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Evidence on precise time-coded symbols and memory of patterns in monkey cortical neuronal spike trains

(brain/coding/triplets/redundancy)

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ABSTRACT **High-resolution examination of pulse sequences generated by single visual cortex cells of the rhesus monkey in response to precisely controlled visual stimuli has disclosed (*i*) that the outputs of such neurons contain highly improbable ($P < 10^{-7}$) numbers of identical triplets of precisely repeating pulse patterns; (*ii*) that the precision of such matches is better than 1/6000th of a second; (*iii*) that there is a similarly improbable high number of precisely matching pairs of triplets and anti-triplets, about half of which are present in symmetrical quadruplets of the form A-B-A, and that precisely replicating quadruplets and quintuplets are similarly generated in improbably large numbers; (*iv*) that identical triplets occur highly preferentially during immediately succeeding presentation of the same stimulus to the eye; and (*v*) that identical triplet (and doublet) patterns occur much more frequently in the responses of the same nerve when the eye receives identical or similar stimuli in different experiments than when dissimilar stimuli are applied. From these findings it is concluded (*i*) that the high precision of pattern replication required for triplets of pulses in time to serve to encode specific inputs and to permit their decoding through spatial summation is met (observations *i–iii*); (*ii*) that stimulus-specific triplets symbolize components of responses to specific stimuli (observation *iv*); (*iii*) that a temporary memory store of previous responses exists (observations *iv* and *v*); and (*iv*) that the mammalian brain uses precise patterns of discharges in time to represent and store specific data, rather than statistical qualities associated with pulse trains to symbolize qualitative stimulus components.**

A previous absence of evidence for precisely replicating patterns of pulses in time in the outputs of mammalian neurons in response to specific stimuli has led to the general assumption that specific information detection involves statistical handling of the numbers of events (an average frequency code) rather than a more precise, economical, and flexible coding system. Sophisticated statistical analyses of the output patterns of neurons (1–7) have failed to provide substantial insights into means neurons use to communicate specific rather than quantitative information to other neurons over substantial distances. The present work was undertaken to reexamine the possibility that nerves cause responses only in selected other nerves neither through specifically "hard-wired" exclusive lines nor through averaging of the effects of excitatory and inhibitory outputs, but rather through the generation of highly specific symbols (patterns of pulses in time), which are also able to elicit the specific response of cells that are able to decode them. It also should be noted that there is no reason to expect patterns to be present only in the form of immediately successive spikes. So, in these studies (in contrast to previously published analyses—except, see refs. 8 and 9) we considered the intervals between all possible pairs of spikes in a train of pulses (provided that they were separated by <100 msec in time) as possible elements in a time coded pattern.

For a specific pattern of pulses in time to be able to symbolize some quality in a form recognizable by a decoding cell, the copies of that pattern generated at different times in response to the same input must be extraordinarily precise. This precision is required if the pattern is to be distinguished in its effect (and "meaning") from other similar patterns. But more importantly, the symbolic coded pattern must be a precise replica of other copies of the same pattern because spatial summation, the most likely (and probably only) logical means to generate a specific response, must occur within 1/6000th of a second if efficient response (decoding) is to occur (10).

For this reason, in these studies we examined the outputs of single (complex) cells in the visual cortex (area 18) of the rhesus monkey (11) to determine whether multiple copies of pulse patterns that meet the required criteria are present. Not only did the results of these analyses provide evidence that extremely precisely identical copies of the same patterns are generated in response to specific stimulus presentations, but they also provided evidence that these responses differ from stimulus to stimulus and that there is a temporary memory of a previous pattern's generation.

MATERIALS AND METHODS

Source of Pulse Interval Data Files. Copies of 55 different intracellular records of neuron (in visual area 18 of a curarized monkey) responses to precisely presented lines (of various intensities, lengths, widths, orientations, and speeds of travel across the visual field) were obtained from Bruce Dow (in 1973, while he was at the National Institutes of Health). The recordings were copied from FM recorder records then existing and were transferred to a SANKYO stereo audio recorder (model STD-1800). On the left channel, a 10-kilocycle sine wave was recorded to provide calibration information in the event of significant distortion of the time base. The conditions of each experiment were also recorded on this channel prior to the records of the pulse trains resulting from these experiments. The pulse trains were recorded on the right channel. (An experiment is defined as the group of responses of visual cortex neurons of a curarized monkey to successive repetitions of identical stimuli in which the stimuli are lines of various intensities, lengths, widths, orientations, and speeds of travel across the visual field.)

