CROSSCORRELATION ANALYSIS OF INTRACOLUMNAR NEURONAL CONNECTIVITY IN AREA 17 OF BINOCULARLY DEPRIVED CATS

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Abstract. Eight cats were binocularly deprived of pattern vision by rearing in masks from the time of eye opening. Twenty five groups of 3 neurons and 28 neuronal pairs were studied in visual orientation columns of their striate cortices. The crosscorrelograms of neuronal discharges were analyzed and the inference of underlying interneuronal connectivity was made. The results were compared with the normal cats data obtained earlier in an identical experiment. Total number of existing interactions was only slightly reduced: from 95% of analyzed pairs in normal cats to 90% in deprived animals. The most pronounced effect of visual deprivation was the reduction of the percentage of neuronal pairs that shared the same source of input from 61 to 34%. The proportion of direct excitatory connections was not affected, while an increase in the number of inhibitory correlations was found.

INTRODUCTION

The response properties of the visual cortex of cat can be modified by elimination of patterned visual input that the animal receives in early life. The changes of receptive field properties of visual cortical neurons produced by binocular deprivation have been described by many authors. To the most noticeable effects belong the reduction in respon-
siveness to visual stimulation and decrease in response strength (1, 5, 16, 18, 22, 23). A loss of direction and orientation selectivity was also reported (1, 9, 16, 18, 22). If cats were raised with sutured eyelids of both eyes, a disruption of binocularity of area 17 units was found (9, 16, 22, 23). These modifications of response properties of cortical neurons can be caused by changes in the efficiency of input to area 17 from subcortical visual centers and/or in the functioning of intrinsic cortical transfer and processing of information.

The crosscorrelation analysis is a sensitive method for demonstrating the detailed organization of a neuronal network. Recent development of this method permitted some insight into the interneuronal ties in the cortex. Studies by Kimura et al. (7), Toyama et al. (19, 20) and Michalski et al. (13) described the principal types of neuronal interactions in the visual cortex of the normal cat. The present paper uses this technique to examine interactions within a cortical column in area 17 of binocularly deprived cats. The data are compared with those obtained for normal cats with the same kind of electrodes and stimulation (13).

METHODS

Results were obtained from eight cats. Animals were binocularly deprived of pattern vision by rearing in masks from the time of eye opening. The masks were made of white double linen. They were changed and washed daily. During the changing the cat's eyes were kept closed by the experimenter and washed with a cotton wool swab soaked in a weak antiseptic solution. The reduction of diffuse retinal illumination by the mask was variable; in a clean one it was only about 1 log unit, in the one stained by the eye secretion it could reach 6.5 log units. Thus, an average reduction produced by the old mask was comparable to that produced by closing the cat's eyelid. (According to our measurements a grey eyelid reduces the illumination by about 5 log units.) The cats were kept in the animal colony together with other normally reared cats. The illumination of the cat's cages depended on weather and time of day and varied from 1.4 to 58 lux (for details of deprivation procedure see (8)). Recordings were done when the cats were 6 to 8 months old.

Cats were anesthetized with Nembutal (Pentobarbital Sodium). The initial dose of 40 mg/kg was given intramuscularly. It was followed by the maintenance intravenous injection of 1.5 mg/kg/h. The femoral vein and trachea were cannulated. After tracheotomy the animals were mounted in the stereotaxic holder. Cats were paralysed with gallamine triethiodide (Flaxedil: initial dose 100 mg, maintenance dose 20 mg/h) and
artificially respirated with room air. End-respiratory CO$_2$ was maintained at 4% by adjusting the tidal volume delivered by the respirator. Temperature was stabilized at 38°C with the servo-controlled heating pad. After several hours of experiment subcutaneous injections of glucose and saline were administered.

An opening in the skull (0.5×1.0 cm) was done unilaterally over area 17. The center of the opening was at the stereotaxic coordinates $P$ — 1.5, $L$ — 1.5. Dura mater was removed and the cortex protected with a thin layer of agar. The electrode was carefully positioned perpendicular to the cortical surface and the opening was sealed with agar.

Lids and nictitating membranes were retracted and pupils dilated with atropine. Corneas were protected with contact lenses with 5 mm artificial pupils. Refractive state of the eyes was estimated by direct ophthalmoscopy.

