sections of tissue incubated for 30 minutes in a buffer where sucrose replaces isosmotically the sodium present in the usual Ling-Ringer phosphate buffer (*10*). Under these experimental conditions, tissue cyclic AMP concentrations are increased fivefold over control values (*11*). As shown in Fig. 2, there is a striking increase in the immunofluorescence seen in the tissue on deletion of sodium from the buffer. The increase in staining, however, is most prominent over muscle, although the epithelial cells also stain more intensely.

The results reported here demonstrate that the cyclic AMP content of all epithelial cells, both granular and MR, increases in intact toad bladder after AVP addition, and that both the biochemical and the physiological effects of AVP are manifest not only in MR but also in the more prevalent granular cells. This result contrasts sharply with the results obtained with separated MR and granular cells (*6*). If the latter results are valid—that is, if the hormone responsiveness of granular cells has not been destroyed during separation of granular and MR cells—then the present results raise the possibility that there may be a rapid and specific transfer of cyclic AMP from MR to granular cells. The transfer would not appear to be extracellular as there is no evidence for leakage of immunofluorescent staining material out of the epithelial cells. The repeated rosette arrangement of four or five granular cells surrounding a central MR cell (*3*) could thus provide the proper anatomical relation for intercellular chemical transfer. While as yet there is no direct evidence for such intercellular communication, the work reported here, as well as previous work by Loewenstein *et al.* (*12*) demonstrating low-resistance electrical pathways between toad bladder epithelial cells, raises this possibility.

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21 October 1974; revised 10 December 1974

Neural Plasticity in Visual Cortex of Adult Cats after Exposure to Visual Patterns

Abstract. *Over a period of 2 weeks, adult cats were twice a day exposed for 1 hour to a visual environment consisting only of vertical stripes and for the rest of the time were kept in darkness. Subsequent investigation of the striate cortex showed a decrease in the number of neurons sensitive to orientations around the vertical relative to those sensitive to horizontal orientations. This indicates that plasticity of functional properties of the cortical neuronal network still exists in adult animals.*

Most of the neurons of the visual cortex of all mammals so far investigated respond best or exclusively if moving contrasts, especially light or dark lines, are used as stimuli. An individual neuron responds optimally only to a specific orientation of the line and a specific direction of movement, the latter being usually normal or nearly normal to the optimal orientation. Most neurons have an orientation "tuning width" of 10° to 30° around the optimal orientation and do not respond at all to stimuli oriented 30° to 90° away from their optimal stimulus orientations (*1*). In adult animals, at least, all orientations are represented equally frequently within the whole neuronal population (*1, 2*). If young kittens are raised, during the critical period from 3 to 14 weeks of age, in a visual environment consisting only of stripes of one orientation, most neurons will be maximally sensitive to orientations within about ±30° of the one to which they were exposed (*3*). A similar effect has not yet been reported in adult animals.

We have done experiments on seven adult cats (more than 1 year old, weight 2.2 to 3.4 kg). The animals were kept for 14 days in a completely dark cage. Twice a day they were put into a cylinder (2 m high, 60 cm in diameter) for 1 hour. The inner wall of the cylinder was covered with vertical stripes of variable widths between 0.5° and 7.5°, measured from the center of the cylinder. The cat was sitting in the middle of the cylinder on a horizontal partition made of clear plexiglass so that the vertical stripes were continuous in the upper and lower visual field. The cylinder was brightly lit from above through a diffusing plexiglass lid, resulting in a brightness of 17 cd/m^2 of the white stripes and 1.4 cd/m^2 of the dark stripes. The animals were observed from time to time through a small hole in the lid.

Four to 12 hours after the last exposure to this visual environment, the animal was anesthetized in the dark with Brevimythal (25 mg/kg) intraperitoneally, and its eyes were carefully covered. The further preparation followed the standard procedure of this laboratory (tracheotomy and artificial respiration, trepanation of the skull over the visual cortex and fixation of a closed chamber, widening of the pupils with atropine, and retraction of the nictitating membrane with Neo-Synephrine). The refraction, which was determined with an ophthalmoscope, was corrected with contact lenses. Except during receptive field testing, the eyes were covered with a black mask. Stimuli were projected onto a screen 1 m in front of the eyes. After muscle relaxation with 2 ml of Flaxedil, an infusion containing 1.0 ml of Flaxedil, 0.3 ml of glucose solution, and 1.7 ml of Ringer solution was continued throughout the experiment (3 ml/hour). The electroencephalogram, electrocardiogram, CO_2 level, and temperature were continuously monitored. All animals were kept under N_2O respiration (70 percent N_2O, 30 percent O_2) throughout the experiment. Recording from single units was done with glass microelectrodes (filled with 1*M* NaCl). Area 17 was penetrated near the midline so that recordings from many "columns" were possible during one penetration. When a unit was found, one eye was uncovered and the receptive field and trigger feature were

