Alteration of Mutation Frequency by Treatment with Actinomycin D

Abstract. The frequency of lethal mutations occurring in *Drosophila melanogaster* was reduced by approximately one-half when irradiated males were treated with actinomycin D, which also inhibited the appearance of melanotic atypical growths in the strain used for the study.

In the course of investigations on alteration of mutations, actinomycin D was administered to a strain of *Drosophila* developing tumors, and mutation frequency and tumor incidence were determined after irradiation. The *st sr e^s ro ca; tu 36^a* strain, which has an unusually constant but low incidence of tumors due to multiple recessive genes, was used in these studies. Preliminary tests on sex ratios in individual matings were used to reduce the likelihood of antecedent lethal mutations. One-half of the males raised on medium containing antibiotic in 1×10^{-3}-percent concentration were irradiated with 3000 r [6 ma, 100 kv (peak), 1.0 mm of Al, 15 cm] at the age of 3 days. The *sc^{81} B InS w^a sc^8* inversion and sequential matings of the Muller 5 type were utilized to detect lethal mutations on the X chromosome (Table 1). Semilethals and visibles are not included in the tabulations. Determinations of tumor incidence in P_2 and F_1 generations (*1*) were also recorded and are presented in Tables 2 and 3. Untreated cultures and cultures treated with actinomycin D alone and irradiation alone were also studied in a similar manner.

Table 1. Effect of actinomycin D on mutation rate in *Drosophila*.

Treatment	Chromosomes tested	Lethal mutations	
	(No.)	No.	%
Control	1375	2	0.14
Actinomycin D	978	1	0.10
Irradiation (3000 r)	1168	67	5.73
Irradiation (3000 r) plus actinomycin D	952	31	3.25

The usual frequency of mutation was found in the control cultures (two lethals among 1375 chromosomes tested), and no significant difference was encountered when actinomycin D was added to the medium. However, the frequency with irradiation, 5.73 percent, was reduced to 3.25 percent when actinomycin D was present in irradiated cultures. In the P_2 generation, any tumors formed had regressed, leaving a pigmented residue before irradiation, so that no difference was expected between irradiated and nonirradiated cultures. The presence of actinomycin D resulted in significant reduction ($p < .05$) in tumor incidence in each group, however. In the F_1 generation, the antibiotic alone and irradiation alone reduced the number of tumors as compared to the control group. Irradiation did not reduce the number of tumors by a significant additional amount when used as a supplement to treatment with actinomycin D.

The current studies show a reduction in the number of atypical growths in *Drosophila* after treatment with actinomycin D, but inhibition of tumors by both the antibiotic and irradiation suggests that in neither case is this effect mediated through the mechanism of mutation. Actinomycin D undoubtedly reduces the number of lethal mutations occurring after x-irradiation ($p < .05$). Whether a similar reduction in the frequency of natural mutations can be demonstrated by extending the scope of the tests remains to be seen. Although the mechanism (*2*) is obscure at present, the implications of the effective reduction of irradiation-induced mutation in metazoa by an agent which can be administered in therapeutic dosage would seem of some interest (*3*).

Table 2. Effect of actinomycin D on incidence of tumors in *Drosophila*.

Treatment	Males			Females			Total		
	Tumors	Total	%	Tumors	Total	%	Tumors	Total	%
Control	133	1644	8.1	127	1520	8.4	260	3164	8.2
Actinomycin D	20	514	3.9	24	493	4.9	44	1007	4.4
Irradiation (3000 r)	88	971	9.1	79	1069	7.4	167	2040	8.2
Irradiation (3000 r) plus actinomycin D	22	502	4.4	11	505	2.3	33	1007	3.3

Table 3. Effect of actinomycin D and irradiation on incidence of tumors in *Drosophila*.

Treatment	Males			Females			Total		
	Tumors	Total	%	Tumors	Total	%	Tumors	Total	%
Control	387	6110	6.3	378	6217	5.38	765	12357	6.2
Actinomycin D	34	1762	1.9	34	1731	1.96	68	3593	1.9
Irradiation (3000 r)	119	4474	2.7	145	4416	3.24	264	8890	3.0
Irradiation (3000 r) plus actinomycin D	29	1113	2.6	20	1115	1.79	49	2228	2.2

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Modification of Cortically Induced Responses in Brain Stem by Shift of Attention in Monkeys

Abstract. The long-latency electrical response of the brain stem evoked by stimulation of the cortex of freely moving monkeys is modified by a change of attention. The modification may be either suppression or augmentation, according to the background activity prior to the shift of attention.