Visual stimuli were displayed on a white tangent screen located 57 cm in front of the cat’s eyes. Stimulus intensity was 10 cd/m$^2$ and background level was 0.5 cd/m$^2$. For each unit the receptive field size and location, the degree to which it responded to stimulation of one or the other eye and the near optimal stimulus velocity was determined with hand-held projector. An electronically driven projector was used during the recordings.

Extracellular recordings were obtained with three-channel tungsten electrodes. Epoxy insulated tungsten wires of 12 μm diameter were mechanically sharpened to remove the insulation over the distance of about 20 μm and obtained pencil-like tips. Three sharpened pieces of wires were glued together to form a bundle. The distance between individual tips varied between 100 μm and 200 μm. The bundle was inserted into a glass pipette and glued to it with electrode tips protruding about 2 mm. Connecting wires were attached to the upper ends of the electrodes. Tip resistance measured for 1 kHz square wave varied between 0.8 and 1.2 megohm.

Signals from each electrode channel were amplified by separate electronics, displayed on a multichannel oscilloscope and recorded, together with stimulus markers on the Unitra M2406 4 track, tape recorder. Crosscorrelograms of the artificial spike trains proved the satisfactory quality of magnetic recordings. Activity of each neuronal group was recorded for at least four different orientations of moving light stimuli: the near optimal orientation of the analyzed column, the null orientation and two orientations between them (with a 45° jump). Axis of the stimulus movement was always perpendicular to the stimulus orientation. The time delay between the first and the last correlogram varied between 30 min and 1 h. Only if the recordings in all channels were
stable the data were considered for analysis. PST histograms were computed during the experiment with Anops analog — digital analyzer. Full data analysis, including triggering procedure, was done off-line from the analog tape with Cromemco Z-2 microcomputer. For most of the data presented here only the largest spike on each wire was detected by a simple threshold circuit, and sent to the computer. All the cases when window circuits were used to separate spikes of different amplitudes from a single wire were clearly labeled.

The following set of measurements was used to analyze each group of simultaneously recorded units:

1. Low and high resolution autocorrelograms (0.5 and 5 ms bin width) for each neuron, for each stimulus orientation.
2. Peri-stimulus-time (PST) histograms for each neuron, for each stimulus orientation.
3. Low and high resolution crosscorrelograms (0.5 and 5 ms bin width) for each pair of neurons for each stimulus orientation.
4. Shift predictors corresponding to all crosscorrelograms computed from stimulated unit activity.

The shift predictor is a necessary control to isolate the correlation effects due purely to the (periodic) stimulus when it affects both neurons. It was calculated by shifting one of the two spike trains by one interstimulus period and then recomputing the crosscorrelogram. Only if the original crosscorrelogram had peaks or valleys not seen in the shift predictor the interactions between neurons were indicated. These methods and their interpretations were extensively discussed by Perkel et al. (17) and Dickson and Gerstein (4).

**RESULTS**

Bundle electrodes allowed simultaneous recording from several neurons in a fairly close proximity. Bundles were always inserted perpendicular to the surface of the lateral gyrus, stereotaxic coordinates of entry varied between P 2.5 and A1 rostrocaudally and between L 0.5 and L 1.5 laterally. Individual tips positions differed by less than 50 μm in the horizontal plane and between 100–200 μm in the vertical plane. To compare the data with those obtained for normal cats with identical electrodes and stimulation, PST histograms were carefully examined in order to determine whether the simultaneously recorded and closely situated cells belonged to a single cortical orientation column. In normal cats only if the preferred orientations of all neurons in a group were found within the same 15° wide angular sector, the data were used for further analysis (13). In deprived animals the reference to the orien-
tion columns was more arbitrary since only 38% of responsive units were orientation selective. However, only the simultaneously recorded neurons that did not differ in their preferred orientations (if present) by more than 30° were analyzed. This less stringent requirement was employed because in deprived animals even the neurons classified as orientation selective had weaker selectivity and weaker response strength, which lowered the accuracy of the preferred orientation measurements.

