test (one-tailed) for each subject tested the hypothesis that the number of seizures per 30-day period did not decrease from the pretraining value. This hypothesis was rejected for subjects T_1, T_3, T_4, ST_2, and ST_3; it was not rejected for subjects T_2 and ST_1.

Wilcoxon tests were used to determine whether (i) time-out time per minute decreased and (ii) the time per minute during which SMR period waves were present increased as a function of training. Each of these variables was averaged over the feedback periods of each training session and over corresponding periods of each pretraining session, and each training session value was compared with the pretraining session mean. Separate tests were done for data from the left and right hemispheres of each subject. Three of the four TO subjects significantly decreased time-out in at least one hemisphere, as did two of three SMR + TO subjects. The SMR time increased in one hemisphere for subject T_1, and increased in both hemispheres for subjects T_4 and ST_1.

These results indicate that SMR training is not necessary for reductions in seizure frequency; they provide no evidence of a relationship between SMR time and seizure rate (*18*). Of the five subjects whose seizure rate decreased, only two showed an increase in SMR time; ST_1 increased SMR time significantly but showed no decrease in seizure rate.

Neither are these results entirely consistent with the hypothesis that reductions in time-out activity are necessary and sufficient for lowering seizure rate. The two subjects who failed to reduce seizure rates (T_2 and ST_1) significantly reduced time-out time, indicating that escaping or avoiding epileptiform activity during feedback training is not sufficient for lowering seizure rate. Furthermore, subject T_4 did not significantly reduce time-out time but did significantly reduce seizure rate.

Thus, SMR conditioning is not necessary for the reductions in seizure rate that have been reported to result from the SMR + TO procedure. Although TO training may be necessary, it is clearly not sufficient. The effective component of the time-out procedure is yet to be identified.

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11. We know of no experimental evidence that would suggest that SMR has any relationship to seizure frequency. Sterman (*12*) has failed to replicate his initial finding (*13*) that seizure latency following the administration of monomethylhydrazine is significantly longer in SMR-trained cats than in normal controls. Furthermore, Kaplan (*3*) used no time-out with two epileptics and found no changes in seizure frequency.
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14. Serum concentrations were checked approximately once a month, and the medication schedule was altered if they were either above or below those prescribed. This system required that the medication schedules of three patients be altered during the experiment. One patient (T_3) had medication reduced three times and increased once, T_4 had medication increased twice, and ST_2 had it reduced once.
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16. Samples were taken every 2 msec; at the amplifier sensitivy used (2 μV/mm), the system had a resolution of 0.33 μV. Therefore, any input to the analog-to-digital converter representing a wave with a smaller peak-to-peak amplitude ≥ 0.33 μV, and having a period such that (71 ± 1) msec $\leq$ period $\leq (83 \pm 1)$ msec was regarded as an SMR-period wave.
17. Extra experimental factors were evident in these two cases. Subject ST_1 became pregnant during the first month of feedback, a condition which had once before grossly exacerbated seizures. Three sharp rises in the seizure rate of subject T_2 occurred at days 68, 114, and 159 from the start of feedback, concomitant with, respectively, the days that T_2 left an uneasy home situation, returned home, and realized that the home situation had not improved. Furthermore, the reliability of T_2's data is questionable, in that her pretraining seizure rate was estimated in her medical file to be eight times the rate she reported during the pretraining period. This subject's neurologist also indicated that he believed T_2 to have many more seizures than were reported.
18. This lack of relationship has also been reported in other published reports (*1*). We are aware of no data, including those reported by Sterman (*5*), demonstrating significant increases in SMR in groups of epileptic subjects receiving SMR training.
19. Supported by Ontario Mental Health Foundation grant 258-69D and Medical Research Council of Canada grant MA 5854. A.C. is an Ontario Mental Health Foundation Research Scholar. We thank Dr. A. Upton for providing medical supervision and assistance throughout this study.