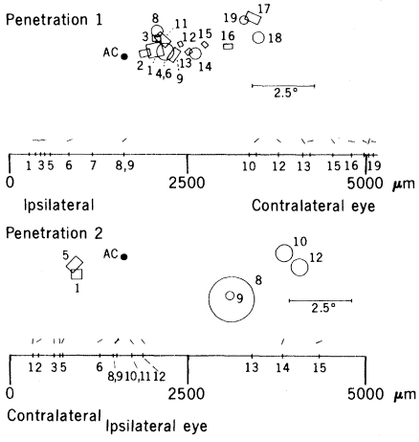

Fig. 1. Two typical penetrations of the medial bank of area 17 in the same animal. Each line on the abscissa indicates a recorded neuron and its depth relative to the first recorded unit. Just above the abscissa, the optimal orientation of each recorded neuron is indicated by an appropriately oriented line. If no line is drawn, the unit could not be driven. At top, the positions of excitatory receptive fields relative to the area centralis (*AC*) are shown (squares: the field was determined exactly; circle: approximate field). Penetration 2 was about 1.5 mm in front of penetration 1. The receptive field position of only some cells was exactly determined during this penetration in order to show the shift of receptive fields within the visual field.

quickly determined with a hand lamp. Then the optimal orientation and the "orientation tuning range" were measured with an optimal stimulator which automatically moved a narrow light bar back and forth over the receptive field at a speed of 2° to 10° per second. Around the range where a consistent response could be elicited, the orientation was changed in steps of 5°, and at each step the stimulus moved across the receptive field several times. This procedure was repeated a number of times, the orientation being changed clockwise or anticlockwise in a counterbalanced order. Two experiments listened to the response over an audio-monitor, and were able to agree to within about 5° to 10° on what they considered to be the optimal orientation. The direction sensitivity of the cell, that is, whether it responded to forward, backward, or both movements, was disregarded (*4*). Only the dominant eye was used for classification. In general, the recordings began 6 to 8 hours after beginning of the anesthesia and lasted for another 10 to 12 hours. In each animal, at least four penetrations were done and a total of 31 to 51 cells were recorded. In all seven experimental animals, 248 cells in 25 penetrations were investigated.

Two typical penetrations from one animal are shown in Fig. 1. The penetrations were about 5 mm long measured from the first recorded cell (abscissa). It can be seen that the positions of receptive fields show the typical "random walk progression" (*5*) from the center of the visual field (*AC*) toward the periphery and that the receptive field diameters are of the usual size. The first cells recorded in a penetration were mostly sensitive to the same or a similar orientation, owing to the fact that the penetration started at a direction normal or nearly normal to the surface.

In contrast to what is known from normal animals (*1, 2*), the different orientations were not evenly distributed in the trained animals. Cells sensitive to vertical or next to vertical orientations, that is, to the stimulus to which the animals were exposed during the previous 2 weeks, were much *less* frequently found than cells sensitive to horizontal or near horizontal orientations (Fig. 2). This was a consistent finding in each individual experiment. In Fig. 3, the number of cells optimally responding to horizontal orientations (open columns) and of those optimally responding to vertical orientation (hatched columns) are shown for each animal. The difference is evident in each experimental animal. It was smallest in animal No. 5, which was the oldest and strongest of all our cats; it weighed 3.4 kg. This animal slept or licked its fur most of the time in the cylinder and thus paid apparently little attention to the surroundings. In the control animal, both orientations were evenly represented, as found in all previous investigations in this laboratory (*2, 5*).