In conscious cats the size of sensory evoked potentials is modified as far down as second-order sensory neurons according to the state of attentiveness of the animal (*1*). This phenomenon is interpreted as due to inhibitory influences descending through the reticular core. While it is obvious that the focusing of attention requires a selective facilitation of certain sensory input above other sensory afferents, it is not clear whether a similar mechanism applies to the situation where a corticifugal impulse is interacting with sensory afferents, or vice versa.

In this study of the central biological action of urine extracts from schizophrenic subjects (*2*), a number of *Macaca* monkeys with a system of 42 to 50 permanently implanted electrodes were used (*3*). In these animals which were kept from over 6 months to 2

years, it has been possible to correlate certain electrophysiological phenomena with behavioral changes.

Two sets of cortically induced long-latency responses in the brain stem were observed. Recording was made through the Teflon–coated bipolar electrode (No. 36 platinum iridium wires) separated 0.5 mm at the tip. One such response was recorded in the subthalamus after stimulation of the sensorimotor cortex in two monkeys, and the other was recorded in the nucleus of the third nerve after stimulation of the parietal cortex in three monkeys. All recording sites have been verified histologically. Stimuli up to 10 volts at 200 pulses per second (pulse duration, 0.1 msec) applied to the subthalamus did not produce any overt behavior except for brief facilitation of sensory evoked potentials in the diencephalic structures following such tetanization. Stimulation of the posteriorly placed, midline electrode in the nucleus of the third nerve produced an elevation of the upper eyelids and an inward rotation of the eyeballs (Fig. 1*B*).

The cortical stimuli were square wave pulses of 6 to 8 v and 0.1 msec duration, applied at a rate of 1 to 2 pulses per second. The animals did not show any overt behavioral change. Only when the intensity of the stimulus was sufficiently raised did the animal lift its contralateral hand as if some sensation were referred to it.

Although the recording and stimulating loci were different in these animals, it was found that the size, shape, and behavior of these evoked potentials were extremely similar. Both responses were characterized by a large negative potential with the latency of its peak about 100 msec and of 200 to 250 msec duration (Fig. 1*C*). This response was most conspicuous when the animal was relaxed, and its amplitude became largest when the animal was drowsy. In the latter case this negative wave was followed by a rather prominent positivity. As long as the animal was relaxed, the amplitude of the response remained consistently high, although it showed a definite tendency to wax and wane. Such waxing and waning were not correlated with any recognizable change of gross electrographic patterns from a number of cortical and deep structures so far investigated.

The brain stem response was significantly depressed when the animal became alert, or it practically disappeared. When the animal looked at its own hand, probably because of an induced paresthesia from stimulation of the sensory cortex, the response was suppressed. Such a modification of the response was also evident when a novel stimulus, such as a click or a flash of light, was introduced or the amount of room light was suddenly changed. However, as the animal gradually adapted to a new situation the amplitude of the response returned to normal (Fig. 1*D*).

So far the most powerful suppressor of such brain stem response has been the visualization of laboratory workers (Fig. 1*A*). When the animal discovered that it was being watched, the response was depressed as long as the animal could see the experimenter through the one-way mirror. Although the animal carefully followed every minor move of the experimenter with its gaze, such an obvious alertness on the part of the animal was not always accompanied by a flattening of the response. However, such flattening regularly occurred whenever the animal realized that the experimenter's gaze was fixed on it. This specific nature of the most powerful suppressor of the response—that is, the direct meeting of the experimenter's gaze and that of the monkey—suggests concentrated focusing of discriminatory attention of a quality necessary for self-preservation in the monkey. Since three recording sites of the response, which fluctuated according to the animal's state of attentiveness, were located in or around the nucleus of the third nerve, and since focusing of attention is usually accompanied by a shift of gaze, eye movement itself as a source of cortical-response modification cannot be ruled out, although it is an unlikely one.

Sometimes the reduction or flattening of the response was found to last for a considerable period of time even though there was no recognizable environmental change in a quiet animal chamber. On such occasions the animal was usually showing a facial expression of "preoccupied relaxation." Whether such a sustained flattening of the response is due to the animal's attention to the effect of cortical stimulation or whether it is due to some unknown change in *milieu intérieur* is not clear.