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Plasticity: The Mirror of Experience

Abstract. *A simple avoidance training procedure during early development produces massive neural traces in visual and somatic cortices of kittens reared in a normal environment. A preponderance of cells in these areas had response preferences for the stimuli used during training. Furthermore, some of these cells exhibited properties never found in normal animals not receiving such training. It appears that, even in an environment in which many other stimuli are present, some early experiences powerfully affect brain development and the way in which other experiences exert their effect.*

One of the most interesting endeavors in neuroscience has been the search for neural modifications induced by experience and their loci in the brain. Lashley (*1*) named this "the search for the engram" and after many experiments concluded that memories had to be distributed because they could not be localized. Wiesel and Hubel have demonstrated in the visual cortex of cats that if sensory deprivation is enforced during a critical period of development that spans 4 to 8 weeks, substantial phenomena of neural plasticity result (*2, 3*). In 1970, Hirsch and Spinelli (*4*) demonstrated that controlled exposure to vertical lines for one eye and horizontal lines for the other during early development causes a preponderance of monocular cells in the visual cortex. Most important, the shape of receptive fields was remarkably similar to the shape of the stimuli viewed during development. Similar results have been obtained by others (*5*). In some of these experiments (*4, 5*), except for the controlled experience, the kittens were kept in a dark room at all times; that is, the exposures are delivered on a background of visual deprivation. We also showed that after our cats from the 1970 experiment acquired normal experiences binocularly for a year and a half, they also acquired new functional properties in some of their visual cortex cells: cells were found with binocular disk-shaped receptive fields (*6*). Cells with line-shaped receptive fields appeared to be still monocular and bound to the early experience.

Our memory hypothesis, that many responsive cells are actually shaped by the experience (*6*), might explain at least some of the effects observed. Alternatively, atrophy from disuse might have

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eliminated apparently missing cell classes. A demonstration that plasticity can be produced without deprivation would be a significant step toward elucidating some of the ways in which experience engraves itself in the brain.

We now report that early experience can produce plastic neural changes in the visual and somatic cortices of animals that are not raised in conditions of sensory deprivation. The experience was a simple avoidance task. By making the experience more "meaningful" to the animals and by allowing them to develop in a "normal" environment, we believe that the results allow more direct inferences concerning the effects of normal experiences gained during development and in adulthood on the behavior of the animal and on the functional properties of the neural substrate.

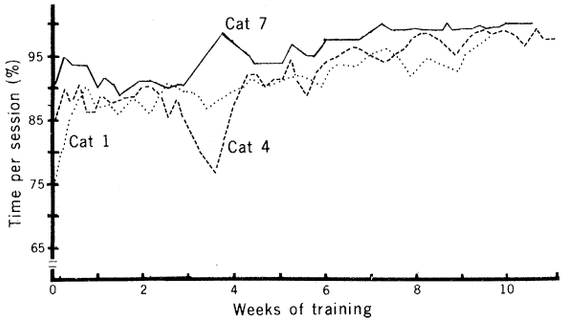

Fig. 1. Performance curves for cat 1 (trained from 11 weeks), cat 4 (trained from 5½ weeks), and cat 7 (trained from 4 weeks). The ordinate describes the percentage of time per session that the forearm was up and shock was avoided.

Kittens were raised with their mothers in a standard animal facility. They were friendly with the experimenters and behaved in all respects as normal kittens; in particular their visuomotor coordination seemed normal. Every day each kitten received 8 minutes of avoidance training. The kitten was suspended in a sling, and each foreleg was attached by string to a microswitch which could be activated by leg flexion. On each forearm, stimulating electrodes were held in place with a rubber band; one forearm received a mild electric stimulus (1-msec, 1.2-mA pulses at a rate of four pulses per second) sufficient to make the kitten withdraw its arm but without being frightening.

