References and Notes

1. P. D. Parkman, E. L. Buescher, M. S. Artenstein, *Proc. Soc. Exptl. Biol. Med.* **111**, 225 (1962).
2. T. H. Weller and F. A. Neva, *ibid.* **111**, 215 (1962).
3. B. Sigurdardottir, K. F. Givan, K. R. Rozee, A. J. Rhodes, *Can. Med. Assoc. J.* **88**, 128 (1963).
4. J. L. Riggs and G. C. Brown, *Proc. Soc. Exptl. Biol. Med.* **110**, 833 (1962).
5. G. C. Brown, unpublished data.
6. Baltimore Biological Laboratory.
7. J. L. Riggs, P. C. Loh, W. C. Eveland, *Proc. Soc. Exptl. Biol. Med.* **105**, 655 (1960).
8. H. F. Maassab, J. A. Veronelli, A. V. Hennessy, American Society for Microbiology, 64th Annual Meeting, May, 1964, *Bacteriol. Proc.* (1964), p. 144.

9. Supported in part by PHS grant AI 05876 from the National Institute of Allergy and Infectious Diseases, NIH, and in part under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board and by the Office of the Surgeon General, U.S. Army, Washington. We thank Mrs. Margaret Jakobovitz for her skillful assistance.

11 June 1964

Poliovirus: Guanidine Dependence and Loss of Neurovirulence for Monkeys

Abstract. *Guanidine-dependent polioviruses are obtained in vitro by subculturing Brunenders, Mahoney, and Sabin strains in the presence of increasing concentrations of guanidine. Mahoney viruses dependent on guanidine lose virulence (as indicated by paralysis) for monkeys inoculated intramuscularly or intracerebrally. Protection against virulent Mahoney viruses is induced by treatment with guanidine-dependent strains, and serum antibodies against the virulent strains are present in the treated animals.*

Guanidine inhibits the growth of poliovirus in HeLa cells (*1*). This property of guanidine was recognized independently by Rightsel *et al.* (*2*) and by Melnick *et al.* (*3*).

Table 1. Guanidine dependence induced in poliovirus 1, Brunenders strain (1S), propagated in HeLa cells. The results are expressed in cytopathic units which were calculated by the end-point method.

Modified poliovirus strain	Guanidine HCl in culture medium (μg/ml)		
	0	200	1000
1S	10^8	10^3	10^3
4G*	10^6	10^6	10^6
28G†	10^5	10^9	10^7
52G‡	10^3	10^8	10^8
108G§	10^3	10^3	10^6

* 4G is 1S propagated once with guanidine HCl at 62.5 μg/ml and three times at 250 μg/ml. † 28G is 4G propagated 24 times with guanidine HCl at 250 μg/ml. ‡ 52G is 28G propagated 24 times with guanidine HCl at 1000 μg/ml. § 108G is 52G propagated 56 times with guanidine HCl at 1000 μg/ml.

Experiments originally designed to clarify the nature of the guanidine inhibition revealed that poliovirus strains, after a number of transfers with increasing concentrations of the drug in HeLa cell cultures, became thousands of times more resistant to guanidine than the original virus. After further transfers the virus became guanidine-dependent, growing only in the presence of high concentrations of guanidine (Table 1) (*4*).

This unique dependency of poliovirus for exogenous guanidine has been confirmed recently by Lwoff (*5*). By repeated subculturing (*4*) we have developed guanidine dependency in a number of poliovirus strains, including the Brunenders, Mahoney, and Sabin strains (Table 2).

Since the concentration of guanidine required for growth by a guanidine-dependent poliovirus is much higher than that present in mammalian cells and fluids, we considered the possibility that the guanidine-dependent poliovirus might be nonpathogenic for mammals. Therefore we infected cercopithecus monkeys (*6*) with a virulent Mahoney strain of poliovirus type 1, or with the same strain made guanidine-dependent.

The results (Table 3) demonstrate the absence of paralysis in monkeys injected intramuscularly with the guanidine-dependent virus. Intracerebral inoculation gave similar results.

The immunizing effect of injection of guanidine-dependent virus is reflected in the development of serum antibodies and protection against subsequent challenge with virulent Mahoney virus. Two monkeys injected intramuscularly once, and two injected twice (28 days apart) with the guanidine-dependent virus (3×10^8 CPU) were then injected (challenged) intramuscularly, 19 days after the last inoculation, with five to ten paralyzing doses of the original, virulent strain. These four monkeys showed no signs of paralysis 1 year from the time of the viral infection. At the same time two control monkeys, which had not received the guanidine-dependent virus, were injected intramuscularly with comparable doses of the untreated strain. Both animals developed paralysis, one 4 days and the other 11 days after inoculation.

This finding prompted us to study the Sabin poliovirus strains 1, 2, and 3. These strains can also be made highly guanidine-dependent in vitro (Table

Table 2. Guanidine dependence induced in various poliovirus strains, propagated in HeLa cells. The results are expressed in cytopathic units which were calculated by the end-point method.

