cochlear nerve and nucleus (*16*) has been effectively criticized for incomplete control of acoustic field variations: with such controls transmission at all auditory relay nuclei below the thalamic level was found to be stable with changing levels of arousal and attentiveness (*17*). Our findings are consistent with these reports and suggest that attention is mediated not by selective gating of inputs at the periphery but by specialized processing of relevant stimuli at higher levels of the sensory system.

Although the efferent olivocochlear pathway when electrically stimulated suppresses click-evoked transmission in the cochlear nerve (*18*), its role in sensory behavior is unknown. Recent evidence suggests that it acts as part of a feedback system involved in frequency discrimination or the detection of signals in noise (*19*). Our present experiments offer no evidence that the olivocochlear bundle is active in several types of human attention.

The negative results of this first direct inquiry into whether attention can influence peripheral auditory transmission in man must be interpreted with certain qualifications. Since only two of the varieties of auditory attention—intensity discrimination and selective binaural listening—were investigated, and these only with click stimuli, it is still possible that discriminations of more complex and significant sounds such as speech might be susceptible to peripheral gating. Most importantly, the response that we recorded at the ear represents the summed activity of many auditory nerve fibers (*20*) and so we cannot rule out the possibility that attention-induced alterations in the responses of some of them might pass undetected. Nevertheless, there seems to be no gross modulation or suppression of the cochlear nerve response with inattention.

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5. All electrodes on the scalp and face were nonpolarizable Ag-AgCl pellets (Beckman Instruments).
6. The Grass 7P5 preamplifiers were set with low-frequency half-amplitudes of 10 hz for the cochlear responses and 0.3 hz for the scalp responses. For the contingent negative variation experiment the latter setting was changed to 0.15 hz. High-frequency half-amplitudes were 3 khz. The cochlear nerve response was averaged with a Fabritek 1052 signal averager, and the vertex AEP and ocular potentials were averaged with a Mnemetron CAT 400A computer.
7. Because of the mild discomfort involved in the insertion of the electrode we did not think it proper to use volunteers as subjects. The use of sophisticated subjects opens the question of whether they can deliberately control their responses to conform with the known experimental predictions. We believe that such effects are achieved by regulating one's level of attentiveness. Whatever the mechanism, there seems to be no voluntary control of the cochlear nerve response.
8. Measurements on all four subjects showed that the intensity of 55-db sensation level yielded a cochlear nerve response that lay on a steep portion of the intensity-amplitude function [see (*4*)], so that any attention-related changes in effective click intensity would be reflected in changed cochlear potentials.
9. Subjects made no movements during the attend condition and their gaze was fixated. In the control condition, however, saccadic eye movements occurred during reading. Since certain eye movements have been shown to reduce the cortical response to clicks in cats [J. S. Ebersole and R. Galambos, *Electroencephalogr. Clin. Neurophysiol.* **26**, 273 (1969)] the possibility exists that the reduced AEP in the control condition is related to eye movement. In our third experiment, however, eye movements were similar in both the attend and control conditions.
10. The intensity discrimination task was reasonably difficult. The percentage of the fainter signals correctly detected by each subject was: T.P., 96 percent; S.H., 82 percent; R.G., 92 percent; D.W., 76 percent.
11. The amplitudes of *N1* and *N2* were measured relative to the baseline at the onset of the wave. The amplitudes of the vertex AEP were measured peak to peak. All statistical significance levels were obtained with one-tailed *t*-tests. Because of the great inter-subject variability, the statistical tests for the overall means were made after converting each measurement into a percentage of the mean reading amplitude for that subject.
12. In one of the subjects, an early positive-negative wave with a latency of 25 to 40 msec was consistently recognizable in the vertex AEP; attention did not significantly change this wave. If this wave represents activity of the primary auditory cortex, its constancy implies that attention is effected after signal analysis is completed through the entire afferent pathway.
13. Confidence intervals are based on the assumption that the difference between the means of attend and control conditions followed a *t* distribution.
14. The mean responses (in microvolts) for the single subject in this experiment were: cochlear nerve response: *N1*, 1.53 (reading) and 1.58 (attending to the clicks); *N2*, 1.50 and 1.47; maximum CNV amplitude, 2.36 and 4.50; vertex AEP to clicks, 7.00 and 11.33.
15. The mean response amplitudes (in microvolts) for the single subject in experiment 3 were: cochlear nerve response: *N1*, 1.60 (attending to the electrode ear) and 1.64 (attending to the opposite ear); *N2*, 1.15 and 1.11; cortical evoked response, N1-P2, 4.53 and 2.95. These results differ from those of D. B. D. Smith, E. Donchin, L. Cohen and A. Starr [*Electroencephalogr. Clin. Neurophysiol.* **28**, 146 (1970)] who found no significant changes in the vertex AEP with selective binaural attention. We believe that our discrimination was more difficult than theirs and required more constant attention.
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21. This research was supported by NASA grant NGR-05-009-08. We appreciate the technical and attentive assistance of David L. Woods.