Electrode penetrations were made in the cortical representation of the area centralis. Receptive fields of responsive units were located within the central 15° of the visual field with a great overlap between them. Twenty five groups of 3 neurons and 28 groups of 2 neurons were recorded, i.e., the total of 131 neurons. Crosscorrelational analysis was done on 103 simultaneously recorded pairs.

Twenty nine units (22%) were unresponsive. All other neurons could be driven by moving bars of light. Standard parameters of all receptive fields were tested and compared with the effects of other deprivation procedures. These data, presented in separate paper (14), resembled results obtained for cats binocularly deprived by eyelid suturing. Twenty three percent of units did not respond to the visual stimulation. Responses of the remaining units were less vigorous than in normal cats: on the average the peak to background ratio in the PST histograms was reduced by 65%. The binocularity of units was impaired: 64% of cells could be driven only by the contralateral eye. Only 10% of cells were influenced equally by both eyes. Orientation selectivity was reduced: only 38% of responsive units were orientation selective. Twenty three percent of neurons were direction selective. Unusually large (more than 10°) receptive fields were found in 10% of units.

The interactions between simultaneously recorded neurons were analyzed and the inference of underlying neuronal connectivity made according to the criteria developed by Dickson and Gerstein (4). Crosscorrelograms were classified in terms of: (i) direct synaptic connection, (ii) shared input, (iii) stimulus dependent coordination. The same criteria were used and discussed in more detail in our previous work on normal cats (13). We also refer the reader to a series of earlier papers that introduced these methods of crosscorrelogram analysis (2, 4, 15, 17).

In 10 cases (10%) the crosscorrelograms showed no interactions at all between the two tested neurons, while in normal cats only 4.5% of pairs were uncorrelated (Table I). The difference between deprived and normal cats in the proportion of pairs showing no correlation is statistically significant \((P < 0.05)\). In 93 pairs (90%) one or more of the several forms of coordination was found.
Direct synaptic connections

Crosscorrelograms indicating the existence of direct synaptic connections between two investigated neurons are characterized by narrow, peaks slightly shifted from the center. This type of interaction appeared in 13 pairs (13%), but not in isolation; in 10 of these cases direct connections were accompanied by the broad shared input waves and in 3 pairs by both shared input components and inhibitory troughs. The differences between the visually deprived and normal cortex did not reach the level of statistical significance. (In normal cats pure excitatory direct connection was found in 5.6% of pairs. In conjunction with the other types of interaction it was observed in 20.3% of cases). Figure 1 shows an example of such mixed coordination. Components could be easily distinguished on the narrow bin crosscorrelogram. Excitatory direct connection is characterized by a narrow, high peak, shifted 2.5 ms from the correlogram center. In the visually deprived cats such peaks varied in height up to nine-fold of the level of background correlogram bins, i.e., the bins not affected by the broad shared input waves. The delay of the correlogram peak from zero varied between 0.3 and 3 ms with a mean of 1.4 ms. Peak width varied between 1 and 10 ms (mean 5.4 ms). The same correlogram in Fig. 1 clearly shows the broad wave of the shared input component and 4 ms wide inhibitory trough. Trough center
is shifted by 1.5 ms from correlogram zero. On the wide bin correlogram only the strong shared input coordination can be seen. Both shift predictors are flat, indicating that the stimulus contributes nothing to the peaks or valleys of the correlograms.

The number of counts under the correlogram peak expressed as a percentage of the total number of spikes of the follower neuron was defined as the contribution of the driving cell \(10, 12\). Such percentage could be used effectively for pairs with direct synaptic connections. If the excitatory connection is tight and if the excitation comes mainly from the driving neuron this "contribution" may directly express the strength of the excitatory drive. It should be mentioned, however, that for the pairs with very tight connections and very low "uncorrelated" activity, as well as for the very broad peaks this coefficient can go beyond 100\%. This is the direct effect of the crosscorrelation algorithm without physiological interpretation. Contribution coefficients in visually deprived animals varied between 2 and 49\% with a mean of 18\%. These values did not differ significantly from the normal cats data. (In normal cats the percentage contributions varied between 12 and 30\% with a mean of 18\%).

