Table 1. Mutations induced in *A. nidulans* by γ-rays.

Dose (kr)	Analyzed strains			Mutations detected in the mutant strains			
	Total	Normal	Mutant	Translocations	Lethals	Translocation lethals	Auxotrophs
30	9	4	5	6	0	1	1
35	3	2	1	0	0	1	0
40	13	3	10	9	1	4	1
50	2	0	2	1	0	1	0

use of the fact that in structurally homozygous diploids all chromosomes can be recovered in the hemizygous condition in mitotic haploids (*1*), or near haploids, produced by the process of mitotic nondisjunction (*2*). The diploid strain used contains several markers which facilitate the selection of such haploids (*3*). It is assumed that single chromosomes which cannot be so recovered carry recessive lethals. Complete linkage between two linkage groups leading to the recovery of two types (reciprocal with respect to the markers on these two linkage groups) instead of four is taken to indicate the presence of a viable translocation [as confirmed in several well-analyzed cases (Käfer, *4*)]. When more than two linkage groups show mitotic linkage, further meiotic and mitotic analysis is needed to decide whether simple translocations affecting a common linkage group or aberration complexes are present.

This type of analysis, therefore, permits the simultaneous determination, in a normally haploid species, of the frequencies of two main types of mutations, recessive lethals and translocations, which has not so far been possible in any microorganism. This will permit a direct comparison of the effects of mutagenic agents on fungus chromosomes with the effects on chromosomes in higher organisms where determination of these frequencies is usually used to analyze genetically the action of different mutagens. The viable nutritional mutants and translocations are automatically mapped as to linkage groups and can be recovered in haploids for further analysis (*5*). Recessive lethals are mapped as to chromosome, but no further isolation and analysis is possible, in contrast to Atwood's method for recovery of recessive lethals from *Neurospora* heterokaryons (*6*). Since the presented method is also somewhat more laborious, it is not very suitable for the detection of rare spontaneous mutations (*7*) but will be of value for the measurement of aberration frequencies after mutagenic treatment of fungus chromosomes.

Twenty-seven strains were analyzed, and the results are summarized in Table 1. The most striking feature of these data (*8*) is the small number of lethals not associated with rearrangements (only one) as compared with viable translocations (16 were detected). Aberrations listed as "translocation lethals" (of which seven were identified) were those cases in which only one homologue of each of two chromosome pairs was recovered. This type of abnormal segregation would also be found if two independent lethals had been induced in a single nucleus, but in view of the low frequency of lethals not associated with a translocation this is not likely for most of these cases. It cannot be decided, however, whether the lethal effect is independent of, or caused by, the translocation.

In either case the frequency of induced viable translocations is very high. These translocations are found in more than half of the diploid nuclei that survived radiation doses no higher than those generally used for induction of markers in microorganisms. However, it has to be taken into account that the treated strains are normally haploid (with a smaller number of chromosomes and chromosome breaks) and are therefore likely to show a lower translocation frequency. All the same, it becomes obvious that strains treated repeatedly with x-ray—for example, with the standard dose of 50 kr in *A. nidulans* (*9*)—have a very high probability of containing chromosomal aberrations.

A further category of induced mutations was discovered in the course of this analysis when survival rates for haploid and diploid conidia were compared to determine suitable doses of irradiation. Counts of visible colonies after 24 hours incubation were very similar, showing about 50 percent survival after treatment with 15 kr and about 1 percent after treatment with 50 kr. Prolonged incubation (up to 8 days), however, yielded at the highest doses, from diploid conidia only, large numbers of abnormal colonies, which exceeded by far the number of normal appearing ones at 50 kr. These mutant types, which often show interesting patterns of segregation and produce normally viable types, appear to contain mutations, probably mostly chromosomal aberrations, with a semidominant lethal effect (*10, 11*). Under conditions of crowding they do not form colonies and are lost along with the dominant lethals.

M. ANNE TECTOR*
ETTA KÄFER

Department of Genetics, McGill University, Montreal, Quebec

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* Present address: Box 213, Graduate Residence Centre, Indiana University, Bloomington.

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Evocation by Fear of a Habit Learned for Electrical Stimulation of the Brain

Abstract. Rats were trained to press a lever for electrical stimulation of the brain. After extinction of the habit, fear-producing stimuli (a buzzer or electrical shock to the feet) recalled the rat to the lever, although its lever pressing on these occasions never produced further intracranial stimulation. Operant levels of the lever-pressing habit were also greatly increased for long periods following the fear-producing stimuli. The phenomenon is most striking in rats trained with tegmental electrodes and is almost completely absent in rats trained with electrodes in the hypothalamus. This suggests that electrical self-stimulation in some sites may be caused by an activation of mechanisms underlying normal fear and escape.