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Recovery of Function after Serial Ablation of Prefrontal Cortex in the Rhesus Monkey

Abstract. *Rhesus monkeys with one-stage or serial ablation of sulcus principalis (prefrontal association cortex) were compared on three spatial tasks. On all tests, the serial monkeys made fewer errors than did the monkeys with one-stage lesions. These results indicate that partial recovery of function can occur after extensive destruction of association cortex in the mature primate brain if the damage is distributed over a number of operations.*

While recovery of function after infant brain damage has often been noted (*1*, *2*), recent investigations suggest that such recovery is also possible in mature organisms following sequential surgery. Thus, rats (*3*) and cats (*4*) with multiple-stage bilateral removals of cortical or subcortical structures are less impaired than are animals with single-stage ablations of identical tissue. These ameliorating effects of serial lesions have been demonstrated also in monkeys after ablation of primary sensory cortex (*5*), but recovery of function following damage to the association cortices involved in cognition and learning has not

been demonstrated at the primate level. The present experiment was designed to study, in the rhesus monkey, the effects of serial ablation of sulcus principalis, a prefrontal association area involved in the mediation of spatial abilities (*6*). Our findings indicate that bilateral serial lesions of sulcus principalis, in comparison with single-stage bilateral ablations of the same tissue, result in less severe deficits on three spatial tasks.

The subjects were ten naive adolescent rhesus monkeys (*Macaca mulatta*) ranging in weight from 3.0 to 4.0 kg. Each of the monkeys was trained preoperatively on a 5-second spatial delayed alternation task (DA). Upon attaining criterion on DA, the animals were designated to receive either a single-stage (SS) or multiple-stage (MS) ablation of sulcus principalis.

Surgery was performed under standard aseptic conditions with Nembutal anesthesia (40 mg/kg). Cortical tissue in the banks and depths of sulcus principalis was removed by subpial aspiration. All SS monkeys received bilateral lesions of principalis in one operation 10 weeks after reaching criterion on DA. In the MS group, sulcus principalis was removed in four operations spaced 3 weeks apart, with the first operation occurring within 1 week of attaining criterion on DA. Each stage of MS surgery involved the removal of one bank of sulcus principalis (for example, operation 1: left hemisphere, lower bank), and on each succeeding operation the hemisphere and bank were changed (for example, operation 2: right hemisphere, upper bank). Two weeks after all surgery had been completed the monkeys commenced postoperative testing for retention of DA followed by training on a 5-second delayed response task (DR) and then on place reversal learning (PR). Following the completion of all behavioral testing, the monkeys were killed and their brains were prepared for histological examination (*7*).

All behavorial testing was conducted in a modified Wisconsin general test apparatus with raisins serving as reinforcements. The animals were tested 5 days a week, with 30 trials for all three tasks.

On DA, the monkeys were presented on each trial with two identical wooden plaques that covered two recessed food wells. One food well was located to the right, the other to the left of the subjects. To attain criterion, the animals had to learn to alternate their spatial response (left-right) on each successive trial. On the first trial of each test session, both food wells were baited with raisins; on the second trial, the food well not selected on the first trial contained the reinforcement. If the monkeys made an error on any given trial, reinforcement remained on the same side until they responded correctly on some succeeding trial (that is, correction procedure). The 30 daily trials included such correction trials. For both preoperative acquisition and postoperative retention, DA training continued until the subjects attained a criterion of at least 90 correct responses in 100 consecutive trials or until they had completed 1000 trials without reaching criterion.