To analyze the trains, the time intervals between each of the successive pulses making up a train were derived from these records. An 8080 microprocessor (using North Star basic and a Sol 20 computer) was used to effect these conversions as follows. The output derived from the audio record on the right channel was used to trigger the sweep of a Tektronix (model 5115) memory cathode ray oscilloscope each time a pulse emerged from the recording. The triggering

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level of the cathode ray oscilloscope was adjusted to ≈50% of the distance from the top of the high frequency low amplitude noise level in a given record and the maximum height of the pulse derived from the same record. The event that signaled the detection of a neuronal discharge was the presence of an output pulse at the z-axis output terminal of the cathode ray oscilloscope. This signal was presented to the parallel input port of the computer, which polled this port at a high frequency (40 kilocycles). At the beginning of each transfer cycle, the depression of a start key initiated an object code program that polled the parallel port and incremented a counter of its own cycles by 1 unit every 1/40,000th sec. When a signal occurred at the input port, the number of cycles transpiring between the previous and the present input was transferred to a portion of memory assigned to an array for storing these values, and then the counter was returned to zero before cycling was resumed. At the end of the analysis of each experiment, the data stored in the array were transferred to a disc file by the proper routines in the program.

ANALYSIS OF DATA

Low-Resolution Two-Dimensional Matrix Histogram Display. After conversion of stored clock intervals to seconds, the series of elapsed times between events were examined (using a computer program) with respect to the presence of specific triplets of intervals (consisting of pairs of sequential interspike intervals and/or the sums of multiple successive intervals in a record). Thereby, a two-dimensional array denoting the first of a pair of intervals and the associated second interval was filled with these pairs of intervals. The plot of these data as points in a graph (100 × 100 msec) gave results like those depicted in Fig. 1. It will be noted that there are several clusters of redundant patterns.

High-Resolution Matrix Display. The data derived from each of 21 such experiments were then examined to determine the precision with which redundant patterns lying near the center of a cluster were replicated. To this end, we selected 25 areas (2 × 2 msec each) centered on local maxima of the 21 two-dimensional histograms (away from the origin, where the accumulation is related to the sharp maxima of the simple interspike distributions). [The 25 areas (cells) selected for further examination were clearly visible as the centers of local maxima in the 21 two-dimensional histograms but did not include the less obvious of such maxima. In the examined cells, the total number of triplet patterns found was 154 (an average of ≈6 per cell).] A typical high-resolution plot of such an area is also shown in Fig. 1 (*Inset*). Most of the high-resolution plots display a striking distribution of the points similar to this one, with very close associations between points, like twin stars. To depict this phenomenon clearly and quantitatively, we plotted (Fig. 2) the distribution of the distances between each triplet and its nearest neighbor in the same plot for all of these 25 selected areas. For the sake of comparison, we ran a Monte Carlo simulation (50,000 trials in total) to determine the distribution to be expected if the same number of points in each plot were scattered at random. The result of this simulation is shown as a solid line in Fig. 2.

Statistical Analysis of the Significance of Triplet Clustering. To determine how much the frequency of occurrence of pairs of triplets that come very close to each other in the high-resolution plots described above is greater than would be expected on a random basis within such clusters of redundant patterns, we computed the probability of occurrence of the

