ber of such sections, cut at random orientations through many small crystals in a pellet, are shown in Fig. 2. Arrays of particles are clearly seen. These micrographs, including others not shown, are strongly suggestive of a cubic lattice. Some, but not all, micrographs show vacancies in the lattice, reminiscent of the vacancies seen in polyoma crystals (*4*). Such vacancies may adversely affect the quality of the diffraction pattern.

The most interesting aspect of SV40 structure is the minichromosome with its constituent nucleosomes. Since the SV40 capsid has icosohedral symmetry, it can be placed in a crystal lattice in 60 equivalent orientations. Since there are not 60 nucleosomes per particle, the nucleosomes themselves cannot have the symmetry of the capsid and must be rotationally disordered in the crystal. Because of this disorder, nucleosomes in the image will appear rotationally averaged, and will therefore be deficient in detail, or perhaps effectively invisible. A low-resolution structure determination, which could be greatly expedited by the use of noncrystallographic symmetry (*16*) would be sufficient to reveal whether any chromatin is likely to be seen.

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References and Notes

1. F. K. Winkler, C. E. Schutt, S. C. Harrison, G. Bricogne, *Nature (London)* **265**, 509 (1977).
2. D. Such, I. Rayment, J. E. Johnson, M. G. Rossmann, *Virology* **85**, 187 (1978). M. Rossman informs me that a 2.8-Å structure has been completed.
3. W. T. Murakami, *Science* **142**, 56 (1963).
4. K. W. Adolph, D. L. D. Caspar, C. J. Hollingshead, E. E. Lattman, W. C. Phillips, W. T. Murakami, *ibid.* **203**, 1117 (1979).
5. T. J. Kelly, Jr., and D. Nathans, *Adv. Virus Res.* **21**, 86 (1977).
6. D. L. D. Caspar and A. Klug, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 1 (1962).
7. F. A. Anderer, H. D. Schlumberger, M. A. Koch, H. Frank, H. J. Eggers, *Virology* **32**, 511 (1967).
8. M. A. Koch, H. J. Eggers, F. A. Anderer, H. D. Schlumberger, H. Frank, *ibid.*, p. 503.
9. W. Phillips, D. Schneider, E. E. Lattman, unpublished results.
10. E-S. Huang, M. K. Estes, J. S. Pagano, *J. Virol.* **9**, 923 (1972); G. Christiansen, T. Landers, J. Griffith, P. Berg, *ibid.* **21**, 1079 (1977).
11. W. Fiers *et al. Nature (London)* **273**, 113 (1978).
12. U. Müller, H. Zentgraf, I. Eicken, W. Keller, *Science* **201**, 406 (1978).
13. R. G. Martin, *Virology* **83**, 433 (1977).
14. T. Friedmann, R. F. Doolittle, G. Walter, *Nature (London)* **274**, 291 (1978).
15. A. McPherson, Jr., in *Methods Biochem. Anal.* **23**, 249 (1976).
16. G. Bricogne, *Acta Crystallogr.* **A30**, 395 (1974).
17. C. J. Dawes, *Biological Techniques in Electron Microscopy* (Barnes & Noble, New York, 1971), pp. 71-73.
18. I thank Drs. Daniel Nathans and Thomas J. Kelly, Jr., for advice and for the use of tissue culture facilities; the MIT Cell Culture Center for large-scale virus production; Dr. L. Mario Amzel for help with thin-sectioning. This work supported by NIH grants AI-14820 and AI-00271, and by an internal grant from the Johns Hopkins University School of Medicine.

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Interaction of Laminae of the Cingulate Cortex with the Anteroventral Thalamus During Behavioral Learning

Abstract. *Neurons in deep laminae of the rabbit cingulate cortex develop discriminative activity at an early stage of behavioral discrimination learning, whereas neurons in the anteroventral nucleus of thalamus and neurons in the superficial cortical laminae develop such activity in a late stage of behavioral learning. It is hypothesized that early-forming discriminative neuronal activity, relayed to anteroventral neurons via the corticothalamic pathway, contributes to the construction of changes underlying the late-forming neuronal discrimination in the anteroventral nucleus. The resultant late discriminative activity in the anteroventral nucleus is then relayed via the thalamocortical pathway back to the superficial cortical laminae, promoting disengagement of cortex from further task-processing.*

A long-standing and intransigent problem in neuroscience has been to identify brain systems mediating learning processes. We now report results of studies of multiple-unit activity recorded from the cingulate area of the cerebral cortex of the rabbit during learning of discriminative avoidance behavior. The results indicate a differential involvement of superficial and deep laminae in mediating acquired discrimination.

