependence on rainfall, and their emission patterns and then arrive at emission factors for the whole basin.

We have been concerned with the role of natural hydrocarbons in ozone production and have studied areas in the Midwest, Northeast, and Southeast, and we do not expect the San Francisco Bay Area to be significantly different. The greatest concentration of terpene we ever observed was in a pine forest in North Carolina, when the α-pinene carbon concentration was approximately 60 parts per billion (ppb), corresponding to ~50 percent of the total nonmethane hydrocarbon found in the canopy. A maximum pinene flux of ~67 μg/m^2-min was observed at this location; the temperature was ~34°C. If a simple line source diffusion model (*3*) is used with a forest 100 km in depth, the α-pinene carbon concentration in a city 1 km downwind will be 60 ppb at a high flux of 100 μg/m^2-min, assuming that the α-pinene is stable—that is, that there is no reaction with O_3 and OH radicals usually found in the atmosphere. With background O_3 and OH levels of 20 to 30 ppb and 10^5 cm^{-3}, respectively, the α-pinene concentration will be much lower. It is clear that even a high flux of 100 μg/m^2-min will not have a significant effect on the hydrocarbon concentration in a city downwind. The EKMA (empirical kinetic modeling approach) model (*4*) predicts that such low levels (60 ppb carbon) could, at the most favorable hydrocarbon/NO_x ratio, produce only approximately 15 ppb ozone.

Sandberg *et al.* state that the highest hydrocarbon levels are observed in the early morning (3 to 4 a.m.) and hypothesize that this is due to katabatic nocturnal drainage from the wooded hills. However, if the biomass is emitting the hydrocarbons in the early afternoon, as the authors suggest, they would be expected to react with ozone, which is present in high levels in the afternoon. This would be expected since most of the natural hydrocarbons are olefinic (isoprene and the terpenes) and would have a very high reaction rate with ozone. Therefore, hydrocarbons involved in katabatic nocturnal drainage would have to be emitted during the night, when biomass emissions are at a minimum. The emission levels given by Sandberg *et al.* are therefore gross overestimates. I suggest that if the authors consider the NO_x values along with the hydrocarbons they will also find the NO_x values elevated early in the morning. These high values are a result of two factors: anthropogenic emissions and the low mixing heights in the nighttime hours.

The authors found a high correlation (.81) between hydrocarbon and CO. Since they found no correlation between CO and rain but did observe a correlation between hydrocarbon and rain, it would appear that their data base changes from correlation to correlation.

Finally, Sandberg *et al.* ignored existing aerometric hydrocarbon data. We have studied a number of cities in the past decade and have never found a significant concentration of biogenically produced hydrocarbons (isoprene and the C_{10} terpenes). Much of the data has been published. All the published data show that the automotive contribution to hydrocarbons between 6 and 9 a.m. can vary from 40 percent (in Wilmington, Ohio, a rural site) to as much as 90 percent (in Manhattan, New York). The remainder is attributable to stationary sources.

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The underlying assumptions that led Sandberg *et al.* (*1*) to the conclusion that there is a significant relationship between biomass increase during wet years and summer ozone excesses require a much more rigorous examination. First, in regard to the data displayed in their figure 1, the authors employed only a limited number of years to support their biomass hydrocarbon–ozone hypothesis. It would provide a much more stringent test of their hypothesis if data available from the whole span were used. We transferred the numbers for the whole span (1962 to 1977) from figure 1 and found a correlation coefficient of only .45 for the 16-year period. We also examined the annual averages of the maximum hourly average oxidant for the San Francisco Bay Area air basin for 1965 to 1974 (*2*). The correlation coefficient obtained for this average and the 2-year precipitation was .12. Thus, the totality of the data hardly justifies the strength of their claim for a rainfall-biomass-ozone interaction and erodes confidence in their suggestion that a single wet winter (1977 to 1978) will test it. The authors speak of ozone excesses, but that is not technically correct for the entire length of the air monitoring record. The instruments commonly in use until the last 3 years did not measure ozone specifically but responded also to other pollutants (positively to the peroxyacyl nitrates and NO_2 and negatively to SO_2); it is appropriate to distinguish between total oxidant and ozone data.

Second, no measurements were made to confirm that the kinds of hydrocarbons in the San Francisco Bay Area actually originated from biomass. The authors used estimates of hydrocarbon emissions based on a study of plants in a

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