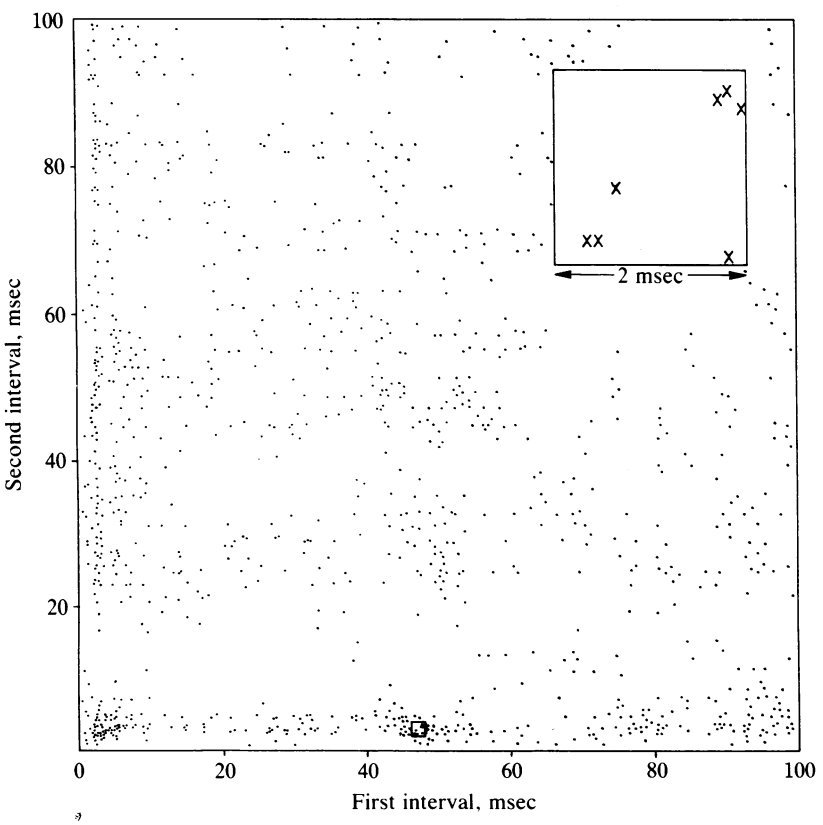

FIG. 1. Two-dimensional representation of all possible combinations of some first intervals and an immediately succeeding one. The distance along the x axis defines the first interval (using sums of interspike intervals, as necessary), and the y axis defines a single or summed intervals immediately following the x interval. No interval longer than 100 msec was considered in deriving the x and y values. (*Inset*) High-resolution shows the results obtained if a 2 msec × 2 msec cell (small square, lower center) is expanded by calculating the precise interval correspondences involved.

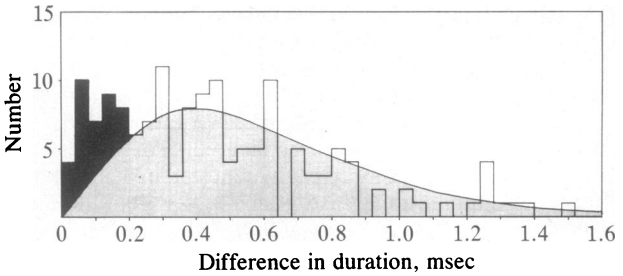

FIG. 2. Differences in duration of closely similar triplets present in the output of a neuron in response to highly specific stimuli—where the difference in duration is defined as the square root of the sum of the squares of the x and y differences in matches between duplicate pairs of time intervals present in the output of a neuron in response to highly specific stimuli. Area under curve describes the differences in duration to be expected on a random basis; heavily shaded area in the histogram shows the excess of very precisely replicating triplets over what is to be expected on a random basis. The significance of this figure is that the matches in duration times are most improbably derived from purely random combinations of intervals, but rather indicate that some mechanism generates these precise patterns, patterns suited to serve as coding symbols.

closest association in each plot, given the number of points entering it, and assuming a flat (random) distribution of x and y coordinate values within the 2 msec × 2 msec "cells." The probability that the observed distribution of the distances in the 25 plots analyzed would result from a random distribution of intervals is only 3×10^{-8}. (In this application of Baye's theorem, the *a priori* probability for randomness of the distribution of coordinates within a given 2 msec × 2 msec cell was set to 1 and the *a priori* probability of realizing each plot was set to 1/2.) (The simple χ^2 test is inappropriate to the calculation of difference significance of the histogram in Fig. 2 because sometimes two points in a given "cell" have the same closest neighbor—for instance, when the pairs of points in question have the absolute minimum distance possible in the plot.)

