light break was placed between 15 and 16 hours after the beginning of the main light component (Zeitgeber time 15 to 16), and the second when placed at Zt 21 to 23. According to Pittendrigh and Minis (*7*), the first peak represents the position of the substrate maximum (or the critical daylength). When the light break is placed later in the light-dark cycle a phase-jump occurs, until at Zt 21 to 23 the light break serves to phase-set the substrate rhythm so that its peak now coincides with the end of the main light component, and diapause is again eliminated.

When this experiment was repeated with the addition of a 4-hour period at 2°C at the beginning of the main light period, the first peak of inhibition, although depressed, was observed to have moved further into the "night" by 2 hours (to Zt 17 to 18) (Fig. 1) (*12*). This is assumed to be because the substrate rhythm is now forced to oscillate within the 20 hours available at suitable temperature (Zt 4 to 24), but its shape remains symmetrical. In other words, the substrate rhythm now has a period of 20 hours and reaches its peak at Zt 17 to 18 instead of at Zt 15 to 16.

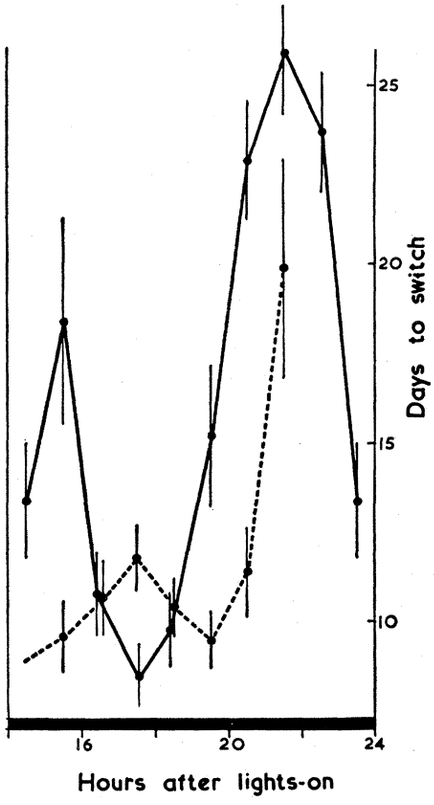

Fig. 1. Effect of 1-hour light breaks (night interruptions) on the inhibition of diapause production by females of *Nasonia vitripennis* in a light-dark cycle of LD 14 : 10 (data given are for the 10-hour dark period). The first peak of inhibition moved to the right by 2 hours when chilling was applied for the first 4 hours of the light component. Each point represents the mean age at the "switch" from developing to diapause larvae for about 20 females; vertical lines, plus or minus twice the standard error. Solid line, in LD 14:10 without chilling; dashed line, in LD 14:10 with chilling at 2°C for 4 hours after lights-on.

This result is interesting for two reasons. (i) It provides an explanation for the results summarized in Table 1. For instance, if a similar period of chilling had occurred at the beginning of the main light period in an LD cycle of 16 : 8, the 2-hour shift in the substrate maximum to the right would have resulted in the conversion of a long daylength to a short one. (ii) This result provides circumstantial evidence for the participation of a circadian rhythm in insect photoperiodism, since chilling in the light affects the position of the first peak in a way that can be predicted from Pittendrigh and Minis' (*7*) "co-incidence model." If an interval timer, which measures the duration of the dark component only, was involved, chilling in the light would not have had such an effect.

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11. These experiments were conducted in Gallenkamp cooled incubators fitted with Philips 8W striplights controlled by Londex time switches.
12. The mean ages at the "switch" for the two groups at Zt 16 are significantly different ($t = 6.187$, $P < .001$); so are those for the two groups at Zt 18 ($t = 3.755$, $P < .001$).
13. I thank Mrs. M. H. Downie for technical assistance, and the Science Research Council for financial support.