The different representation of vertical and horizontal orientations by the neuronal population is also evident if one only counts the orientations found at the beginning of each penetration. This figure may give an indication of the distribution of ori-

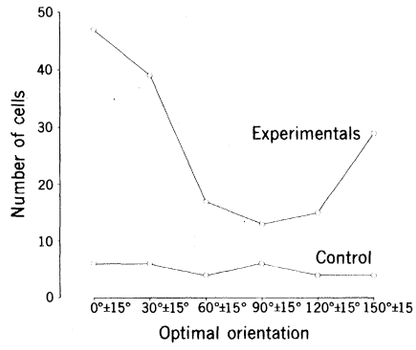

Fig. 2. Number of cells found in the different orientation classes in six experimental animals and one control animal. Values from animal 5ˣ in Fig. 3 are not included. 0° = horizontal orientation, 90° = vertical orientation of bar. The bars were moved normal to their orientation. Total number of cells, 248; cells with defined optimal orientation, 167; cells with undefined optimal orientation, 81; penetrations, 25.

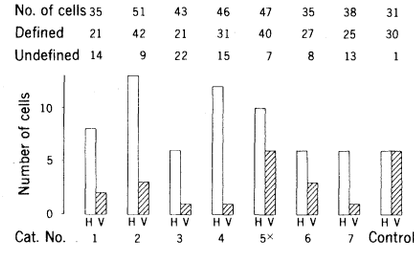

Fig. 3. Number of cells in the horizontal (0° ± 15°) (open columns, *H*) and in the vertical orientation class (90° ± 15°) (hatched columns, *V*) in each animal. For comments on animal 5ˣ see text. The numbers at the top give the total number of cells recorded in each animal (*No. of cells*), the number that could be driven by visual stimuli (*Defined*), and the number that could not be classified by visual stimulation (*Undefined*).

entation columns [or their equivalent, see (*5*)]. The mean optimal orientation of the first three units was horizontal (±15°) in eight penetrations, and only in two was it vertical (±15°).

The tuning widths, that is, the total angular range over which a consistent response could be elicited, were about the same in all orientation classes (mean values between 41° and 67°, range 15° to 100°). Some cells with very narrow tuning widths (≦15°) were all found in the optimal orientation classes ±45° around the horizontal, but these few cells did not affect the mean values of tuning widths in the different orientation classes significantly.

The number of cells which were unresponsive, in our hands, to visual stimuli was higher (about one-third of the whole population) than we usually found in normal cats. In some penetrations, such unresponsive units were found in clusters, but this was not the rule.

Our experiments have thus demonstrated that the exposure of adult animals to a simple monotonous visual environment (vertical stripes) may alter the functional organization of the visual cortex at least temporarily. This finding supports the view that the cortex may be organized as an adaptive neuronal network rather than as a fixed wired system (*6*). Our results are also strikingly different from the effects of a similar experimental procedure in young animals during the critical period (3 to 14 weeks). In these cases units responding to the adaptation orientation seem to be found exclusively or predominantly (*3*), while in our experiments the reverse tendency was found. Our findings are more similar to those of Maffei and Fiorentini (*7*), who describe in kittens a reduced sensitivity of cortical evoked potentials, as well as of lateral geniculate mass responses, to gratings of a spatial frequency to which the animals had previously been exposed.

Changes of the type we found in adult animals are actually suggested by perceptual negative aftereffects such as the waterfall, the tilt, the spatial frequency adaptation, and the McCollough effect, in which the aftereffects are complementary to the adaptation conditions. While most of these effects seem to last only for short periods, some seem to last longer. The McCollough effect, for example, has been reported to last for hours, especially if no visual stimuli are presented in the interval between adaptation and testing (*8*). It was proposed that these aftereffects may be due to increased inhibitory synaptic linkage between neurons excited by such stimuli, and that similar mechanisms may be involved in memory (*9*). The present experiments are in line with these psychophysical phenomena insofar as they offer, for the first time, a mechanism by which the nervous system becomes less sensitive to the stimulus to which it was exposed. Our experiments do not clarify, however, whether the relative decrease of units responsive to the trained stimulus is due to an "adaptation" or "habituation" of excitatory connections or whether it is due to increased effectiveness of inhibitory connections within the cortical network (*10*).

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4. It should be noted in this context that, since most cortical neurons respond best or even exclusively to moving stimuli, the description of the fundamental property of a cortical neuron by its "optimal" orientation is therefore incomplete. With this reservation in mind it may nevertheless be used, since a complete description of the fundamental properties of cortical neurons is still a matter of dispute.
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18 November 1974; revised 27 December 1974

Electrostatic and Steric Effects in the Selective Complexation of Cations in Nonactin