However, it was under such circumstances that a novel afferent sensory

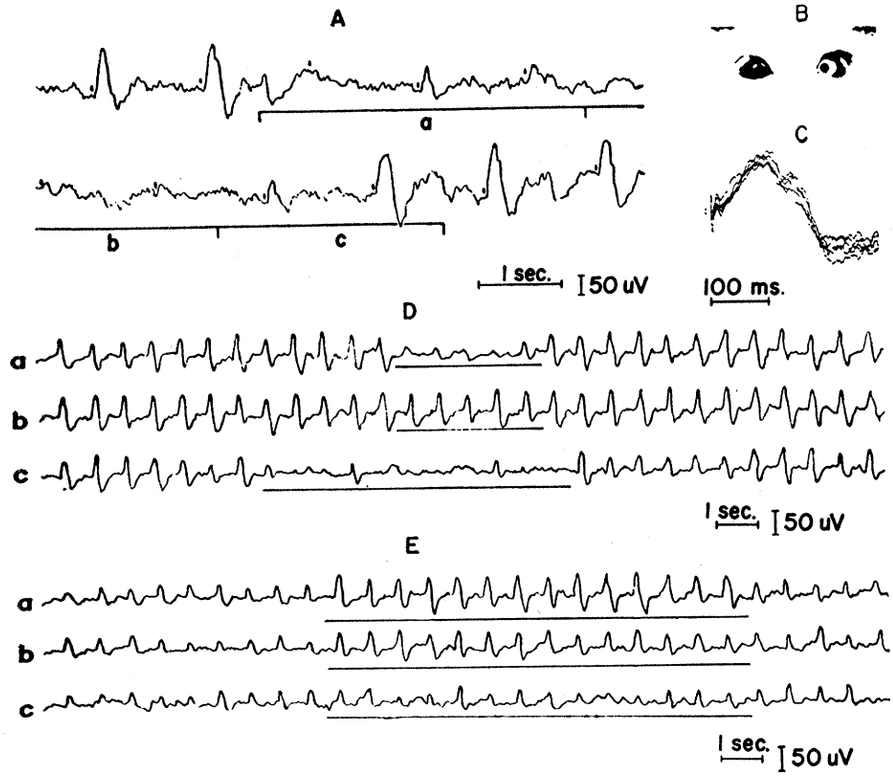

Fig. 1. Electroencephalographic records of two monkeys. *A* and *B*, monkey "Shiro"; *C*, *D*, and *E*, monkey "Goro." (*A*) The records are continuous. The short vertical marks indicate cortical (parietal) stimulation. The recording site is the nucleus of the third nerve. The bar indicates illumination introduced by the experimenter: (*a*) the experimenter not watching the animal; (*b*) the experimenter's gaze fixed; (*c*) the experimenter's gaze turned away from the animal. (*B*) Inward rotation of the eyeballs on stimulation of the recording site. (*C*) Five consecutive cortically induced (sensorimotor) responses in the subthalamus (negative up). (*D*) Bars indicate the first (*a*) and the fifth (*b*) presentations of clicking at five clicks per second and (*c*) sudden dimming of the light in the animal chamber. (*E*) Bars indicate the first (*a*), the third (*b*), and the seventh (*c*) presentations of clicking at 20 clicks per second.

input sometimes produced an entirely different effect on the response (Fig. 1*E*). As soon as a clicking noise was introduced, the amplitude of the response increased significantly, and it remained high throughout the clicking. However, such augmentation or restoration of the response became less and less marked as the novelty of the stimulus gradually decreased through repetition. This type of response modification thus appears to be due to the shift of attention from some previous subject of preoccupation to the novel stimulus given.

If we are to conceive of the flattening of the response by an afferent input as an inhibitory process, the sudden reappearance or restoration of the response by some afferent could be considered a manifestation of "disinhibition." Disinhibition may result from interaction between a previously operating inhibitory process and the novel stimulus. The novel stimulus evokes a shift of attention which in itself produces an inhibitory process. Since this latter inhibition is not observed, the two inhibitory processes must have cancelled each other out. External inhibition in classical Pavlovian conditioning may be a somewhat analogous process, since "unexpected" input preceding the conditioning stimulus inhibits the usual effect (*4*).