Goggles were fitted on the eyes for stimulus viewing. The position of the forearm connected to the shock controlled the state of the visual stimulus. Forearm down meant shock for the dorsal aspect of the forearm and vertical or horizontal lines for one eye, forearm up meant no shock and lines of the orthogonal orientation for the other eye. In other words, there was a "safe" stimulus and an "unsafe" one, a situation commonly encountered by normally developing kittens in normal environments. All conditions were randomized between kittens. For one of the kittens, the visual stimuli and the shock were not contingent upon a response (yoked condition).

Experimental groups were started at 4 weeks (N = 4, including the yoked kitten), 5½ weeks (N = 4), and 11 weeks (N = 1) of age. All kittens were trained until the day before recording and learned the task uneventfully (Fig. 1). After about 10 weeks of training, the kittens were prepared under Pentothal anesthesia for single-cell recording by exposing the somatic cortex (*7*), primary visual cortex (between stereotaxic coordinates anterior-posterior −2 to +2 mm, 1.5 mm lateral to the midline) and visual "association" cortex (−2 anterior-posterior, −2 to +2 mm; medial-lateral, +4 to +8 mm) (*8*). The animal was placed in a stereotaxic apparatus that leaves the visual field free. Long-acting local anesthetic was used at all incisions and pressure points. The trachea was intubated through the mouth, and the femoral vein was cannulated (with a Cathlon IV catheter) without dissection. Care was taken to maintain the kittens without pain and in optimal condition. Recordings from single units were made with tungsten microelectrodes (*9*), beginning from the postcruciate cortex 7 mm lateral to the midline about 1.5 mm posterior