Pure inhibitory direct connections were not found in deprived animals. (In normal cats it was found in one neuronal pair). In 19 pairs (18\%) we observed the inhibitory direct connections mixed with other types of coordination, as typified in Fig. 1. In 8 cases the inhibitory trough was accompanied by excitatory shared input wave, in 3 pairs both the shared input and the excitatory direct connection were observed and in 8 pairs the shared input and the stimulus dependent effect were added to the inhibitory direct connection. The inhibitory direct connections occurred in the deprived cortex with significantly higher frequency than in normal animals \(P < 0.01\). (In normal cats inhibitory direct connections, including mixed types, were observed in 7.9\% of cases.) Figure 2 shows an example of long-lasting inhibitory effects. Narrow bin correlogram shows only a weak, broad excitatory wave. Inhibitory trough is visible on the wide bin correlogram. The trough is 30 ms wide and shifted by 30 ms from correlogram center. Shift predictors do not show any stimulus dependent effects. The widths of the inhibitory troughs varied between 1 and more than 100 ms, but it is possible that the relatively rare long-lasting effects should be analyzed separately, because in 15 pairs trough widths varied between 1 and 7 ms with a delay from correlogram center shorter than 3 ms, as it is shown in Fig. 1. Trough depth sometimes reached 80\% of the surrounding correlogram bins.
Fig. 2. Long lasting inhibitory interaction combined with weak excitatory component. Conventions the same as in Fig. 1. Neurons were recorded with separate wires. Both neurons were stimulated with moving slit of light.

Shared input

As in the normal cats, the most common type of correlogram in deprived animals was the medium width peak centered on the origin. In theoretical studies such peaks were explained in terms of either shared excitatory or shared inhibitory input from unobserved to observed neurons (15). Shared input was found in 35 pairs (34\%) including correlograms that suggested the presence of additional processes. The decrease in proportion of neuronal pairs showing shared input coordination in the deprived cortex was statistically highly significant (\( P < 0.001 \)). (In normal cats the shared input coordination was found in 60.8\% of cases). In 10 pairs the shared input was accompanied by excitatory functional interaction and in 19 pairs by inhibitory trough. Shared input peaks in visually deprived cats did not differ from the waveforms observed in normal cats. Figure 3 shows the most common forms of shared input crosscorrelograms. Pair A demonstrates a broad wave topped with a narrow peak; the peak is most likely of the same nature as the basal wave, because it is centrally located and matches the overall shape of the waveform. In B the centrally located peak is only 1.5 ms wide and is clearly distinguishable from the basal broad wave. Examples C and D show the broad waves without high peaks in the center. All the shift predictors are flat. This means that the presentation of stimuli made no contribution to the peaks shown in the left column. The widths of the shared input peaks in the deprived cats varied between 10 and 200 ms (mean = 24.6 ms). Peak heights were up to 6 times that of the background bins.

An unambiguous inference of the shared input can be made only if the correlogram peak straddles the origin (4). This type of correlogram
Fig. 3. Typical shared input waveforms demonstrated in driven activity of neuron pairs A, B, C and D. Each pair is characterized by four histograms in the row: 0.5 ms bin width crosscorrelogram, 5 ms bin width crosscorrelogram, 0.5 ms bin width shift predictor and 5 ms bin width shift predictor.
indicates also that the input signals come to both tested cells almost simultaneously. Units in a pair had a tendency to fire roughly at the same time but with no preferred order. Small differences in latencies, due to the conduction time differences are usually lost if the correlogram peaks are broad and flat topped. Peak position could not be detected with high accuracy in these cases. Large difference in latency indicate that one or both cells receive the input from a common source indirectly through one or more interneurons. In these cases the cross correlogram peaks are shifted from the center. The inference of connectivity is ambiguous here because different complicated arrangements of neuronal connections may lead to similar correlograms. In visually deprived cats off-center peaks were observed in 14 pairs (14%). In all these cases shifts were greater than 3 ms and often exceeded 10 ms. Proportion of broad, shifted peaks in the deprived cortex did not differ significantly from the result obtained on normal animals. (In normal cats 8.7% of such pairs were found).

