it feeds and then is transferred through pupa to adult (*11*). Similarly, senecio alkaloids are incorporated by moths from food plants of the genus *Senecio* (*12*), and cardenolides are accumulated in grasshoppers and butterflies from milkweed plants (*13*). In *Romalea*, sequestration may account for more than just the 2,5-dichlorophenol in the froth. The other components all occur as such or in similar form in many species of plants (*14*), and the grasshopper may simply incorporate them with slight or no change from the diet. But 2,5-dichlorophenol would still be unique because of its apparent ultimate derivation from an exogenous source recently unleashed upon the ecosystem by man himself.

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1. Pieces of fresh integument (25 mm²) of *Romalea*, treated with droplets (1 to 2 μl) of froth, were ignored by ants (*Paratrechina longicornis*) when placed in their foraging trail. Untreated controls were carried away. Tethered *Romalea* exposed on soil to foraging ants (*Pogonomyrmex badius*) discharged froth (and regurgitated crop fluid) when attacked, thereby repelling their assailants. After depletion of froth (and crop fluid) the grasshoppers were vulnerable.
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Aplysia californica: Analysis of Nuclear DNA in Individual Nuclei of Giant Neurons

Abstract. *The nuclei of the giant neurons of the marine mollusk* Aplysia californica *can contain more than 0.2 microgram of DNA. This is more than 200,000 times as much DNA as the haploid amount found in* Aplysia *sperm. On the basis of nuclear DNA content, the giant neurons R-2, P-1, and L-6 of adult animals can each be divided into at least two populations. The mean DNA content of these two populations (0.067 and 0.131 microgram of DNA) are approximately related by a factor of 2. This suggests that much and perhaps all of the genome replicates repeatedly (up to 16 times) during the growth and development of these neurons and that each replication is synchronous. The enormous amount of DNA in these cells opens up the possibility of characterizing the DNA and other constituents of chromatin from individual but phenotypically different neurons.*

The giant neurons of *Aplysia* represent one of the largest somatic cell types in the animal kingdom. These neurons, which range in size up to 1 mm in diameter, each contain a single nucleus which comprises approximately 30 percent of the volume of the cell body. The large size of the nucleus and the staining characteristics of the nucleoplasm with the Feulgen stain led Coggeshall (*1*) to suggest that the giant cell nuclei contain considerably more DNA than the diploid amount for *Aplysia*. This possibility was supported by an unpublished observation of Strumwasser (*2*) that the largest neurons contain 50,000 times as much DNA as mammalian cells. We have quantified the nuclear DNA from individual giant neurons and have found that the amount of DNA in *Aplysia* giant neurons is many thousands of times greater than the haploid value for these animals.

One of the remarkable features of the *Aplysia* nervous system is that individual neurons can be easily recognized in the abdominal ganglion; over 30 of these cells have been characterized electrophysiologically and morphologically (*3*). We chose to study cells R-2 and L-6 of the abdominal ganglion [nomenclature of Frazier *et al.* (*3*)] and the single giant cell of the left pleural ganglion, which we have abbreviated P-1. Cells R-2 and P-1 have been called the colossal cells and are the largest in the *Aplysia* nervous system; L-6 is somewhat smaller.

The giant cells are surrounded by thousands of glial satellite cells and contain a large complement of mitochondria. Therefore, in order to quantify the nuclear DNA of the giant neurons, it seemed necessary at the outset of these experiments to isolate the nuclei from the giant cells and rule out any contamination from glial or mitochondrial DNA. This precaution was justified in light of the finding that the glial cells and neuronal cytoplasm contribute 33 percent of the DNA to the intact giant cell (*4*).