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Volatile Factor in Culture of Insect Spermatocytes

Abstract. The in vitro differentiation of insect spermatocytes is critically dependent on the water content of the medium. The postulated "volatile factor" is accounted for in terms of a loss of water from the cultures.

When spermatocytes of diapausing pupae of the Cecropia silkworm are cultured in single hanging or sitting drops of the blood of a developing adult, they develop into spermatids and spermatozoa (*1*). It has been reported (*2*) that these striking cytological events take place only if the cultures are enclosed in a tightly sealed chamber. An examination of this phenomenon (*2*) suggested that the developing spermatocytes produce a "volatile factor" which is necessary for the growth and survival of spermatocytes. The nature of this hypothetical factor has been examined in the present investigation.

Table 1. Effect of daily ventilation of culture chambers on development of spermatocytes.

Cultures per chamber	Gas mixture (%)	Cysts counted (No.)	Developing cysts (%)
	*Cyst suspension I**		
4	Air†	804	22 (18–25)
4	O_2(20), N_2(80)‡	708	26 (12–33)
4	O_2(20), N_2(80)	901	26 (15–37)
4	O_2(10), CO_2(5), N_2(85)	606	34 (25–42)
8	Air†	1220	38 (36–46)
8	O_2(20), N_2(80)‡	1287	33 (24–40)
8	O_2(20), N_2(80)	1519	37 (35–41)
8	O_2(10), CO_2(5), N_2(85)	1659	41 (35–48)
16	Air†	1061	36 (28–55)
	Cyst suspension II		
16	Air†	260	53 (46–58)
16	O_2(2), N_2(98)	391	60 (55–62)
16	O_2(2), CO_2(5), N_2(93)	584	58 (53–63)

* Blood diluted with insect Ringer. † Not ventilated. ‡ Not saturated with water vapor.

The cultures were prepared essentially by techniques which have been described (*2, 3*). Each culture chamber consisted of a Lucite ring sandwiched between two glass plates, the joints being sealed with melted paraffin. The internal volume of each chamber was 16 cm^3. In experiments where the chamber was ventilated, the Lucite ring was provided with inlet and outlet tubes. Up to 16 sitting-drop cultures were placed in a grid pattern in each chamber. The drops were of uniform size, but the number of cysts (*4*) in each drop varied between 50 and 300. In each case the experimental and control chambers were prepared from the same suspension of cysts. After 7 days at 25°C, four or more drop cultures were photographed. The degree of development was assessed from the photographs in terms of the percentage of cysts that had completed the meiotic divisions and had begun to elongate.

Table 1 is a summary of the results obtained in cultures ventilated daily with 125 cm^3 of the specific gas mixture given. The mixtures were saturated with water vapor before they were passed into the chamber, except in the two cases noted. The results in Table 1 show no indication of any harmful effects of the ventilation procedure.

One culture was ventilated continuously for 7 days with a gas mixture of 3 percent oxygen, 5 percent carbon dioxide, and 92 percent nitrogen equilibrated with insect Ringer's solution. The flow rate was 30 to 80 cm^3/hr. The average development in two unventilated control chambers was 25 percent, whereas the ventilated chamber showed an average development of 28 percent.

The concept of a "volatile factor" was initially derived from the observation that single drop cultures undergo little or no development if placed in a chamber in the absence of other cultures (*2*). This finding has been confirmed in the present study. Thus, a single culture enclosed in the 16 cm^3 chamber invariably degenerates. If, however, the single culture is placed in a chamber with 7 or 15 drops of blood, it may show development comparable to a drop culture enclosed in a chamber with 7 or 15 other cultures (Table 2).

In this culture technique, water always evaporates from the cultures and condenses as a visible film on the walls of the chamber. The distillation from the drop cultures is attributed to temperature gradients within the chambers, especially when the latter are examined under the compound microscope. I have also been able to show that the blood of the developing adult is ordinarily at the upper limit of concentration that can be tolerated by spermatocytes;

Table 2. Development of single drop cultures accompanied by drops of blood, compared with development of 8 or 16 cultures of the same cyst suspension.

Cultures per chamber	Cysts counted	Developing cysts (%)
8	1393	29 (25–35)
1*	217	32
8	1220	38 (34–46)
1*	201	30
16	402	43 (31–59)
1†	92	66
16	282	30 (12–53)
1†	289	18

* One drop culture plus 7 drops of blood.
† One drop culture plus 15 drops of blood.