The cingulate cortex is reciprocally interconnected with the anteroventral (AV) nucleus of thalamus (*1*). Our results also shed light on the interaction between the cortical laminae and the AV nucleus during learning.

The details of procedure have already been presented (*2*). During avoidance training, we successively presented (in a random order) two tone stimuli, the positive conditional stimulus (CS+) and the negative conditional stimulus (CS−). For each rabbit, the CS+ was either a 1- or an 8-kHz tone (80 dB relative to 0.0002 dyne/cm^2, with a rise time of 3 msec); the CS− was the other tone. The assignment of frequencies to rabbits was counterbalanced. Onset of the CS+ was followed after 5 seconds by constant-current footshock (1.5 mA) delivered to the rabbit through the grid floor of a rotating wheel apparatus (*3*), and terminated by locomotion. Locomotion during the CS+ terminated it and prevented footshock. The CS− was never followed by footshock. Thus, the rabbits learned to avoid footshock by locomoting to the CS+, and they learned not to respond to the CS−. Forty-six rabbits were each given 120 trials daily (60 with each stimulus) until behavioral discrimination reached criterion (*4*). A subset of 29 rabbits received additional sessions of training (overtraining). Before training, each rabbit received a preliminary training session in which tones and the footshock were presented unpaired to provide control data for evaluating training-induced acquisition of discriminative neuronal and behavioral activity (*5*). In order to observe the relationship between neuronal activity and behavioral acquisition, we focused exclusively on neuronal results obtained at certain behaviorally defined stages of acquisition. The stages were preliminary training (PT), the first exposure (FE) to conditioning, the session of first significant (FS) behavioral discrimination (*6*), the session in which the stringent criterion (Cr) of behavioral discrimination was attained, and overtraining. Significant behavioral discrimination did not occur during FE.

Each of the rabbits had a single permanently indwelling metal microelectrode (range of tip lengths, 10 to 60 μm) in cingulate cortex, and 14 of the rabbits had such an electrode in the AV nucleus. Throughout behavioral training, unit activity was fed into high-pass active filters (bandwidth, 500 to 10,000 Hz) and subsequently into pulse-height discriminators set to pass only the three or four largest neuronal spikes. Outputs of the discriminators were fed into a computer programmed to process the neuronal data and to control the behavioral experiment. The computer calculated numerical scores for each session, representing the average frequency of neuronal firing at various periods after CS onset, normalized with respect to the pre-CS baseline. This report concerns the scores representing neuronal activity in the second through the sixth periods of 100 msec. Results of a fine-grained analysis of scores for the first 200 msec will be presented elsewhere (*7*).

Inspection of the brain sections containing the electrode tracks revealed that the recording tips were localized in each of the six laminae that have been distinguished within the cingulate cortex (*8*). An established principle of brain organization states that the superficial laminae (I to IV) of the cerebral cortex are regions which receive axonal input from other brain regions, whereas the deep cortical laminae (V and VI) are regions of origin of axonal outflow to other regions (*9*). Recent anatomical research (*1*)

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indicates that the interconnections of rabbit cingulate cortex and AV nucleus conform to this principle. Twenty-eight of the 46 cortical records were in one of the deep (output) laminae, and 18 were in one of the superficial (input) laminae.

The neuronal scores obtained from each rabbit were classified with regard to presence versus absence of acquired neuronal discrimination, defined as a training-induced enhancement of the neuronal response to the CS+, relative to the response evoked by the CS− (*10*). Of the 46 cortical records, 13 did not show discriminative activity in any stage of acquisition. Of these, seven were in the superficial laminae and six were in the deep laminae. Of the remaining 33 (discriminating) records, early-discriminating neuronal records (during FE or FS) predominated in the deep laminae, and late-discriminating records (during Cr or overtraining) predominated in the superficial laminae (Fig. 1). The association between stage in which first neuronal discrimination was manifested, and position in the cortex was significant [χ^2 (1) = 11.50, $P < .001$].