Estimate of the Number of Replicating Triplets To Be Expected as a Result of Juxtaposition at Random of Existing Replicating Doublets. (A replicating pattern is defined as one in which the duplicate intervals involved do not differ from each other by more than 0.14 msec.) If interspike intervals, whether simple or summed, repeat themselves with high precision it appeared possible that random juxtaposition of such intervals could account for replicating triplets as well. At the outset of these studies, therefore, we computed for each of the 21 experiments the expected number of replicating triplets that should result from such random juxtaposition of the observed replicating doublets (if scattered at random through the course of an experiment). It was found that the expected number of replicating triplets in the 21 experiments is 236.9, compared with the 333 observed triplets. Such an excess of observed triplets could only be produced by chance once in 2.5 billion times, assuming they are produced independently of each other. We are therefore compelled to conclude that the occurrence of these complex replicating patterns must have another origin. The same conclusion also holds for the occurrence of replicating quadruplets (63 times) and quintuplets (15 times), the number of which is also incompatible with their formation through random addition of doublets to triplets or quadruplets.

Detection of Anti-Triplets That Match Triplets. The same records contain 545 instances in which the occurrence of a triplet is accompanied by a pattern in which the time intervals in the triplet are precisely matched but the order is inverted. Of these triplet–anti-triplet pairs, 256 are associated with each other as symmetrical quadruplets of the type A-B-A, while the remaining 289 triplets and anti-triplets are time-shifted with respect to each other and have the forms A-C and C-A. The expected number of such shifted triplet–anti-triplet pairs should, on a random basis, have been very similar in number to the expected number of duplicate triplets in the records (236.9) if they had resulted from random association of replicating doublets. They are, in fact, much higher than expected, as shown.

DISCUSSION AND CONCLUSIONS

A key class of events necessary if a device such as a mammalian brain is to carry out the processing of information presented to it is the selective excitation of only certain nerves in response to the outputs of other nerves. The coding of such outputs provides a means through which great selectivity may be exercised as to which nerve(s) is to respond and which is not, provided that the code is itself precise (12).

Average frequency coding mechanisms and their variants (13) are well-suited to generate graded quantitative responses by the receivers of such information (e.g., contracting muscles), but they are ill-suited to transmit representations of qualitative properties efficiently essentially because of statistical overlaps between adjacent average frequency bins (channels). Even using very large numbers of events, the number of channels possible is very limited and these channels lack versatility and require the integration of many events if even moderate numbers of channels are to be used. The alternative to an average frequency code is one based on specific patterns of events in time—i.e., a coding system in which some highly precise and reproducible pattern structure is used when a given coded signal is to be transmitted (12). Such precision will permit the system to make use of spatial summation to evoke specific responses by specific neurons—because, if a series of excitatory pulses constituting a symbol arrive simultaneously (within ≈1/10,000th sec) via separate pathways at a receiving neuron, that neuron will respond, provided, of course, that the summated effects of the multiple pulses in the pattern exceed the receiving cell's discharge threshold.

The significance of the discovery here reported that the precision with which replicated copies of the same pattern match each other is 1/6000th to 1/20,000th sec is that nerve cells are indeed able to generate the same patterns with that remarkably high precision required. This finding is predicted by a temporal code for qualitative features that consists of at least two coordinated time intervals (a triplet of pulses) as previously proposed by one of us (B.L.S.). However, if information in triplet-coded form is to be used by neuron-based systems (i.e., is specifically detected at certain locations but not at others), then there must also be a very precise match between the delays needed for decoding and the delays between successive pulses that make up a pattern. This match (10) must be better than 0.15 msec (in differences in times of arrival of the separate pulses making up a pattern at separate synapses on the same cell). We found experimentally that the cut-off point that maximized the ratio of observed to expected numbers of nearly identical triplets was 0.14 msec difference in duration of both the first and second intervals, and it is this cut-off for differences in durations of intervals that has been uniformly applied for detection of replicating patterns in the present analysis.

It is important to realize that if the redundant copies of the same pattern are codes for the same property or quality (symbols derived from the input sources), then the different redundant copies must each match the decoder with this precision and therefore must also match each other with the same precision (<0.15 msec). That a disproportionately high fraction of similar triplets replicate each other with striking precision is shown in Fig. 2. In fact, for triplets, the frequency

of time differences below 0.2 msec is maximal at ≈0.07 msec. Thereby, a second prediction of the theory (that useful redundant triplets must match each other with the above precision if they are to be decoded by the same delay-line network) is also confirmed.

