material extracted from the brain of tolerant animals. However, for reasons still unknown, brain extracts identically prepared from donors subjected to the same treatment show widely varying potencies. To demonstrate the effect of low potency extracts, higher doses have to be given with a longer interval between administration of the extracts and testing of their effect. Not all tests are equally suitable for showing the transfer of tolerance: graded responses are less satisfactory than quantal effects. The shift in the mortality curve of morphine, although less sensitive than the analgesic effect, is particularly suitable for an easy demonstration of the transfer of tolerance. Purification of the transfer factor, although still incomplete, has reached the point where administration of 0.1 to 0.2 μg of material per mouse induces significant tolerance to the analgesic effect of morphine.

1. Ungar, G. and Cohen, M., Intern. J. Neuropharmacol. **5**, 183 (1966).
2. Tirri, R., Experientia **23**, 278 (1967).
3. Smits, S. E. and Takemori, A. E., Proc. Soc. Exptl. Biol. Med. **127**, 1167 (1968).
4. Bianchi, C. and Franceschini, J., Brit. J. Pharmacol. **9**, 280 (1954).
5. Litchfield, J. T. and Wilcoxon, F., J. Pharmacol. Exptl. Therap. **98**, 305 (1949).
6. Ungar, G., *in* (A. Schwartz, ed.), "Methods in Pharmacology" Appleton, New York, in press.

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Lack of Effect of Paralyzation with Gallamine and Decamethonium on Duration of Hippocampal and Amygdaloid After-Discharge (33540)

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Paralyzation with gallamine triethiodide (Flaxedil), but not with decamethonium, increases the duration of cortical after-discharge (1–3). We have previously interpreted this effect of gallamine as being due to an attenuation of the accompanying motor seizure (1). Further, we have suggested that the difference in effect between gallamine and decamethonium lies in their differential effect on muscle spindles (3).

The purpose of the present investigation was to examine the effect of gallamine and decamethonium on the duration of after-discharge elicited from the hippocampus and amygdala. These experiments were suggested by the fact that after-discharge from these brain areas is not accompanied by a motor seizure, at least during early trials (4). Thus it was reasoned that paralyzation with gallamine should not have an effect on their duration.

Methods. Twelve adult cats of both sexes were used in these experiments. The animals were prepared under pentobarbital sodium (25 mg/kg) anesthesia. Bipolar electrodes, consisting of parallel strands of 32-gauge stainless steel wire, bared for approximately 0.5 mm at the tip, were inserted into the dorsal hippocampus of 6 cats and into the lateral amygdala of 6 other animals. Stainless steel screws were threaded into the skull for recording the electroencephalogram (EEG). The electrode leads were externalized via a Winchester plug which was cemented to the skull with dental acrylic.

Preliminary trials to determine the after-discharge threshold were begun after a 2-week recovery period. Each cat was stimulated with a 5-sec train of 1 msec, monophasic square wave pulses at 25 pulses/sec (delivered from a Grass S-4 stimulator via a Grass SIU-4B stimulus isolation unit and Grass CCU-1A constant current unit) starting from 0.5 mA and with increments 0.5 mA. The current was increased until after-discharge was recorded from the site of stimulation. At

least 5 min elapsed between stimuli. The threshold value was used in each subsequent trial. Throughout all trials the animals, restrained only by the confines of their cage, were stimulated only once daily.

The stereotaxic coordinates (5) of the hippocampal electrode were A = 2.0; L = 8.0; Hc = +5.0. Those for the amygdaloid electrode were A = 11.5; L = 11.0; Hc = −5.5. The electrode positions were confirmed following sacrifice of the animals. The EEG was recorded from the medial ectosylvian, posterior sigmoidal, and posterior lateral gyri. All recordings were made on a Grass model III-D electroencephalograph.

After the trial to trial stability of the after-discharge duration was determined (see "Results"), the paralyzation experiments were begun. The animal was given gallamine triethiodide (5 mg kg) or decamethonium bromide (1 mg/kg) intravenously. Artificial respiration (Harvard respirator set at 16 strokes/min) was immediately started. The tidal volume used for each animal was based on a Harvard ventilation graph and was based on the animal's weight.