Next, we computed analysis of variance on data of the discriminating cortical records, using depth of electrode placement as a two-level factor. The analysis yielded a significant interaction of training stage, stimulus, and cortical depth [F (12, 372) = 1.76; P = .05]. Separate analysis of the data from the AV nucleus yielded a significant interaction of stage and stimulus [F (3, 36) = 13.11; $P < .001$]. Subsequent least significant difference tests (*11*) of the interaction means revealed that the mean neuronal response to the CS+ was greater than that to the CS− in the deep laminae during FE, FS, and Cr (Fig. 2A). In the superficial laminae and in the AV nucleus, significant discriminations occurred at all periods in Cr but not during FE or FS (Fig. 2, B and C). There were no significant neuronal discriminations in preliminary training, except for the significant "reverse" discrimination during period 5 in the superficial laminae (Fig. 2B). Each brain locus manifested significant discrimination at all periods in each stage of overtraining. Thus, results from the analysis of variance corroborated the conclusion derived from classification of individual records. Deep cortical laminae manifested early-forming neuronal discrimination, whereas superficial laminae and the AV nucleus manifested late-forming discrimination during the course of behavioral discrimination learning.

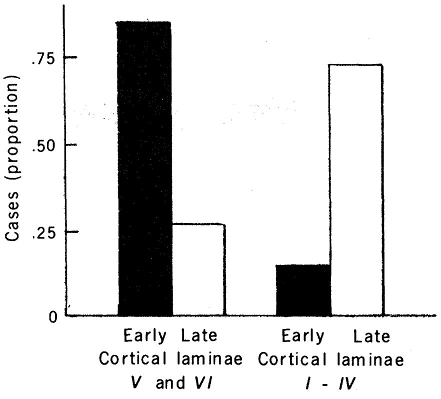

Fig. 1. Relation of cortical layer to first discrimination.

The prime intent of this report is to convey the cortical laminar differences in the acquisition of neuronal discrimination during behavioral learning. We have tentatively assumed that early neuronal discrimination formed in the deep (output) laminae of the cingulate cortex is relayed to the AV nucleus via the corticothalamic pathway. After the discriminative input from cortex acts upon principal neurons of the AV nucleus for some time, the latter set of neurons form their own discriminative response, which is immediately relayed back via the thalamocortical pathway to the superficial (input) laminae of cortex.

Because the discriminative activity in the deep laminae was acquired during FE, prior to behavioral discrimination, we hypothesize that the deep laminae may be loci for acquisition of the neuronal code for the associative significance of the conditional stimuli. The acquired code is presumed to be critical for the rabbit's ability to use the CS+ as a cue for the avoidance response. This hypothesis receives support from studies indicating that acquisition of stimulus-cued aversively motivated behavior is impaired in rats and cats with induced damage to the cingulate cortex (*12*).

Because behavioral discrimination predated neuronal discrimination in the AV nucleus, the activity of the AV nucleus may not be viewed as a possible mediator of behavioral acquisition. Rather, it is more reasonable to suppose that the late-developing discriminative activity of the AV nucleus may have reflected processes relevant to retention of the neuronal code for stimulus significance. This interpretation is consistent with observations of deficient retention