On the DR task, the animals were initially allowed to observe the investigator bait and cover one of the two food wells and then to respond immediately (zero delay, no screen). Upon reaching criterion (at least 27 correct responses in 30 consecutive trials) at this nondelay interval, delay procedures were introduced by lowering an opaque screen between the subjects and the manipulanda immediately after baiting of a food well. At first, the screen was lowered and raised as quickly as possible (zero delay, with screen). When the monkeys reached criterion at this zero-second delay (with screen), the interval between the lowering and the raising of the screen was increased by 1-second intervals each day until the monkeys demonstrated criterion performance with a 5-second delay period. If at any given delay (for example, 3 seconds), the animals failed to obtain 27 correct responses in 30 trials, the interval was decreased by 1 second on the following day (thus it would be 2 seconds). Testing was terminated if an animal failed to reach criterion at the 5-second delay interval within 1000 trials.

Training in PR required the subjects to reverse a previously learned spatial position habit. Thus, after learning to respond to the right food well to obtain reinforcement, they had to reverse this habit by responding to the left food well for raisins. Four such reversals, each to the same criterion (at least 27 correct responses in 30 trials), were used in this experiment.

The behavioral data were analyzed by the Fisher randomization test (*8, 9*). This test for two independent samples is based on an extension of the logic of the Fisher exact test for situations involving continuous measurement and is preferred to the classical *t*-test since it has been shown to be 100 percent as efficient but does not make restrictive parametric assumptions.

Table 1 shows the number of errors on DA, DR, and PR. To assess the monkeys' overall spatial performance, the total number of errors on all three spatial tasks was calculated for each animal. The MS made significantly ($P < .05$) fewer total errors than did the SS monkeys.

On DA retention, the MS monkeys

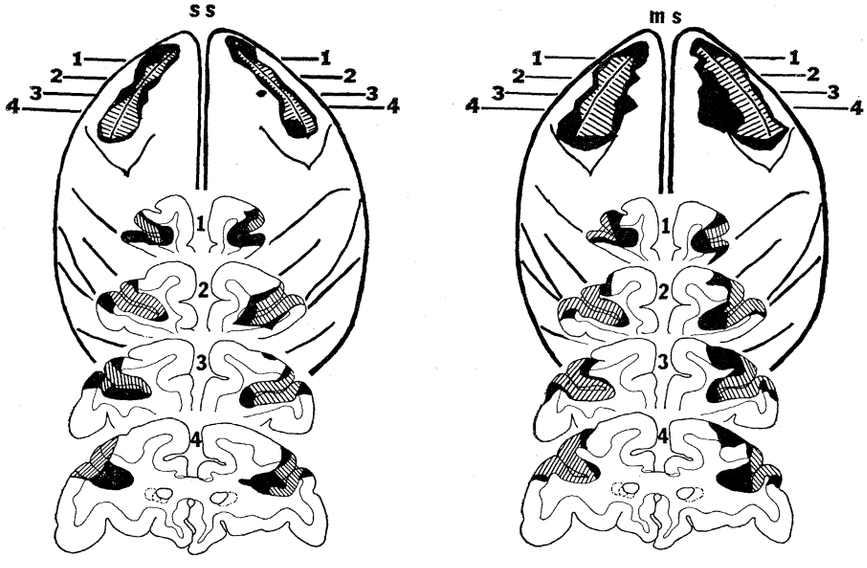

Fig. 1. Dorsal views and representative coronal sections of the maximum and minimum extent of frontal damage in the single-stage (SS) and multiple-stage (MS) surgical groups. The hatched areas indicate the minimum insult observed, while the addition of the solid portions represent maximum damage.

made fewer errors than did the SS animals, but this difference does not approach statistical significance. However, in both surgical groups one monkey relearned DA within 1000 trials while the other four failed to do so. When the performances of monkeys who failed to relearn DA are compared, significant differences do appear. While the MS ($\bar{X} = 193$ errors) and SS ($\bar{X} = 213$ errors) monkeys perform almost identically for the first 500 retention trials, the MS ($\bar{X} = 124$) made significantly ($P < .05$) fewer errors than did the SS ($\bar{X} = 191$) monkeys during the last 500 retention trials. Also, while the MS monkeys show considerable improvement during the 1000 retention trials (78 percent correct responses during the last 200 trials), the SS monkeys reveal little improvement (60 percent correct during the last 200 trials).

On DR, the MS monkeys made fewer errors than did the SS monkeys ($P < .07$). All five MS animals reached criterion within 1000 trials while two of the SS monkeys failed to do so.

The performances of the two groups on the first two spatial reversals were not significantly different, but on both the third and fourth reversals the MS monkeys made significantly ($P < .05$) fewer errors than did the SS monkeys. Again, like their performance on DA, the PR performance of the MS monkeys improved with practice while the SS monkeys failed to demonstrate such gains.