Abstract. *The ester carbonyl stretch frequencies of complexes of the macrotetrolide nonactin with Na^+, K^+, Rb^+, Cs^+, Tl^+, NH_4^+, NH_3OH^+, and $(NH_2)_2CNH_2^+$ have been measured. For the larger alkali cations and the polyatomic cations, the ester carbonyl stretch frequency is linearly proportional to the cation–ester carbonyl electrostatic interaction energy. This constitutes direct evidence that the cation-nonactin interaction is primarily electrostatic, rather than mechanical (steric).*

The biological mechanisms of ion complexation and ionic selectivity have been studied extensively using ionophorous antibiotics (*1*). The macrocyclic antibiotic nonactin is particularly interesting because it selectively complexes with a wide variety of cations (*2*). Crystallographic and nuclear magnetic resonance (NMR) studies show that the cation is located near the middle of the nonactin molecule (*3*) (see inset, Fig. 1a) and suggest that cation binding proceeds primarily through the ester carbonyl oxygen atoms.

While it is generally agreed that electrostatic forces are primarily responsible for the stability of antibiotic-cation complexes (*4–6*), several distinct models for the ionic selectivity of nonactin have been advanced. In one (*4, 5*) the selectivity is ascribed entirely to differences in the electrostatic free energy between the solvated ion in solution and the unsolvated ion bound to the antibiotic, while in another (*6*) it is suggested that "the ability [of carrier ligands] to distinguish among the cations of one group of the periodic system by forming complexes over a large range of stabilities is largely due to intra-ligand steric interactions...."

We report the first laser Raman spectroscopic studies of nonactin and its complexes. By correlating the observed ester carbonyl stretch frequencies with known properties of the cations, we have obtained information on the physical nature of the forces that perturb the carbonyl and tetrahydrofuran oxygen atoms in the nonactin complex.

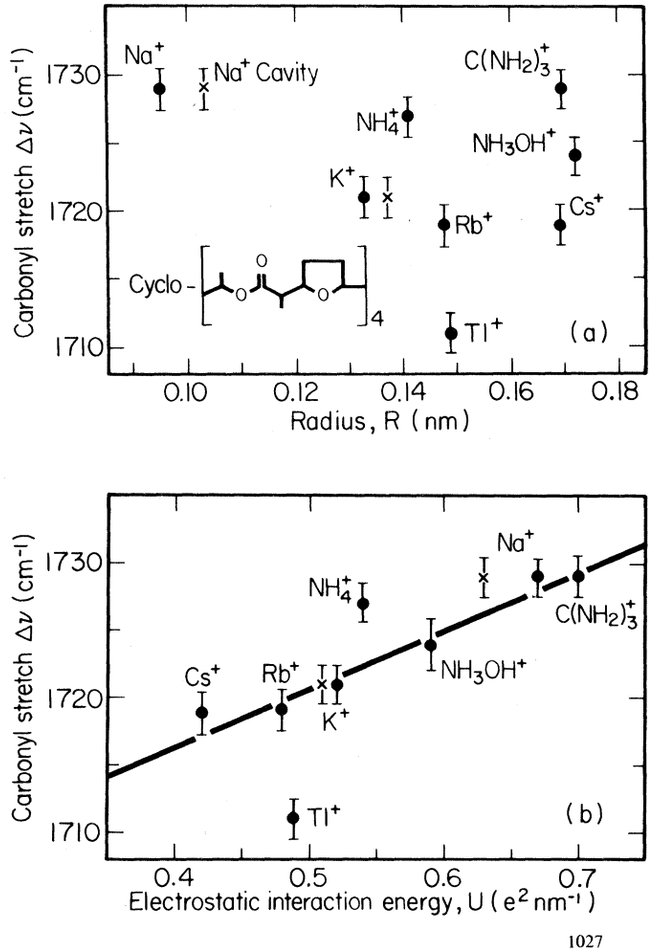

Fig. 1. (a) Ester carbonyl stretch frequency as a function of ionic radius for nonactin-ion complexes. For Na^+ and K^+ the ionic radius and the radius of the cavity in the complex are different; both radii are plotted. (b) Ester carbonyl stretch frequency as a function of electrostatic interaction energy for the nonactin-ion complexes. The electrostatic interaction energy for each complex is calculated by Coulomb's law from the charges and positions assumed (Fig. 2), supplemented by the available x-ray structural data. For the guanidinium and hydroxylamine complexes, where steric interactions between the ion and some of the carbonyl groups are important, the indicated electrostatic energy is for ester carbonyls not subject to steric interactions. Note particularly the positions of the Cs^+, NH_3OH^+, and $C(NH_2)_3^+$ ions in (a) and (b).