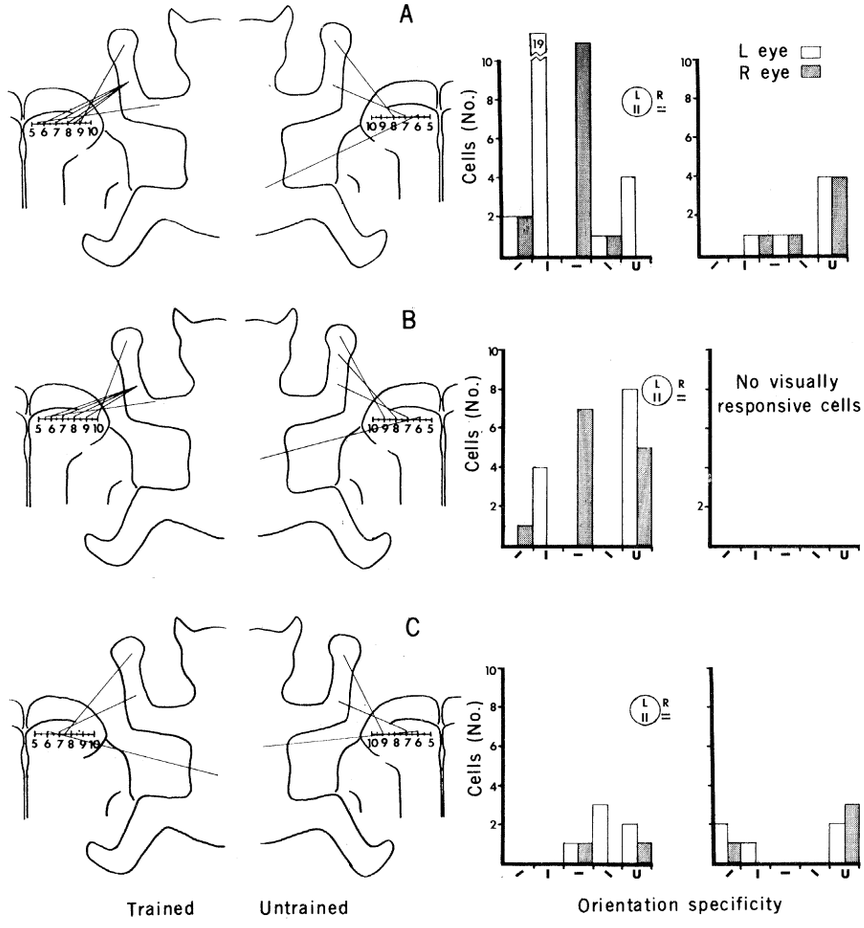

Fig. 2. On the left are cortical representations of the trained and untrained sides of the body for all cats. Electrode penetrations are marked (in millimeters lateral to the midline) along the postcruciate cortex 1.5 mm posterior to the cruciate sulcus. Lines are drawn from each penetration to the part of the body it represents on the figurine. On the right are histograms of orientation specificities of units in the somatosensory cortex. Angle of oriented line stimulus to which the units were responsive is indicated on the abscissa. Left and right histograms are from the hemispheres corresponding to the trained and untrained body sides, respectively. (A) Cat 5, trained from 4 weeks; (B) cat 6, yoked condition, trained from 4 weeks; and (C) cat 1, trained from 11 weeks. Stimulus types used during training for each eye are indicated next to each pair of histograms, and that stimulus that was concurrent with the forearm shock is circled.

to the cruciate sulcus, which previous experience had shown to be the center for the locus of the forearm representation, normally having a diameter of 0.5 to 1 mm. Subsequent penetrations were spaced medially and laterally 0.5 to 1 mm until the edge of the locus was encountered, that is, until cells were found that responded to nearby parts of the body. Cell responses were elicited by light skin strokings, hair brushing, pressure, or joint movement. Because cells in this area also respond to other sensory modalities (*8*), we tested for auditory and visual polymodal responses. Auditory responsitivity was determined by presenting clicks, handclaps, and other sounds to each ear separately. Visual responses were tested by displaying edges, bars, and flashlight stimuli to one or both eyes. Orientation sensitivity was determined by the use of a black bar moved by hand against a white cardboard screen. Comparison of the findings in the hemisphere contralateral to the shocked forearm immediately reveals that the size of the shocked forearm locus is many times larger than the size of the unshocked one, in general 2 to 3 mm in diameter around the natural locus versus 0.5 to 1 mm (Fig. 2). These changes are brought about by a total of about 10 to 29 minutes (cumulative errors over all training sessions) of mild shock and are present in all kittens with the exception of the one trained from week 11.

Another major and obvious difference between the trained and untrained loci has to do with the number of polymodal cells present in the two: about the same percentage of cells respond to auditory stimuli in both loci (25 percent); for visual stimuli, however, the percentage is much higher (75 percent) for the trained than for the untrained side (30 percent). This was true in all cats except the one that had started training from week 11. In this cat approximately 25 percent of cells exhibited auditory response, as is the case with cells that exhibited visual responses, and the percentages were about equal on the trained versus the untrained side (25 percent).

In the enlarged forearm locus, most of the polymodal cells that exhibited orientation sensitivity and responded optimally to visual stimulation of each eye did so for the stimulus orientation that had been presented to that eye during training (Fig. 2). The percentage of all cells responsive to visual stimuli in the contralateral, untrained locus is much less, and orientation preferences seem randomly distributed (*10*). Thus, the somatosensory cortex seems to have recorded the sensory aspects of the experience in a straightforward fashion. Notable aspects of these results concern the large percentage of cells controlled by the eye paired with the shock. The kittens that learned the avoidance earliest had only about 10 minutes total viewing of the unsafe stimulus and 250 minutes of the safe one. The kitten that had the slower rate of learning viewed the unsafe stimulus for a total of 29 minutes, the safe one for 300. Yet in all cats, the eye paired with shock controlled the larger number of cells. Among the younger two groups, the physiological results mirrored the performance results; the better performers had shorter exposures to the "unsafe" stimulus, yet showed the larger percentages of cells that responded to it and to forearm stimulation. If one assumes that neural changes drive behavioral ones, the interpretation simply becomes that faster or greater accumulation of "expert" cells results in accelerated improvement of performance (*11*).