An abbreviated description of the procedure for removing the giant cell nucleus from the cell body follows. A more complete description will be published separately (*4*). The abdominal and left pleural ganglia were removed from the animal and immersed in Millipore-filtered seawater (*p*H 7.4). The ganglia were incised under observation with the dissecting microscope, and selected cells were removed by severing the single axonal process. The neurons were transferred to a depression slide containing Millipore-filtered seawater. A small hole was made in the giant cell membrane, and the nucleus was extruded from the hole by gentle squeezing of the cell. The nuclei of the largest neurons are polymorphic and take on many odd shapes. However, when the nuclei are extruded into sea-

water, the nucleus swells and tends to become rounded. It was important to ensure that the contents of the nucleus were not lost during the isolation procedure. Damaged nuclei were characterized by either a clear hole in the nuclear membrane, or the presence of gel-like nucleoplasm outside the nuclear membrane (or both). Even slight damage to the nucleus resulted in the presence of nucleoplasm outside of the nuclear envelope. The nucleoplasm is extremely sticky and adheres to the forceps or glass slide. The nucleus was considered to be undamaged if no clear holes were observed in the nuclear envelope and no nucleoplasm was present outside the envelope. A sample of several nuclei that were judged to be undamaged was inspected by electron microscopy. These nuclei retained intact inner and outer nuclear membranes, and very little cytoplasm adhered to the outer nuclear membrane (*4*).

Undamaged nuclei were transferred from the depression slide to a micro test tube containing glass-distilled water (*p*H 6.2 to 6.6). All but 5 ml of water was removed from the tube, and the nucleus was stored frozen. We assayed DNA by the fluorometric method of Kissane and Robins, in which diaminobenzoic acid is used (*5*). Free nucleotides and lipids which interfere with the analysis were removed by a microextraction procedure (*6*). Salmon sperm DNA was used as a reference standard, and standards covering the sample range were run with each set of samples.

Analysis of more than 140 nuclei from cells R-2, L-6, and P-1 (of animals weighing 17 to 600 g) indicated that the amount of DNA per nucleus was 0.02 to 0.27 μg. When the data was plotted in the form of a frequency histogram two major populations of cells were found with respect to nuclear DNA content (Fig. 1). Comparable histograms were produced when the data for cells R-2 or P-1 were plotted independently. The mean DNA contents of these two populations are 0.067 and 0.131 μg of DNA. If the DNA content of *Aplysia californica* sperm (1.0×10^{-6} μg) is taken as the haploid amount (*4*), then the two classes of giant neurons contain, respectively, 67,000 and 131,000 times as much DNA as the haploid value. In contrast, the polytene giant cells of the salivary glands of *Chironomus tentans* (order Diptera) contain up to 0.336×10^{-2} μg of DNA per nucleus or 16,400 times the haploid level for the species

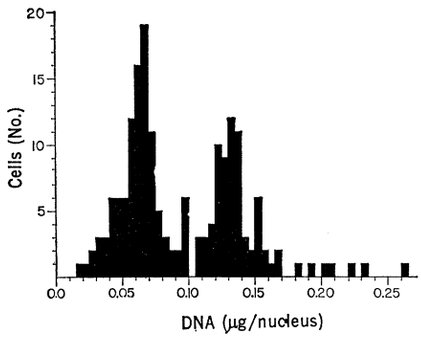

Fig. 1. The DNA content of individual nuclei from cells R-2, L-6, and P-1 is plotted in the form of a frequency histogram at intervals of 0.005 μg of DNA. Nuclei were extruded from giant neurons, and DNA was analyzed by fluorometry. The cells fall into at least two classes with respect to DNA content; means of the two groups are 0.067 and 0.131 μg of DNA.

(*7*). Further increases in the sizes of chromosomes of some of the giant cells of Diptera have been associated with infection of these cells by protozoa or virus (*8*). The increase in DNA content of these infected cells can be greater than eight times the normal level. It should be noted that the giant neurons of *Aplysia* differ from the polyploid cells of the Diptera in which the chromosomes are polytene. Our observations on sectioned giant neurons, nuclear squashes, and whole-mounted nuclei indicate that the chromosomes of *Aplysia* giant neurons are not polytene. The nuclear contents of the giant neurons resemble those of diploid cells during interphase (*1*).