Animals will learn to perform habits for electrical stimulation of certain parts of the central nervous system (*1*). The relation of such phenomena to those of normal drive and reinforcement is suggested by the hunger and sex differential effects reported by Brady (*2*), and by Olds (*3*). A similar but clearer relationship with normal drive mechanisms

Table 1. Effect of brain stimulation in rats on lever pressing. The probability of the difference between the tegmental group and others in the second row is less than 0.004. There is a rank order correlation of 0.9 between degree of recall and electrode placement on the anterior-posterior axis. Each prolonged press in excess of 10 seconds during which the animal "froze" on the lever was scored as ten presses.

Animal No.	Lever presses (No.)			Electrode placement* (de Groot coordinates)		
	After first buzz or shock	Av. No. per recall stimulus	Av. No. in similar control period	A	H	L
			Tegmentum			
27	20	12.0	0.5	3.4	−3.0	0.5
28	14	8.2	.1	3.4	−3.2	1.5
26	18	7.5	.0	2.0	−2.4	0.8
15	16	6.2	.9	4.0	−4.2	1.0
14	6	4.2	.0	3.4	−2.5	1.0
			Hypothalamus			
21	7	2.5	.8	4.8	−3.0	1.75
20	0	1.3	.4	5.0	−3.0	1.5
23	8	0.5	.9	5.8	−3.8	1.0
19	0	.2	.5	6.0	−3.0	2.0
			Septum†			
13	0	.3	.0	8.4?	−1.0?	0.5?

* Histologically verified. † Unfortunately, the anterior part of this animal's brain was destroyed during sectioning.

is shown by our own work which indicates that a habit learned under conditions of electrical self-stimulation can afterwards be evoked independently of such stimulation, solely by fear.

Ten albino Sprague-Dawley rats, of 200 to 300 grams body weight, received implants of bipolar electrodes made of Teflon-coated 0.01-inch Nichrome wire. These were secured in place with stainless steel jeweler's screws and dental cement. Each animal had at least two electrodes, only one of which was used initially in our experiments. The electrodes sites were determined by a Trent Wells stereotactic instrument with reference to de Groot's atlas (*4*) of the coordinates of the rat's brain. The accuracy of our placements was checked histologically. After recovery from the operation, the rats were trained to press a lever in a modified Skinner box. Each press delivered a 60-cy/sec stimulus through a 0.3 megohm resistance in series with the implanted electrode. The duration of the stimulus was limited by a cutout device to half a second for each press. After high rates of stimulation were attained, the rats were repeatedly withdrawn from the lever by hand and released quickly elsewhere in the box. This training was to ensure that the rats learned where the lever was in relation to the rest of the box. Five rats were trained in this way for a tegmental stimulus, four for a lateral hypothalamic stimulus, and one for a stimulus in the basal septum.

After the initial training was complete two types of test were performed. In the first test, after the intracranial stimulation had been discontinued and the rat had lost interest in the lever, a loud buzzer was sounded. If this did not frighten the animal after repeated presentation, the sound was paired with a 300-μa electrical shock to the feet. Both buzzer and shock were of approximately a half second duration. Of the tegmental animals only one was frightened by the buzzer. This animal ran to the lever as soon as the sound of the buzzer stopped and pressed it six times. The other tegmental animals seemed less frightened of the buzzer but returned to the lever following their first experience of shock to the feet. The animals that were trained on the other placements showed no such clear evocation of the lever-pressing habit either by buzzer or shock, but an increase of activity following the shock produced some extra lever pressing in some of them.

The data for this experiment are shown in Table 1. No difference could be detected in the initial rates of lever pressing, between the tegmental and nontegmental animals. The average number of lever presses in control periods without buzz or shock is also given in Table 1. Fear had no significant effect on the rate of reinforced lever pressing.

Lever pressing in the tegmental group might have been a way of avoiding contact with the floor of the cage which had recently acquired aversive properties, but animal No. 26 pressed the lever with its chin and did not lift its feet from the floor bars, while animal No. 14 ran to the lever following the buzzer alone, before it had experienced any shock to its feet.

The second test involved measuring the animals' total number of lever presses during 30 to 45 minutes of extinction. The effect of fear on such prolonged extinction was determined for four of our animals. Fear was produced by three presentations of the buzzer-shock combination. This test was used because we noticed that the lever-pressing habit of the tegmental animals became extremely resistant to extinction after they had been frightened. So far we have quantitative data on only two tegmental and two hypothalamic animals. The ratio of lever presses in the frightened state compared to the unfrightened state was 4.6 and 3.5 for the tegmental animals but 1.8 and 0.98 for the hypothalamic animals. There were also considerable qualitative differences. The tegmental animals tended to "freeze" with a paw pressed down continuously on the lever during extinction. In this state they would always return to the lever and press again when manually removed from it. With time the animals seemed to become less frightened and began to move about the cage again. At this stage the rate of discrete lever pressing tended to rise again in a most dramatic fashion. Such behavior was never observed in the nontegmental animals.

Our results can be explained in terms of Deutsch's theory of learning and electrical self-stimulation (*5; 6*).

J. A. DEUTSCH
C. I. HOWARTH

Departments of Psychiatry and *Psychology, Stanford University, Stanford, California*

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