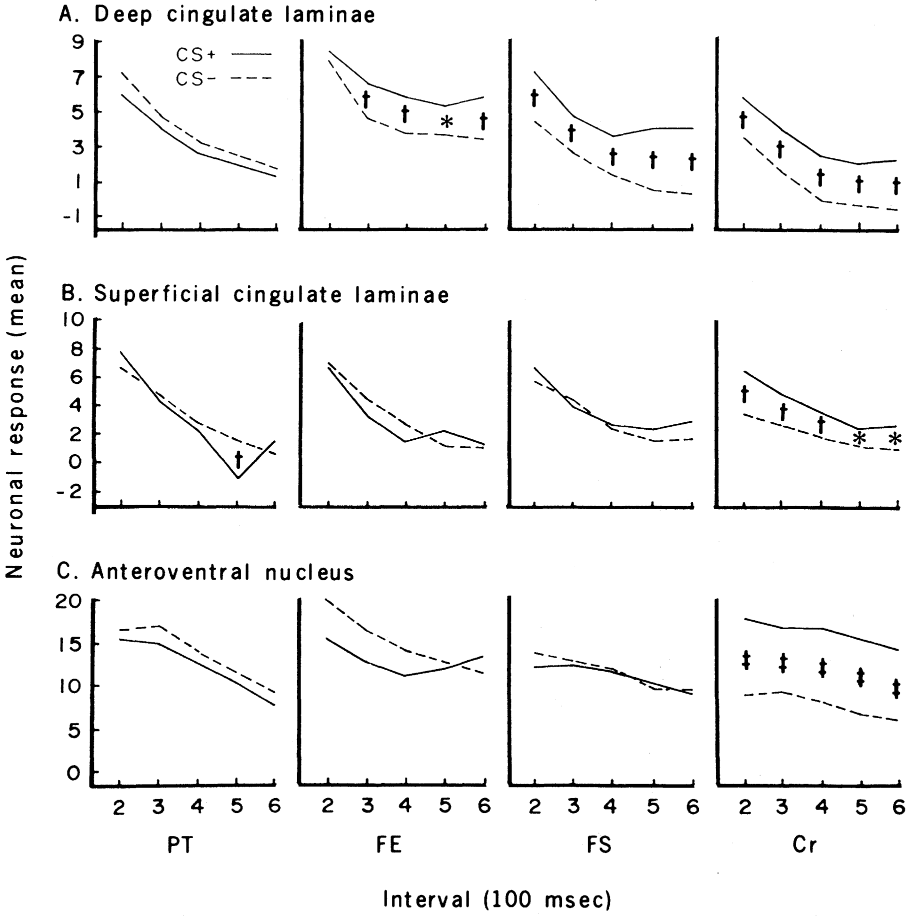

Fig. 2. Mean neuronal response to CS+ and to CS− in successive stages of behavioral discrimination training. Interval 2 represents the time from 100 to 200 msec; interval 3, from 200 to 300 msec; and so forth. Significance of the differences in response to CS+ and CS−: *$P < .05$; †$P < .01$; ‡$P < .001$.

in humans and animals after damage to the AV nucleus and related structures (*13*).

Finally, we hypothesize that the late-forming neuronal discrimination relayed from the AV nucleus back to the superficial laminae may inhibit ongoing neuronal discrimination acquired in the deep laminae in the early stages of acquisition. Thus, once neurons of the AV nucleus are able to produce their own discriminative activity, feedback from the AV nucleus may promote disengagement of the cingulate cortex from the discrimination process.

Two sources of evidence suggested such disengagement. First, 19 of the 22 cortical records that indicated acquired neuronal discrimination at early stages of behavioral acquisition showed its loss in the late stages. Second, only neurons in the AV nucleus manifested reversal of the original discriminative activity during behavioral reversal training (*7*). Thus, relegation of the discriminative response by neurons in the deep laminae to the AV nucleus may free the deep laminae to process new discriminative problems.

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References and Notes

1. T. W. Berger, T. A. Milner, G. W. Swanson, G. S. Lynch, R. F. Thompson, *Soc. Neurosci. Abstr.* **5**, 270 (1979).
2. M. Gabriel, J. D. Miller, S. E. Saltwick, *Physiol. Psychol.* **4**, 124 (1976); *J. Comp. Physiol. Psychol.* **91**, 423 (1977).
3. W. J. Brogden and E. Culler, *Science* **83**, 269 (1936).
4. The criterion required that the percentage of CS+ trials in which locomotory response occurred exceed the percentage of CS− trials with locomotion by at least 60, in two consecutive sessions.
5. G. A. Kimble, *Hilgard and Marquis' Conditioning and Learning* (Appleton-Century, New York, 1961), pp. 44–76.
6. The FS was the first half-session in which the percentage of conditioned responses to the CS+ exceeded the percentage to the CS− by 25 or more. This value approximates the minimum required to produce a significant χ^2 (P = .05) for a difference between correlated proportions [H. M. Walker and J. Lev, *Statistical Inference* (Holt, New York, 1953), p. 101].
7. K. Foster, E. Orona, M. Gabriel, R. W. Lambert, in preparation; M. Gabriel, E. Orona, K. Foster, R. W. Lambert, in preparation.
8. M. Rose, *J. Psychol. Neurol.* **43**, 353 (1933).
9. S. W. Ranson and S. L. Clark, *The Anatomy of the Nervous System* (Saunders, Philadelphia, ed. 10, 1959).
10. Acquired neuronal discrimination was judged to be present in a given training session if two or more of five consecutive scores obtained from the CS+ histogram exceeded their respective CS− scores by a minimum of three units. This criterion had to be met after the corresponding score difference from preliminary training was subtracted from the difference in training. The five scores were those for the second through the sixth periods of 100 msec after CS onset.
11. B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), p. 210.
12. R. A. McCleary, *Prog. Physiol. Psychol.* **1**, 210 (1966); G. J. Thomas, G. Hostetter, D. J. Barker, *ibid.* **2**, 230 (1967). Rats and cats have little difficulty in reacquiring an avoidance habit when cingulate lesions are induced during the retention interval, after behavioral acquisition is complete [R. Y. Moore, *J. Comp. Physiol. Psychol.* **57**, 65 (1964); B. M. Slotnick, *Neuropsychologia* **9**, 61 (1971); B. Eckersdorf, *Acta Physiol. Pol. Engl. Transl.* **2**, 105 (1974)]. Our data suggest an explanation for this outcome: AV nucleus, but not cingulate cortex, mediates discrimination of task stimuli after acquisition is complete.
13. I. Rich and R. Thompson, *J. Comp. Physiol. Psychol.* **59**, 66 (1965); W. H. Sweet, G. A. Talland, F. R. Ervin, *Trans. Am. Neurol. Assoc.* **84**, 76 (1959).
14. Supported by funds provided by the Spencer Foundation, the Biomedical Sciences Support Committee of the University of Texas at Austin, and by NIMH grants 26267 and 31351.