The following data indicate that the values obtained for DNA by the fluorometric method reflect the true DNA content of these cells: (i) previous incubation of the nuclei with deoxyribonuclease reduced the fluorescent values to nearly background levels, whereas ribonuclease had no effect; (ii) analysis of DNA in nuclei by acid extraction and ultraviolet absorption resulted in values comparable to those reported here (*4*); (iii) measurements of DNA on nuclear lysates by CsCl density gradient centrifugation were also comparable (*9*); and (iv) the results of analysis by quantitative microspectrophotometry on sectioned ganglia are on the same order of magnitude (*10*).

The increased amount of nuclear DNA in neurons is not a phenomenon peculiar to *Aplysia*. Some of the large neurons of the mammalian nervous system (for example, anterior horn cells, cerebellar Purkinje cells, sympathetic ganglion cells, and Betz cells of cerebral cortex) are tetraploid or even octoploid (*11, 12*); and neurons in the cerebral ganglia of *Drosophila virilis* contain as much as 16 times the haploid amount of DNA (*13*). Although more information will have to be obtained on other species, it may be a general phenomenon that the largest neurons of the nervous system are associated with increases in nuclear DNA (*12*). In fact, a plausible explanation for the enormous amount of DNA found in the giant neurons of *Aplysia* is that the giant neurons produce extra copies of the genome in order to achieve their tremendous size. That is, an increase in cell volume beyond a certain point is accompanied by a concomitant increase in the number of available transcription sites in the nucleus. We have evidence that the DNA content of the *Aplysia* giant neuron bears some relationship to the size of the animal and to the size of the neuron (*4*).

Several possible mechanisms can be suggested by which transcription sites might be increased: (i) replication of the entire genome by repeated doubling as apparently occurs in the polyploid cells of the salivary glands of flies (*7, 13*), (ii) replication of the genome from a master copy or copies, and (iii) amplification of a select portion of the genome which has specific value to the neuron (*14*). The first of these possibilities is supported by the fact that the two classes of cells which have been identified are related by a multiple of 2 (0.067 and 0.131 μg of DNA per nucleus). Furthermore, if the diploid value for *Aplysia* is taken as 2.0×10^{-6} μg of DNA on the basis of twice the DNA content of a haploid *Aplysia* sperm (*4*), then 16 doublings of the diploid amount results in a value of 0.131 μg of DNA. This value is comparable to that found for the highest class of giant neurons. The data of Coggeshall (*10*) also indicate that the amount of DNA in the giant neurons increases incrementally by multiples of approximately 2. Although this circumstantial evidence supports the polyploid thesis as an explanation for the large amount of DNA found in the giant cells, this question can only be settled by characterization of the DNA of the giant neurons.

The enormous quantity of DNA which is present in the giant neurons of *Aplysia* further enhances the value of this already useful experimental system to the neurobiologist. It is notable that the nervous system of *Aplysia* contains more than 30 giant neurons which

have been identified and characterized. Apparently, each of these neurons is phenotypically unique. Therefore, it may be possible to compare the molecular composition of the chromosomes of neurons that are developmentally closely related but which have differentiated into functionally unique individuals.

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Rhesus Monkey Vestibular Cortex: A Bimodal Primary Projection Field

Abstract. *Single units in the rhesus monkey* (Macaca mulata) *cortex responded to both vestibular and proprioceptive somatosensory stimuli. This bimodal response characteristic is unlike the modality specificity noted for other primary sensory fields. The vestibular field is located, contrary to previous opinion, within a distinct cytoarchitectonic area outside of area 2.*

A limited projection for the vestibular nerve has been found on the cerebral cortex of the cat (*1, 2*) and the rhesus monkey (*Macaca mulata*) (*3*) with the use of the evoked potential technique while the animals were under deep barbiturate anesthesia. This implies the existence of a primary cortical vestibular field comparable to the primary fields of other afferent systems (somatosensory, auditory, and visual).