The histological findings (*10*) are graphically represented in Fig. 1. While all SS lesions were confined to the banks and depths of sulcus principalis, MS ablations were observed to extend beyond principalis onto the surrounding lateral frontal cortex. This additional damage was unintended and appeared to be related to the multiple surgical entries into the same region. As a consequence of this additional damage, however, the MS monkeys had significantly larger total lesions ($P < .01$) than did the SS animals. The histology did not reveal a significant difference between the two groups in terms of the amount of damage to the banks and depths of sulcus principalis. There was no significant ($P > .10$) correlation between the extent of principalis damage and the postoperative performance of either the MS or SS subjects ($\underline{P}$, + .24 and +.21, respectively). Finally, the histological data did not provide an explanation for the atypical behavior of MS-36 or SS-41, the animals that relearned postoperative DA with considerable savings. Their lesions, both in terms of size and location, were indistinguishable from those of other members of their respective groups. Thus the performance of MS-36 and SS-41 was included in all postoperative analyses, with the exception of the comparison concerned with those animals that failed to attain criterial performance on DA retention.

Table 1. Errors compiled by MS and SS monkeys on DA retention, DR acquisition, PR learning, and the total errors.

Monkeys (No.)	Tasks						Total errors
	DA	DR	PR1	PR2	PR3	PR4	
			MS monkeys				
36	5	107	59	16	14	11	212
52	384	68	20	16	17	6	511
46	345	34	19	15	7	3	423
39	308	86	17	8	5	3	427
62	330	20	25	11	6	2	394
$\bar{X}$	274	63	28	13	10	5	393
			SS monkeys				
41	76	123	17	13	7	6	242
42	542	31	11	11	89	33	717
38	479	245	68	101	41	30	964
58	360	178	22	27	28	10	625
59	445	97	36	13	11	6	608
$\bar{X}$	380	135	31	33	35	17	631

As in previous reports (*3, 4*) the present investigation indicates that serial, in comparison to one-stage, ablation of cerebral cortex in the mature monkey is followed by recovery of behavioral functions. This study, however, extends the serial lesion effect in the monkey to a cortical association area involved in the learning and organization of spatial abilities. Although the MS animals had larger prefrontal lesions than did the SS monkeys, the MS monkeys made fewer total errors on the three spatial tasks, and on DR and the third and fourth place reversals their performance was unimpaired. In comparison with their scores on DR and PR, the MS monkeys' overall DA performance showed less recovery of function, but the MS animals still made fewer errors than did the SS monkeys on the second 500 DA retention trials.

A number of possible explanations can account for this limited recovery of DA. (i) The DA task may involve abilities other than spatial, and these other capacities may not recover after serial lesions. (ii) Delayed alternation may be a more difficult spatial task than either DR or PR. Thus, if recovery is only partial with the conditions employed in this study (that is, four operations spaced 3 weeks apart), then the simpler spatial tasks should demonstrate more recovery than the more difficult tasks. (iii) Serial lesions may not spontaneously lead to a reorganization of structure and function, but rather may permit the more rapid reacquisition of spatial functions with extensive practice. The present findings that the MS monkeys approached normality on the second and third spatial tasks (DR and PR) and demonstrated significant improvement on the second 500 trials of the first task (DA) are consistent with this possibility.

Finally, it is interesting to note that these results parallel recent developmental findings (*1*) that demonstrate that removal of prefrontal cortex within the first few months of life is followed by complete compensation of DR performance and partial recovery of DA. Goldman *et al.* (*1*) found that relatively mature monkeys that had been subjected to prefrontal lobectomies in infancy manifested only a minimal impairment on the acquisition of DR but were incapable of achieving criterion on DA within the limit of 2000 training trials. While it has been suggested (*11*) that such partial recovery of function in the monkey is highly unlikely if surgery is performed after 12 months of life, it is now apparent that partial compensation also occurs in mature monkeys after MS surgery.

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Cytochalasin B: Microfilaments and "Contractile" Processes

Wessels and co-workers (*1*) have presented the basic hypothesis that "sensitivity to the drug [cytochalasin (CB)] implies the presence of some type of contractile microfilament system." Some of their evidence is consistent with this hypothesis. Some of their results are preliminary and do not justify a rigid interpretation, as they suggest, which dismisses alternative explanations.