**Stimulus dependent coordination**

Activities of all analyzed neuronal groups were recorded under different stimulus conditions. Spontaneous activity was usually too low to produce a sufficient number of spikes in a reasonable time. We recorded from the cortical representation of area centralis where the great overlap between receptive fields is well known. As most neurons recorded simultaneously in one penetration belonged to one column, they were very often simultaneously responsive. Such simultaneous modulation of both units firing rates may in itself produce the deflections on their crosscorrelograms. The stimulus dependent coordination was found in 25 pairs (24%). Only in four cases the shift predictor matched exactly the original crosscorrelogram, indicating pure stimulus dependent coordination with no additional mechanisms. In 13 pairs the shared input and stimulus dependent coordinations were observed. In eight pairs the stimulus dependent phenomena were accompanied by both the shared input wave and inhibitory trough. The difference between visually deprived and normal cortex was insignificant here. (In normal cats stimulus dependent coordination was found in 19.5% of pairs.)

**DISCUSSION**

Table I summarizes the results of our experiment on visually deprived cats and compares them to the normal animals data (13). The interneuronal coordination in the visual cortex of normally reared cats has been recently studied with the crosscorrelation technique by Toya-
ma et al. (19, 20) and Michalski et al. (13). These studies, although performed under different anesthesia and with different types of electrodes, yielded very similar results (for comparison see (13)). We compared the results obtained for visually deprived cats to the data of Michalski et al. (13), because the experimental conditions were the same; however, the comparison of our data with that of Toyama et al. (19) gives similar results. It should be stressed that the neuronal connectivity in visually deprived cats was studied within a column and compared with the same type of data from normal cats. Due to the way the electrode was constructed the neuronal pairs we studied belonged to one cortical layer or to adjacent cortical layers maximum distance between recorded neurons being 400 μm. Hence all the changes we describe are in short distance, vertically oriented connectivity.

We found that among the changes of cortical neuronal interactions due to prolonged, binocular pattern deprivation, the most pronounced is a decrease in the number of neuronal pairs driven simultaneously with practically no delay. This type of correlation indicates the existence of common excitatory or inhibitory input to both tested cells. Shared input type of coordination is the most common among visual cortical cells in normal cats (13, 19). Interactions of this type were very common in cortical layers III–V (19, 20) which receive most of the monosynaptic LGN input (6, 11). Considering that the visual deprivation reduced the number of shared input interactions by half, we can speculate that the effect was due either to a decrease in number of geniculocortical synapses, or to a decrease in their efficiency. Of course other common inputs (commissural, intracortical, from other ipsilateral cortices etc.) could give the same effect.

Reduction of synapses density per neuron was found in the visual cortex of deprived kittens by Cragg (3). This result was not confirmed by Wienfield (24) for a single adult cat he examined. These studies did not differentiate between afferent and intracortical synapses. It was

<table>
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<tr>
<th>Type of coordination</th>
<th>Percentage of recorded pairs</th>
<th>x² test</th>
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<tr>
<td></td>
<td>Deprived cats</td>
<td>Normal cats</td>
</tr>
<tr>
<td>Direct excitatory</td>
<td>13.0</td>
<td>20.3</td>
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<tr>
<td>Direct inhibitory</td>
<td>18.0</td>
<td>8.0</td>
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<tr>
<td>Shared input</td>
<td>34.0</td>
<td>60.8</td>
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<tr>
<td>Complicated interactions</td>
<td>14.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Stimulus dependent</td>
<td>24.0</td>
<td>19.5</td>
</tr>
<tr>
<td>Not correlated</td>
<td>10.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Number of recorded pairs</td>
<td>103</td>
<td>286</td>
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found in our laboratory that prolonged binocular pattern deprivation by rearing in masks produced, a considerable (60%) decrease in the number of afferent synapses in area 17 (21). Thus, the loss of afferent synapses can at least partly underlie the observed decrease of shared input type of interactions. The possibility of decreased efficiency of synaptic junctions cannot be excluded by these studies and should still be considered. The decrease of shared input interactions interpreted as a loss of geniculate input confirms the report of Singer and Tretter (18) about reduced safety factor in geniculocortical transmission in the deprived cortex. The decrease of instances of simultaneous excitation of cortical neurons by the main visual input source may be responsible for the decreased visual responsiveness and low response strength observed in pattern-deprived animals (14, 18, 23).