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Interaction Between Posture, Color, and the Radiative Heat Load in Birds

Abstract. *The effect of the angle of incidence on the radiative heat load was determined for light and dark plumage. As the angle of incidence to the solar radiation source increases the difference in heat transfer between light and dark plumage disappears. Thus, by postural adjustment, a dark bird may become thermally white with regard to the radiative heat load.*

Cartwright and Harrold (*1*) proposed that plumage coloration could be important to thermal regulation in birds, since color should influence the absorptivity of solar radiation. Several investigators (*2*, *3*) have conducted metabolic studies in the laboratory to show that birds when below thermal neutrality can use solar radiation to allieviate thermal stress. Monteith (*4*) pointed out that "reflectivity is an important discriminant in the heat balance of animals but the relationship between coat color and radiative heat load is complex." There is still a question as to whether dark or light plumage is more effective in the utilization of direct solar radiation. Several studies (*2*, *5–7*) have shown dark coloration to be more effective in the use of solar radiation, while others (*8*) have shown light coloration to be more effective in radiative heat gain. Further, convective cooling (*9*) differentially affects the radiative heating of dark and light plumages, so that at low wind speeds black plumages acquire a greater radiative heat load than do white plumages, but the heat loads of black and white plumages rapidly con-

Table 1. Heat flow through feathers as a function of color, angle, and air temperature; G, gray; W, white.

T_a (°C)	Color	W m^{-2}	T_{skin} (°C)	T_r (°C)*	$T_{surface}$ (°C)	N
			90° angle			
27.0	G	119.1 ± 10	40.0	37.3	69.7	3
27.0	W	61.2	39.5	37.6	62	2
20.0	G	102.6 ± 11	40.2	36.7	69.5	4
20.0	W	46.0 ± 10	38.8	37.0	57.2	4
20.0†	G	54.6	39.5	36.5	67	2
20.0†	W	14.5	36.7	36.5	48.5	2
10.0	G	71.0 ± 5	38.5	36.6	62	3
10.0	W	35.5	38.2	36.5	49.5	2
			135° angle			
27.3	G	50.6 ± 6	38.4	37.0	52.5	3
27.0	W	32.9	38.1	37.1	43.9	3
20.0	G	33.5 ± 4	38.1	36.8	48.0	3
20.0	W	21.0 ± 7	38.1	37.1	41.0	3
20.0†	G	25.6	37.7	37.0	45.0	2
20.0†	W	9.9	36.5	36.5	36.5	2
10.0	G	10.5	36.9	36.3	44.0	2
10.2	W	7.2 ± 1	36.2	35.5	27.0	3
			160° angle			
27.9	G	21.0 ± 9	37.4	36.9	43.7	4
27.0	W	17.8 ± 5	37.1	36.8	39.5	4
20.0	G	2.0 ± 1	36.8	37.0	36.5	3
20.0	W	4.0 ± 5	36.6	36.8	35.7	4
20.0†	G	0	35.0	37.0	35.0	2
20.0†	W	0	36.0	37.0	29.5	2
10.0	G	0	35.0	36.7	32.0	2
10.0	W	0	36.4	36.5	32.0	3

*Radiation passed through 1 cm of water. †Temperature of reservoir.