23 January 1967

Synaptic Loci on Parietal Cortical Neurons: Terminations of Corpus Callosum Fibers

Abstract. *Dendritic spines disappear when the synaptic terminals impinging on them are destroyed. Terminal synaptic sites of axonal projections can be mapped by interrupting the afferent system and then comparing the density of dendrite spines in the suspected recipient area with controls. The corpus callosum was sectioned in five rabbits at birth. When the rabbits were 30 days old, the loss of dendrite spines was limited to the oblique branches of apical dendrites in the parietal cortex.*

It has been well established by means of both light microscopy (*1–3*) and electron microscopy (*4–6*) that the dendritic spine or thorn is an essential part of the postsynaptic membrane of most neurons. Gray showed that the spine receives a special class of presynaptic terminals [Gray, synaptic type I (*4*)], while Colonnier's study indicates that spines are resorbed if the presynaptic terminals are lost (*7*). We have reported (*8*) that after interruption of the visual afferent system (enucleation, lateral geniculate lesion), there is moderate to marked loss of spines along the central third of the apical shafts of pyramids in the visual cortex. The present report deals with results of interruption of another cortical afferent system, the callosal projection.

The corpus callosum was sectioned in five newborn rabbits in the following manner. Under light ether anesthesia, a curved cutting needle threaded with 2-0 silk was forced through the skull just to the right of the midline between the eyes and swept deep and posteriorly in such a way as to emerge just anterior to the posterior fontanelle. The course taken by the suture material was parallel to and to the right of the sagittal suture and below the corpus callosum. The suture was then

tightened while gentle counterpressure on the curvature of the skull was maintained. Preparations stained according to the methods of Nissl and Weil showed that, with this procedure, the corpus callosum is severed with only slight damage to the underlying thalamus and overlying cortex. The test animals and their littermate controls were killed at 30 days. A block of cortical tissue (2 by 3 mm) was removed from the left hemisphere, just anterior to the most anterior portion of the lateral sagittal sulcus, which is within the parietal area of cortex (*9*). The tissue was prepared for study by the rapid Golgi method with the use of osmic acid–potassium dichromate fixation for 4 to 6 days; this was followed by impregnation with silver nitrate (0.75 percent) for 24 hours (*8*).

As a result of the section of the corpus callosum, there was a massive loss of fibers within the white substance. This loss extended to the white matter beneath the area where the counts were performed. (Coding the slides has not been used as a technique for achieving a blind study, because during the search for countable pyramids, the investigator would observe this loss of fibers, noticeable in part by chains of osmophilic degeneration granules, and realize that the section was from an experimental animal.) To reduce the possibility of biased selection of pyramids for spine counting, the slides were first scanned at low magnification for completely impregnated pyramids. The criterion for selection was completely stained basilar, oblique, and apical dendrites that gradually tapered to their endings without abrupt termination. This selection was completed before the slides were observed at a magnification sufficient to estimate spine densities. Ten pyramids were studied in each of the control and experimental animals. Exact counts were made at a number of stations along the entire basilar and apical dendrite trees of each neuron (see Fig. 1B). In addition, a much larger group of pyramids in both categories were studied with the use of qualitative techniques to estimate the degree of spine loss, if any. The dendritic spine is characterized by a round terminal attached to a narrow root which results in a club-shaped outline approximately 2.5 μ long. Although numerous artifacts may occur in Golgi preparations, none resemble the distinct structure or have the same location as dendrite spines. The shape, size, and distribution of these spines have been confirmed by a number of workers (*4–7*) with the use of the methylene-blue technique of Ehrlich (*1*) and with electron micrography.