The third *a priori* prediction (12) is that the detection of a pattern through spatial summation should result in the output of both the detected triplet and its complementary anti-triplet (as a symmetrical quadruplet of the form A-B-A) under certain conditions—specifically, if the threshold of the post-synaptic neuron is reduced from a requirement for three simultaneous inputs from different synapses to a requirement for only one such input—in the period following the detection of a pattern and the cell's response to it. (When the match between the pulses in a triplet and a set of three synapses occurs, a pulse is generated, a second is generated when the middle pulse passes over the last synapse, and the two other pulses are generated when the last pulse crosses the last two synapses.) Alternatively, such a quadruplet could also arise if a pair of pulses separated by interval B were to traverse an axon that made contact with dendrites of a given nerve successively, provided that the time interval between such contacts is A, and that the threshold has been reduced to 1 (see Fig. 3 for description of these alternative mechanisms). We have found 258 patterns of this kind, but from the present data we are unable to determine which mechanism leads to their production.

If a temporary facilitation of the readout of specific triplet patterns were to occur consequent to the decoding of a time-inverted complement of it (its anti-triplet), this would also provide evidence for the existence of a short-term memory. To decide whether the responses observed reflect such short-term memories of the receipt of triplet codes, we have determined whether precisely replicating triplet patterns occur randomly in time throughout the course of presentation of sequential repetitions of the same stimuli or whether they preferentially occur in close time proximity to each other. It was found, as shown in Fig. 4, that there is a very strong tendency for identical triplets to occur close to each other in time. In these experiments, the image of a slit of light moved across the retina first in one and then in the opposite direction during each successive cycle of stimulation (11, 14). That is, every other burst of cell activity represents the same direction of movement of the slit. That there is very little similarity in the response of the cells to movement in opposite directions is dramatically shown by the 50-fold decrease in occurrence of identical triplets in consecutive responses. [Measurement of the number of stimuli (phrases) that occurred between copies of specific triplets disclosed (*i*) that the greatest number (≈50%) are generated during the presentation of a single stimulus; (*ii*) that an interposed, but different, stimulus causes essentially no copies (only 3 of 333) of triplets generated by the previous (but different) stimulus to occur, and (*iii*) that identical stimuli, separated in time, generate copies of the same triplet patterns (see Fig. 4). From these findings, it follows that a single stimulus of a given kind is able to cause more than one copy of a precisely duplicated triplet to be generated and that the triplets produced in response to different stimuli differ from each other. For statistics on data presented here, see Table 1 and its legend.]

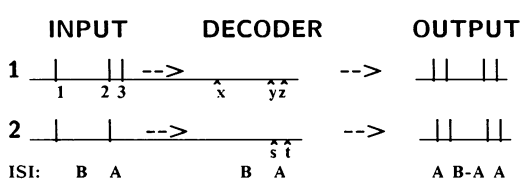

FIG. 3. Alternative means for production of symmetrical quadruplets. See text for details.

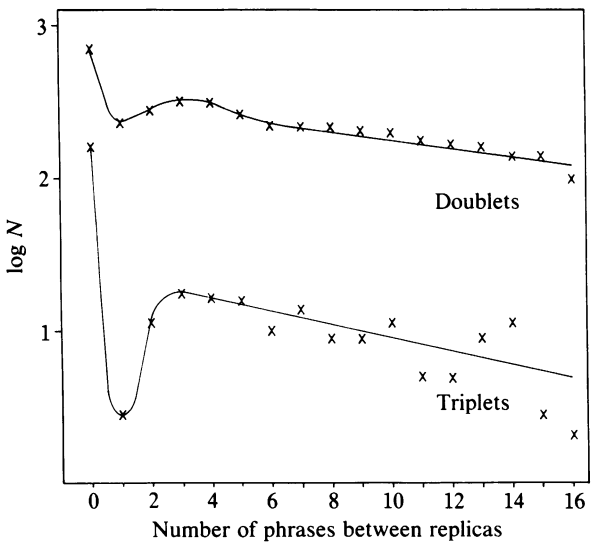

FIG. 4. Memory duration of specific triplet and doublet symbols and stimulus specificity of interval patterns. There is a high probability that identical copies of a given pattern of intervals will appear during a single presentation of a stimulus (see point 0). The extreme depression of matches between patterns resulting from consecutive stimuli evidently occurs because the movement of a slit in the opposite direction from that which elicited the generation of a pattern is very unlikely to generate the same pattern. When two phrases (defined as successive trains of pulses separated by an inactive time >100 msec) are separated by two such inactive periods (e.g., 0 and 2 on the graph), there is a high probability of generating the same pattern, although lower than the probability of generating that pattern during a given phrase. There may be a decline in probability of identical pattern outputs over greater periods between successive stimuli, but this may result at least partly from the fact that there are fewer opportunities for comparisons of phrase similarities as the times separating compared phrases become larger.