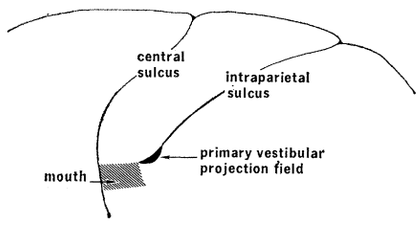

Fig. 1. Rhesus cortical units that responded to vestibular stimulation were located in the black region. Vestibular units were recorded from the depth of the intraparietal sulcus as well. Immediately rostral to this is the primary projection field of the mouth in S1 area 2.

Single units within primary sensory fields have heretofore been found to be strictly modality specific, that is, influenced only by the modality that evoked a slow cortical potential under deep barbiturate anesthesia. This also holds true for all specific relay nuclei. The vestibular system does not appear to fit this scheme of sensory modality specificity, for in the primary relay station, the vestibular nuclei, 80 percent of the units are also influenced by kinesthetic afferents (*4*). Central convergence of these two modalities of "proprioceptive" afferents is apparently essential not only for lower reflex mechanisms but also for the conscious perception of position and movement (*5*). It would be reasonable, therefore, also to expect the primary vestibular cortical field to be an exception to the rule of modality specificity.

The modality specificity of single units within the primary cortical vestibular field in the rhesus monkey (*3*) was qualitatively tested. Single units in the cortex at the lower lip of the distal end of the intraparietal sulcus (Fig. 1) were monitored extracellularly with glass micropipettes (3 to 10 megohms, filled with 2*M* potassium citrate) by the closed-chamber technique in awake monkeys (local anesthesia and Flaxedil). Sensory stimuli included (i) vestibular: bipolar d-c labyrinthine stimulation (0.2 to 1.5 ma) delivered through silver–silver chloride electrodes (round window against bone near posterior semicircular canal); (ii) somatosensory:

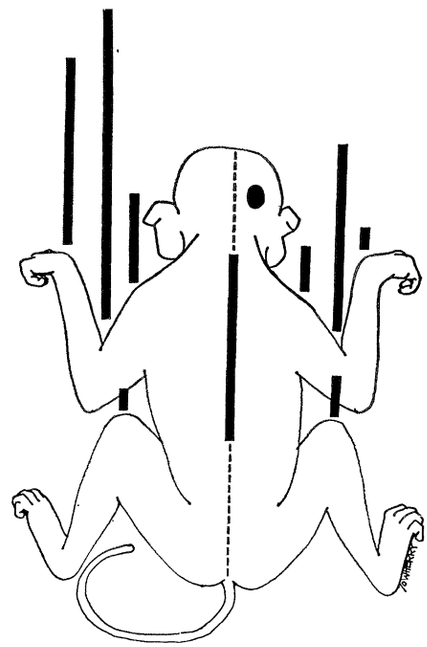

Fig. 2. Joints specifically influencing vestibular units within the primary cortical vestibular projection field. Bar length symbolizes the relative frequency with which the respective joints influenced neuronal activity. The black dot indicates the side of the parietal cortical recording site.

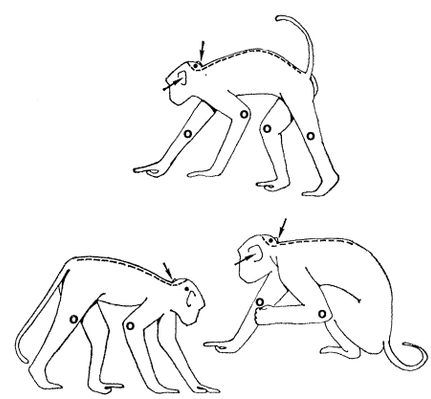

Fig. 3. Three examples of coordinated kinesthetic afferent patterns influencing one cortical unit. Open circles, arrows, and black dots represent, respectively, the effective joint, the labyrinth stimulated, and the cortical recording site. The illustrated joint position produced unit activation, whereas the reciprocal position caused inhibition.