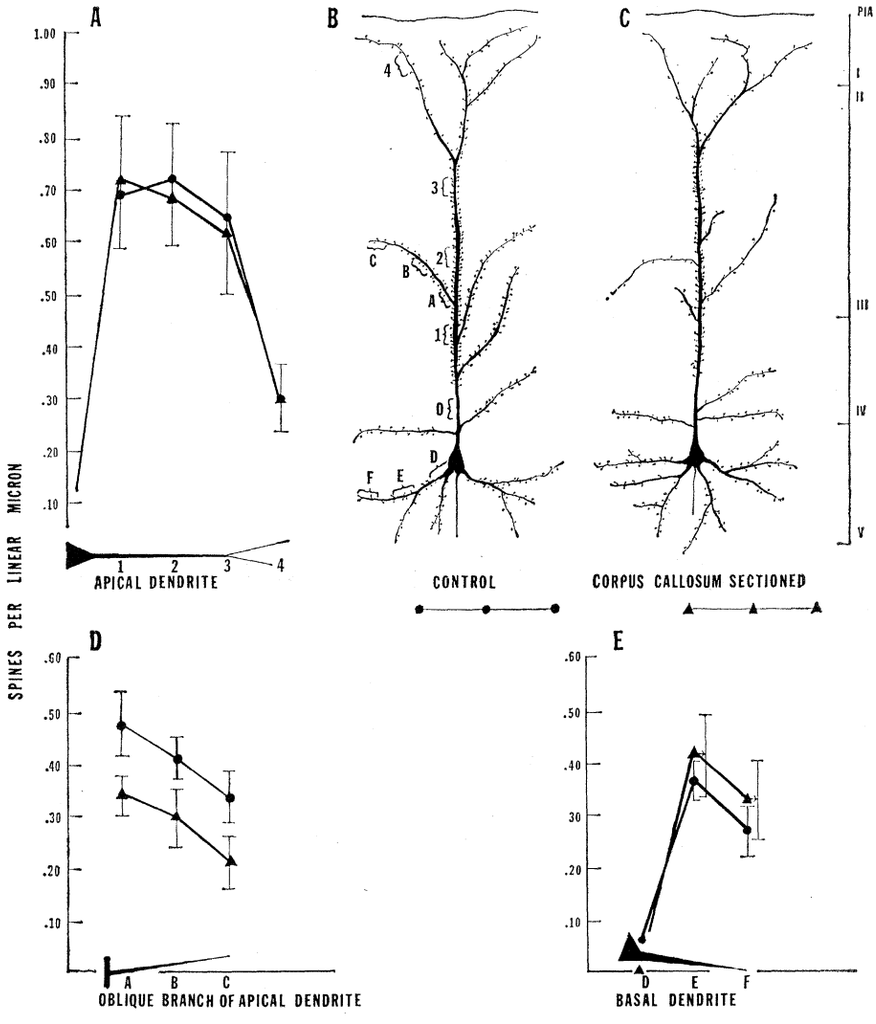

Fig. 1. Diagrammatic and graphic representations of the effect of section of corpus callosum on pyramids in parietal cortex. Parts B and C were drawn from Golgi impregnations of parietal cortex in littermates. Part B represents a cell from the normal control and includes the heavily spined apical shaft (stations *1*, *2*, and *3*); the somewhat less heavily spined oblique branches (stations *A*, *B*, and *C*); the sparsely spined basilar branches (stations *D*, *E*, and *F*) and apical arches (station *4*), and the unspined initial segment of apical dendrite (station *0*). Part C represents a cell from an animal with section of corpus callosum. There is a marked loss of spines on the oblique branches. Graphs A, D, and E contrast spine counts in control (filled circles) and callosum-sectioned (filled triangles) animals along apical dendrites, oblique branches, and basal dendrites, respectively. Brackets indicate area of ± 1 standard deviation.

Thick sections (100 μ) were examined at a magnification of 600. The density of spines on pyramids was estimated in the following manner. The apical dendrite was marked off in quarters and the oblique branches and basal dendrites in thirds (Fig. 1B). All the spines with a 25-μ segment were counted, and the spine frequency per linear micron was calculated.

Study of the soma-dendrite complex of pyramids in animals whose corpus callosum was sectioned immediately after birth revealed two findings: (i) shortening of many of the oblique branches by a factor of about 30 percent, and (ii) loss of approximately one-third of the spines along these branches (Fig. 1C). All other portions of the dendritic complex appeared unchanged.

Normally, apical dendrites have spine densities of 0.75 spines per

micron; oblique branches and subpial arches, 0.40; and basal branches, 0.30. Figure 1 graphically represents these values averaged at each counting station (filled circles in parts A, D, and E). It also shows the values for pyramids taken from rabbits with operative interruption of the corpus callosum at birth (filled triangles in parts A, D, and E). The difference in the means of the spine densities along the oblique branches of the apical dendrite are significant ($P < .02$) on the Student t-test. Along the apical dendrite itself and the basal dendrites, the same test was not significant. Thus, the spine density is reduced along the oblique branches (Fig. 1D) by section of the corpus callosum.