These last facts lead to two important conclusions: First, that the ability to produce identical triplets lasts only a brief time—one or a few stimulation cycles; second, that different kinds of stimuli do not cause the same response and cause the output of triplets characteristic of a different stimulus. In other words, the temporary memory of a response is not caused to be read out by a different stimulus than that which caused the initial response. Further evidence for stimulus specificity of precisely replicating triplets is provided by comparing the similarity of triplets generated during separate experiments (in which the stimuli were identical) with the similarities of triplets obtained when the stimuli were different. It was found that the number of identical matches is a direct function of stimulus similarity. We generally found 2–3 times more additional duplicating triplets in different experiments with identical or very similar experimental conditions (stimuli) than we found in analyses in which responses to dissimilar stimuli were compared. Details of these comparisons will be presented elsewhere.

To our knowledge, the earliest suggestion that specific time intervals may provide coded representations of information was by R. Lorente de No (15), a proposal based on his studies of the anatomical connectivities within the brain. This and later proposals along this line (8) did not result in validation or disproof, although certain publications strongly suggested that triplet patterns are specific information carriers in the pigeon optic nerve (color coding). Triplets were first suggested as efficient means for coding and decoding neural messages in 1969 (12).

Table 1. Statistics on specific pattern occurrences

Event	Number
Experiments	21
Spikes	2721
Intervals	5316
Precisely replicating doublets	4721
Triplets	
Expected	236.9
Found	333
Shifted anti-triplets	289
Symmetrical quadruplets	256

The number of events of different kinds produced by the presentation of different kinds of stimuli to the same system is shown and includes results obtained with two separate neurons. The probability of obtaining such a difference between the expected and observed number of precisely replicating triplets is 4×10^{-10}. The probability of obtaining the difference observed between the expected number and the number of precisely replicating triplets found is 4×10^{-10}. The method of calculation of expected pairs of triplets used is based on the following procedure: Before the analysis of an experiment is carried out, the total number of summed interspike intervals (<100 msec total duration) is derived from the record. Then the number of matching pairs of doublets contained in this list of intervals is calculated. The latter divided by the former gives the probability, P, that any given interval is matched by another interval. Following this, each record is examined sequentially and the location of any interval that is duplicated later in the record of an experiment and the second copy is identified. The sequence of intervals following each such pair is then studied and a series of summated intervals (e.g., if an identified pair of intervals A and A′ are followed, respectively, by intervals B, C, D . . . and B′, C′, D′ . . . then the sets of intervals following A and A′ are B, B + C, B + C + D, . . . and B′, B′ + C′ . . .). If the number of intervals (<100 msec) in the first case is N1 and N2 in the second, then the number of combinations of possible matches is N1 × N2. This product multiplied by P (see above) gives the expected number of precise interval matches following any given matching interval. Repetition of this procedure for all detected matching pairs and summating the results in an experiment gives the total expected in that experiment. The sums of numbers expected from all experiments were obtained and were compared with the observed number using a χ^2 test.

The findings reported here provide strong confirmation that (*i*) nerve cells generate the precisely replicating triplet patterns needed for qualitative information transmission by using a highly specific time-based code; (*ii*) these patterns are almost certainly decoded by other nerve cells and cause the generation of corresponding patterns as outputs; (*iii*) a temporary memory exists of the occurrence of a given pattern; and (*iv*) the nature of the triplets produced is stimulus correlated and probably stimulus specific. The brain therefore functions primarily, during the processing of qualitative information, not as a statistical machine, but as a deterministic one and evidently is able to store representations of the time intervals that make up a meaningful symbol. The duration of these symbolic representations would be expected to be greater in those parts of the cortex in which permanent memories are stored than it is in the portions, such as that studied here, which are involved in early stages of the processing of sensory inputs.

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