A lower number of centrally located shared input peaks in visually deprived animals (34% v.s. 60.8% in normal cats) and a higher number of off-center peaks (14% v.s. 8.7% in normal cats) indicate that if two cells in the visually deprived cortex share the same source of input signal, the connecting pathways are often indirect (with interneurons) and more complicated than in normal cats. On the other hand, the centrally located shared input peaks tend to be even narrower and higher in the visually deprived cats (mean width: 24.6 ms in deprived v.s. 43.9 ms in normal cats; height: 6 X background in deprived and 5 X in normal cats). Thus in the case of simple shared input connections in the visually deprived cortex, the synchronization tends to be even more precise than in normal animals. This may suggest that only the strongest connections are preserved in the deprived cortex.

The number of direct excitatory or inhibitory connections within the orientation columns (about 30%) was not altered by visual deprivation. However, the proportion of excitatory to inhibitory connections was changed. The percentage of direct excitatory interactions was lower in the deprived animals, but the difference did not reach the level of statistical significance. In the normally reared animals 5.6% of pairs revealed pure excitatory functional interaction, which was not found at all among the unit pairs in the deprived cortex. The proportion of inhibitory interactions was significantly higher in deprived cats (18% vs 8%). It should be stressed, however, that the statistics was done on a very small sample (19 and 15 pairs). The increase of inhibitory connectivity in the deprived area 17 would be very surprising, since it is often assumed that the losses of selectivity of deprived cortical neurons are due to a decrease of specific inhibition sharpening the receptive field features. The percentage of direct inhibitory interactions found with the crosscorrelation method was low also in normal animals (8% found by Michalski et al. (13) and 5% by Toyama et al. (19)).
This may, in part, be due to the shortcoming of the crosscorrelation method. Statistical methods, in general, hardly allow the separation of different sources of firing coordination if they are simultaneously active. The statistical signs of weak direct inhibitory connections could be just masked by massive positive waves of shared input coordination. For example, wherever there was evidence that strong direct interaction and shared input coordination were simultaneously present, the shared input almost always vastly predominated. Therefore the increase of inhibitory interactions in the deprived cortex may have been caused by a decrease of shared input type of interactions, which unmasked the existing inhibition. A low number of direct inhibitory connections, even in normal animals, may also indicate that most inhibitory interactions occurred in the inhibitory shared input circuits. Unfortunately the inhibitory shared input tends to synchronize the postsynaptic cells firing in a very similar way to the excitatory shared input, thus generating the positive rather than negative waves near the crosscorrelogram origin. In general, inhibitory shared input tends to produce the broader correlogram peaks than excitatory shared input (15, 17). Although it was not possible to distinguish between the inhibitory and excitatory shared input, it should be noted that the mean shared input peak width was lower in the visually deprived cortex. This may indicate that the inhibitory shared input interactions were more sensitive to visual deprivation than the excitatory shared input interactions.

It should be mentioned that the way the bundle electrode was constructed could have introduced a possibility of a bias for revealing modifications of connectivity between neurons which were situated close to one another (within a volume of cortex about 400 μm deep) or modifications related to afferents that end at the same level. With this kind of bias the shared input interactions would be the most apparent. However, the study of Toyama et al. (19) where variable distances between electrode tips were used yielded similar percentage values of different types of neuronal interactions to those we observed.

In 10% of neuronal pairs no interneuronal coordination was detected: this was a significantly higher proportion than in the normal group.

The proportion of neuronal pairs showing stimulus dependent type of coordination did not differ in the normal and deprived cortex. As in normal cats, the stimulus dependent coordination was in most cases accompanied by other types of neural interaction.

In summary, it was found that the deprivation of pattern vision changes the normal pattern of interneuronal connectivity within the orientation columns of the striate cortex. The total number of interactions between area 17 neurons was slightly reduced, but 90% of tested pairs still showed one or several different forms of coordination. The
number of shared input interactions was reduced by half. Direct connections were much less affected. If our interpretation is right, the results indicate that the visual deprivation was more damaging to the functioning of afferent connections than to the intracolumnar network within the cortex.

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