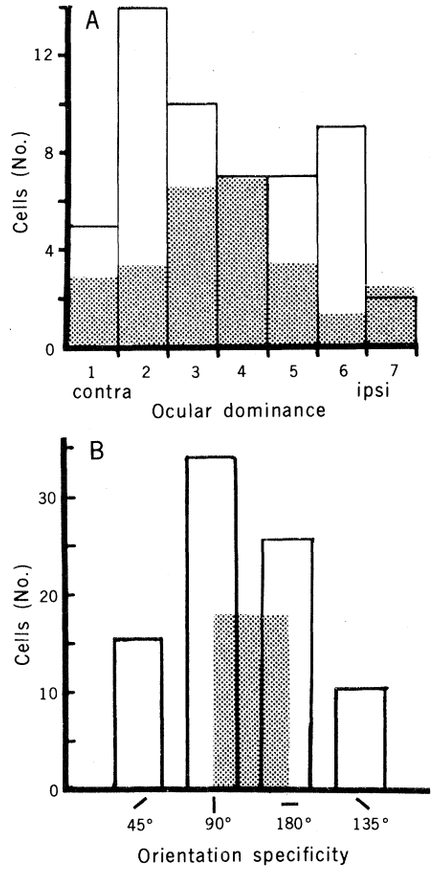

Fig. 3. (A) Ocular dominance histograms, normalized, pooled from all cats trained from 4 and 5½ weeks. Cells in group 1 were driven only by the contralateral eye, cells of group 4 were equally driven by either eye, and group 7 cells were driven only by the ipsilateral eye. Other groups indicate intermediate eye dominance. (B) Orientation specificity pooled from all cats trained from 4 and 5½ weeks. The bars represent classes of cells that were responsive to horizontal, vertical, and diagonal line orientations. The shaded area corresponds to the population of binocular cells with different orientation sensitivities for the two eyes.

Recordings from the visual association area show a preponderance of cells responsive to bars of orientation similar to those used during training. Here cells are primarily visual but exhibit also polymodal responses: often these cells responded to stimulation of the trained forearm.

Some of the most interesting findings were obtained in the primary visual cortex (Fig. 3A). Comparison with normal data [the shaded overlay is from Wiesel and Hubel data (*2*)] indicates a large shift toward monocularity ($\chi^2 = 7.25$, $P < .01$); further, these cells are controlled predominantly by the eye associated with the shocked forearm. The bar graph (Fig. 3B) shows cells classed in terms of orientation sensitivity. In the normal cat, these classes are roughly equal [(*3*), and also in our experience]. Verticals and horizontals—the orientations used in the training—strongly dominate in all cats with the exception of the one whose training was started at 11 weeks of age ($\chi^2 = 6.72$, $P < .01$).

One aspect of the results in the visual cortex that stands out (as comparable findings are never present in normal kittens) is the presence of cells responsive to vertical orientations for one eye and horizontal orientations for the other. The percentage of these cells (shaded area, Fig. 3B) is about 60 percent of the total number of cells responsive to vertical or horizontal bars. Paradoxically, a substantial fraction of these cells responded to vertical bars when tested with the eye that had been trained with horizontal bars, and to horizontal bars when tested with the eye that had been trained with vertical. We called this the inversion phenomenon (*12*).

The interpretation of these findings hinges on many factors, some of which are obscure at present. Even so, it seems clear that as the kittens learn the task, some neural correlates of it must be present in their brains; thus, it is not surprising that the somatosensory cortex, which is rich in polymodal responses and is heavily involved in the task, should be one such area. As more is demanded of the trained locus and as the kittens continue to develop, more neural resources are allocated to this area possibly displacing lower priority ones: the magnification of the locus leads to a magnification of the number of cells that show neural changes produced by the task and makes those cells detectable. It is encouraging for future work that these cells

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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