The after-discharge duration (from the end of the stimulus artifact) was determined from the EEG record. Analyses were performed on the data using a randomized complete block analysis of variance with subsequent testing of the means by Duncan's new multiple range test or by use of a Student's t test (6). For all statistical comparisons, $p < 0.05$ was selected as the significant probability.

Results. After-discharge threshold. The mean current required to elicit an after-discharge from the site of stimulation in the hippocampal group of cats was 1 mA (SE = 0.1). The mean current in the amygdaloid group of animals was 3 mA (SE = 0.8).

Overt seizure pattern. In the early trials no motor seizures, e.g., facial clonus, etc., were observed in either group of cats. Rather, the behavioral pattern during the after-discharge consisted of an arrest of spontaneous activity accompanied by intense staring. In the hippocampal group of cats, vocalization frequently either accompanied or began immediately after the after-discharge.

However, upon repeated trials, motor seizures began to occur in some animals in both groups of cats. Upon their initial appearance they usually consisted of only facial clonus. In later trials, generalized clonic seizures were consistently observed.

In the remainder of this report those trials in which no motor seizure occurred, and the paralyzation experiments associated with them, will be referred to as Phase I experiments. Those trials in which motor seizures were observed, and the paralyzation experiments associated with them, will be referred to as Phase II.

After-discharge duration (Phase I studies). During the 10 trials which preceded the Phase I paralyzation experiments, the after-discharge duration varied little from trial to trial in either the hippocampal or amygdaloid group. The analysis of variance of each of these groups of data showed no evidence for a difference in duration among the trials. However, there was a significant difference among the cats in both groups.

Table I documents the effect of paralyzation by gallamine or decamethonium on the after-discharge duration. As shown, such paralyzation had no effect on the duration of after-discharge elicited from either brain area (Student's t, paired comparison, $p > 0.05$).

After-discharge duration (Phase II studies). Following the development of motor seizures in the hippocampal group, an apparent increase occurred in the trial to trial variability of the mean duration. This apparent increase in variability may be attributed to the fact that motor seizures did not begin to occur in all cats at the same trial. When all of the animals consistently showed generalized clonic seizures, the variability decreased. Statistically, this change in the pattern of mean duration was reflected as evidence being found in the analysis of variance for a difference among trials. However, upon subsequent testing of the means by Duncan's new multiple range test, there was no evidence for a difference between any of the means of the last 11 trials. It was only in these 11 trials that all of the cats consistently exhibited motor seizures.

TABLE I. Effect of Paralyzation by Gallamine or Decamethonium on After-Discharge Duration.

	Phase I experiments[a]				Phase II experiments[a]	
	Hippocampus		Amygdala		Hippocampus	
	Gallamine	Decamethonium	Gallamine	Decamethonium	Gallamine	Decamethonium
Control[b,c]	33 ± 8.0	37 ± 7.1	19 ± 6.5	19 ± 3.2	56 ± 14.7	60 ± 17.9
Paralyzed[b]	33 ± 6.8	36 ± 7.5	19 ± 7.2	22 ± 4.3	52 ± 11.8	69 ± 21.5
N	6	4	4	4	3	3

[a] See text for definition.
[b] Values are mean duration (sec) ± SE.
[c] Trial immediately preceding that in which animal was paralyzed.

The effect of paralyzation during the Phase II experiments is shown in Table I. Again paralyzation with neither agent had any effect on after-discharge duration.

Similar experiments were not performed on the amygdaloid group of cats due to the marked variability in their response. After motor seizures developed in this group, the animals frequently failed to show any after-discharge in a given trial even if the stimulating current was increased up to fourfold over the threshold current. However, in the next trial they would exhibit after-discharge accompanied by a generalized clonic seizure at the previously used threshold current. As the trial(s) in which no after-discharge could be elicited could not be predicted, it was felt that paralyzation experiments would not be meaningful.

Discussion. The lack of effect of paralyzation with decamethonium on the duration of hippocampal and amygdaloid after-discharge is similar to that previously reported for cortical after-discharge (3). However, the lack of effect of gallamine is perhaps surprising for two reasons.