The rigorous limitation of changes in the spine count to the oblique branches of apical shafts strongly suggests that the callosal afferent system, coming largely from the homotopic locus in contralateral cortex, terminates on these branches. The fact that spine loss is partial suggests that other afferent systems may also synapse along these oblique elements.

These data, in conjunction with our previous findings which relate the terminals of the visual afferent (geniculocalcarine) radiation to the central third of the apical shafts of pyramids (*8*), emphasize the topographical specificity of presynaptic terminals along the postsynaptic surface of cortical neurons.

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21 March 1967

Behavioral Compensation with Monocular Vision

Abstract. *If an object is arranged so that when viewed monocularly it appears centered in an aperture it is displaced from the position in which it appears binocularly centered. Before and after viewing the responding right hand with the left eye through a small aperture, observers were required manually to indicate a point in line with the center without visual guidance. Apparent displacement of the limb due to monocular viewing resulted in a change in the direction of responding. This change in response direction (termed behavioral compensation) is similar to that which occurs when the spatial properties of stimulation are modified with an optical system.*

When light from an object is refracted through a prism before entering the eye, the object's apparent position is changed. Manual pointing or reaching responses directed at the object during such spatial transformation change in direction and this change persists if, after the transformation period, responses are made without visual (or auditory) guidance (*1*). Because of the possible significance of these findings for theories of space perception and perceptual-motor coordination these compensatory changes have been extensively investigated and alternative explanations have been proposed (*2*).

In experiments involving visual transformations a common procedure for indexing and measuring the effects is to compare responses made without visual guidance before and after an exposure period in which the limb is viewed through an optical system. The effect of the intervening optics is to alter the apparent position of the limb so that there is discordant information from the visual and kinesthetic systems. So far all experiments have involved an optical, usually prismatic, system between eye and viewed object. The purpose of the experiment we report was to show that similar compensatory changes occur with monocular parallax: a change in the apparent location of an object with change in the eye used to view it. As far as we know changes in responding attributable to monocular vision have not been reported previously.

The viewing arrangement is shown in Fig. 1. If an observer views his hand resting on the surface *OP* through an aperture *XZ* with the right eye *R*, then, in order to appear centered in the aperture at *Y*, the hand must be placed at *P*. If, however, the right eye is occluded and the hand is viewed with the left eye *L*, then, to appear centered, the hand must be moved from *P* to *O*. This is an instance of parallax: a change in the direction of an object resulting from a change in the observer's viewing position. In this case the change in position is consequent upon which eye is used to view and is referred to as monocular parallax.

If with this viewing arrangement an observer is required to respond frequently by marking a point on *OP* so that it appears in line with the center of the aperture, changes would be expected to occur in consequence of the new visual-kinesthetic relationship, as happens with an optically modified relationship. If visually guided centering

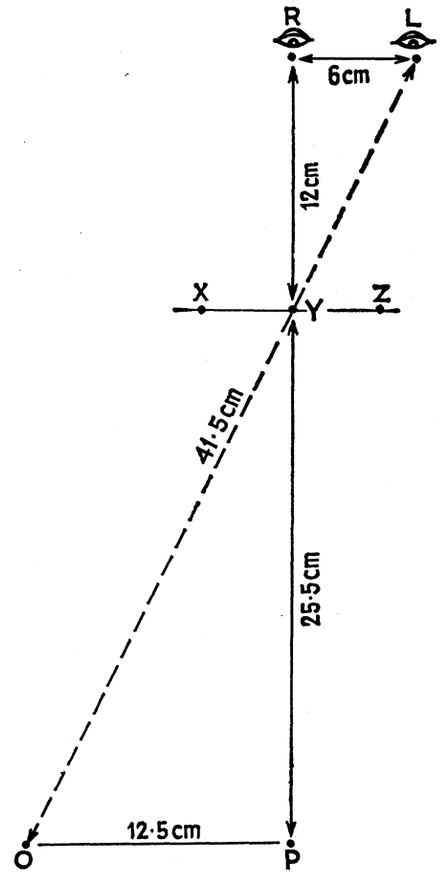

Fig. 1. Monocular viewing system used in experiment. For an object on the surface *OP* to be seen with the left eye (*L*) as centered (*Y*) in aperture *XZ* it must be located at *O*.