Microfilaments have been associated with many cellular phenomena including cytokinesis (*2*), cellular motility (*3*), and morphogenetic movement (*4*). Contractile proteins have been demonstrated in cells (*5*) and are thought to be actin-like (*6*). The relation between the microfilaments and contractile proteins is not defined; specifically, there is no case in which microfilaments have been proved to be contractile elements. Obtaining such proof is difficult because of the small size of the filaments, their irregular organization (as compared with thin filaments in muscle), and their heterogeneity. A drug specifically affecting microfilaments in general or a subgroup of such filaments would assist in determining their function; but, in our opinion, some of the evidence presented by Wessells *et al.* that CB is such a drug is open to criticism.

We ask some questions concerning the relation between CB, microfilaments, and contractile processes:

1) When alteration of the morphology of microfilaments occurs, does it precede alteration of proposed microfilament function? In no instance has this been shown.

2) Do the effects of CB, microfilament, ultrastructural alteration, and loss of contractile processes always occur together? Wessells *et al.* imply that they do but fail to mention or emphasize exceptions. Although Schroeder has observed lack of furrowing in sea urchin eggs (*7*) and HeLa cells (*8*), Carter (*9*) as well as Krishan and Ray-Chaudhuri (*10*) have noted that furrowing takes place in mouse L cells in the presence of CB. The furrowing of fertilized *Xenopus laevis* eggs is normal in time of onset and progression for the first three "cleavages" (*11*); but the furrows reverse after formation, and cytokinesis does not occur (Fig. 1). A single brief exposure to CB (5 μg/ml) at or just before first cleavage begins is sufficient to inhibit cytokinesis (*11*) to blastula stage: furrows are produced but subsequently reverse. At doses of CB of 1 to 3 μg/ml, a partial reversal takes place (see Fig. 2) which represents partial completion of cytokinesis. Thus, some contractile functions such as furrowing presumed to be due to microfilaments are not inhibited.

Wessells *et al.* describe disturbances of "contractile" processes unaccompanied by alterations in one group of microfilaments making up the "sheath" of glial cells and heart fibroblasts. Interestingly enough they say that the "sheath" microfilaments are the more likely ones to have myosin-binding properties.

Schroeder (*7*) has described furrowing unfertilized activated sea urchin eggs in the absence of microfilaments. He does not indicate whether or not such furrowing is sensitive to CB.

3) Are changes in microfilaments and inhibition of "contractile" function related as cause and effect? It is conceivable that the mechanism which changes microfilament structure and inhibits cytokinesis (or other processes) is the same but that the two events are otherwise unrelated. Both cytokinesis and microfilament structure may depend upon intracellular concentration of some vital ion and the mechanism of CB action may be alteration of transport of such an ion. The relation between CB action, microfilaments, and contractile processes is therefore by no means well understood.

In table 1 Wessels *et al.* list processes sensitive to CB without defining CB "sensitivity." We believe that definition should depend on: (i) rapid onset of *complete* inhibition of function; (ii) continuation of other functions such as karyokinesis, increase in size of cells, exclusion or incorporation of vital dyes, respiration, and incorporation of precursors into DNA, RNA, protein, or membrane; (iii) recovery of function after removal of the agent.

Satisfaction of the second and third criteria are important for excluding the "toxic" effects described by others. Carter (*9*) indicated that a dose of 10 μg/ml was toxic to mouse L cells after a few hours. We have found that a dose of 10 μg/ml is toxic to a number of cell lines in culture, both "fibroblast" and "epithelial" cell types (*12*), after 10 to 12 hours of exposure. In addition 10 μg/ml is toxic to *Xenopus laevis* eggs (applied as for Fig. 1) in 20 to 30 minutes (*13*).

Applying these criteria to the list in table 1 of Wessels *et al.* we find that inhibition of the "beating" of cardiac myoblasts fails to meet the three criteria. Inhibition of Ca^{2+}-induced cortical contraction in *Xenopus laevis* embryos fails to meet the last two criteria, and our toxicity data for fertilized eggs suggest that at least the cells at the surface of such embryos may be dead and unable to respond to CB.

Wessells *et al.* have listed processes "insensitive" to CB but do not provide a definition of "insensitivity." We believe that such definition must be based on the following considerations. In those systems in which some process or processes are affected, those which are unaffected can be considered insensitive to CB. For example cytokinesis is "sensitive" while karyokinesis is "insensitive." However, in those cases in which no inhibition is observed, the criteria for establishing insensitivity are far more severe. Inadequate drug absorption or transport, or both, as well as inactivation or breakdown of drug (or combinations thereof), must be excluded as reasons for the absence of drug effect. This exclusion requires