First, Andy and Akert (7) have reported an increase in the duration of hippocampal after-discharge following paralysis with *d*-tubocurarine. Thus one might expect an increase in duration following paralysis with gallamine as these two agents are usually considered to exert similar pharmacological effects. However, the conclusion of Andy and Akert (7) may be more apparent than real as (a) they did not state the number of animals in which this effect was observed, and (b) no statistical analysis was applied to their data.

Secondly, we have previously speculated (3) that the mechanism through which gallamine prolongs cortical after-discharge is via blockade of inhibitory phasic activity arising in the muscle spindles during a motor seizure. If such is the case, one would expect that an increase in duration would have occurred following paralyzation with gallamine in the Phase II trials, i.e., those in which generalized motor seizures occurred.

However, the brain mechanisms and/or sites involved in a motor seizure are, at best, imperfectly understood. Previous investigators (cf. 8, 9) reported that generalized motor seizures occur in animals with lesions in pyramidal pathways. Thus, it was concluded that the main pathways for the development of a motor seizure are via extrapyramidal structures.

However, studies demonstrating that the same pathways are involved in generalized motor seizures elicited from different sites are lacking. It is not unreasonable to suspect that they may differ depending on the site of seizure origin as it is known that seizures elicited from different brain areas can spread preferentially to different subcortical sites (10). If such is the case a neuronal feed back mechanism from the periphery may not affect motor seizures of cortical and hippocampal origin in the same manner. Thus, blockade of such a mechanism would not affect the duration of all motor seizures. It is

obvious, however, that further definitive studies are necessary to understand what the peripheral mechanisms and/or paralyzation with neuromuscular blocking agents may play in regulating seizure duration.

Summary. The effect of paralyzation by gallamine or decamethonium on the duration of after-discharge elicited from the hippocampus or amygdala was examined in a chronic cat preparation. Neither neuromuscular blocking agent had any effect on the duration of after-discharge elicited from either brain area in the absence of a motor seizure. In addition, paralyzation with either agent did not affect the duration of at least hippocampal after-discharge in the presence of motor seizures.

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1. Straw, R. and Mitchell, C., Intern. J. Neuropharmacol. 5, 323 (1966).

2. Halpern, L. and Black, R., Science 155, 1685 (1967).

3. Straw, R., Electroenceph. Clin. Neurophysiol. 25, 69 (1968).

4. Delgado, J. and Sevillano, M., Electroencephalog. Clin. Neurophysiol. 13, 722 (1961).

5. Jasper, H. and Ajmone Marson, C., "A Stereotaxic Atlas of the Diencephalon of the Cat." Res. Council of Canada, Ottawa (1954).

6. Steel, R. and Torrie, J., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).

7. Andy, O. and Akert, K., J. Neuropathol. Exptl. Neurol. 14, 198 (1955).

8. Mettler, F. and Mettler, C., J. Neurophysiol. 3, 527 (1940).

9. Walker, A. and Richter, H., Arch. Neurol. 8, 581 (1963).

10. Poggio, G., Walker, A. and Andy, O., Arch. Neurol. Psychiat. 75, 350 (1956).

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Studies on the Infectivity of Rat Virus (RV) in BALB/c Mice* (33541)

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In 1959, Kilham and Olivier (1) reported the isolation from tumor-bearing rats of three serologically identical strains of a new virus resembling polyoma in many respects. This agent, designated as rat virus (RV), has subsequently been shown to be a small DNA virus, closely related to members of the H-virus group, which were also recovered from malignant tissues (2). The biologic properties of RV have been extensively investigated in various species of experimental animals, and although the rat is apparently the natural host for this virus, the most pronounced effects have been observed after infection of suckling hamsters. In the hamster, RV may cause either (a) an acute fatal disease, (b) mongolism and developmental anomalies, or (c) an inapparent latent infection (3, 4). A similar range of reactivity has been observed after infection of A × C strain rats (5, 6). Dental abnormalities in hamsters (7) and rats (6, 8), and cerebellar hypoplasia in hamsters (9), rats (10), and cats (11) are also known to occur after infection with RV. However, there have been no reports of either induced disease or confirmed RV infection in the mouse. In their original report (1), Kilham and Olivier noted that no apparent illness resulted from injection of RV into suckling or weanling Swiss or C3H mice, and to the best of our knowledge, this subject has not been pursued further. Consequently, it is the purpose of this paper to describe recent

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