Modified poliovirus strain	Guanidine HCl in culture medium (μg/ml)		
	0	200	1000
Polio 1 Bruenders			
Control	10^8	10^3	10^3
108G*	10^3	10^3	10^6
Polio 1 Mahoney			
Control	10^8	10^3	10^2
25G†	10^4	10^5	10^8
Polio 1 Sabin Lsc 2 ab‡			
Control	5×10^6	10^8	10^2
40G§	10^3	10^6	10^7
Polio 2 Sabin P 712, Ch, 2 ab‡			
Control	10^6	10^2	10^2
40G§	10^3	3×10^5	10^7
Polio 3 Sabin Leon 12 ab‡			
Control	10^6	10^3	10^2
40G§	2×10^3	10^5	3×10^7

* 108G is 52G propagated 56 times with guanidine HCl at 1000 μg/ml (see Table 1). † Polio 1 Mahoney propagated twice at each of the following concentrations of guanidine HCl: 10 μg/ml, 20 μg/ml, 40 μg/ml, 100 μg/ml, 200 μg/ml, 1000 μg/ml; and 13 times at 2000 μg/ml. ‡ Virus strains obtained by the Instituto Superiore di Sanità. § Polio Sabin propagated once with guanidine HCl at 10 μg/ml and once at 20 μg/ml; twice at concentrations of 40 μg/ml, 100 μg/ml, 200 μg/ml, and 500 μg/ml; and 30 times at 1000 μg/ml.

Table 3. Lack of neurovirulence of guanidine-dependent (g.d.) poliovirus 1 Mahoney injected into 25 monkeys (*Cercopithecus aethiops*). The dosages, expressed in cytopathic units (CPU), were calculated by the end-point method. Numbers in parentheses indicate the number of monkeys injected with each dose; i.m., intramuscular injection; i.c., intracerebral injection.

Treatment of strain injected	Onset of paralysis (days after last injection)	Virus isolated from spinal cord*	Reciprocal of serum antibody titer†	
			27–28 days after 1st injection	19 days after 2nd injection
3×10^6 to 3×10^8 CPU, i.m. (7)				
No	4 to 11	+		
3×10^5 CPU, i.m. (1)				
No	None‡			
3×10^8 CPU, i.m. (5)				
25G, g.d.	None‡		5 to 25	
Two, 28 days apart, of 3×10^8 CPU, i.m. (2)				
25G, g.d.	None‡		125; 625	3,125; 15,625
10^3 to 5×10^4 CPU, i.c. (4)				
No	8 to 9	+		
Dose: 10^2 CPU, i.c. (1)				
No	None‡			
4×10^5 to 4×10^6 CPU, i.c. (4)				
25G, g.d.	None‡		5	
4×10^6 CPU, i.c. (1)				
25G, g.d.	12	+		

* Monkeys with paralysis were killed 1 to 4 days after paralysis developed. † Antibody tests, performed on the preinoculation specimens, gave no titer. ‡ No paralysis after 1 year's observation.

2). Guanidine-dependent viruses may prove useful in producing immunization against poliomyelitis.

B. LODDO, G. BROTZU, A. SPANEDDA
G. L. GESSA, W. FERRARI
Departments of Pharmacology, Hygiene, and *Microbiology, University of Cagliari, Cagliari, Italy*

References and Notes

1. B. Loddo, *Boll. Soc. Ital. Biol. Sper.* **37**, 395 (1961); ———, W. Ferrari, G. Brotzu, A. Spanedda, *Boll. Ist. Sieroterap. Milan.* **41**, 111 (1962); *Nature* **193**, 97 (1962).
2. W. A. Rightsel, J. R. Dice, R. J. McAlpine, E. Timm, J. M. McLean, Jr., G. L. Dixon, F. M. Schabel, *Science* **134**, 558 (1961).
3. J. L. Melnick, D. Crowther, J. Barrera-Oro, *ibid.*, p. 557.
4. B. Loddo, *Boll. Soc. Ital. Biol. Sper.* **38**, 1043, 1047 (1962); ———, W. Ferrari, A. Spanedda, G. Brotzu, *Experientia* **18**, 518 (1962).
5. A. Lwoff and M. Lwoff, *Compt. Rend.* **256**, 5228 (1963).
6. *Cercopithecus aethiops.*
7. We thank B. B. Brodie for his interest in this work and for excellent advice.

14 January 1964

Synchronous Sensory Bombardment of Young Rats: Effects on the Electroencephalogram

Abstract. *Rats were exposed to intense five-per-second synchronous clicks and flashes from birth to adulthood. Electroencephalographic recordings showed an abnormally high incidence of high-voltage burst activity in the visual cortex and thalamus of the animals; wave frequency within the bursts was five per second instead of the normal seven per second.*

It is nearly a century since the electrical activity of the brain was first studied, and, although considerable progress has been made in the last 35 years, some of the basic phenomena are not yet well understood. For instance, we still do not know the exact basis of the oscillations in recorded potential, nor do we know why the oscillations occur at the rate they do. This report is concerned with the second of these problems.

It is usually assumed that the frequency of brain rhythms depends almost entirely on the intrinsic organization of the nervous system. We have tried to find out what happens when rats are subjected to strong repetitive synchronous bombardment of two sensory systems during their early life. Such treatment might be expected to control the activity of large groups of neurones in the sensory areas and in other parts of the brain, and, by keeping these cells busy, prevent them from being integrated into the normal temporo-spatial organization. We were particularly curious to know whether we could establish a persistent rhythmic activity which would be determined by the rate of our synchronous stimulation.

Twelve hooded rats were reared from birth in a white box measuring 60 by 45 by 45 cm where they were constantly exposed to 10-msec flashes of light and synchronous clicks. The flashes were produced by a Grass model PS-2 photic stimulator, and the synchronous clicks were made by amplifying the pulses from the monitored output of the stimulator and passing them to a speaker attached to the box. The stimulation rate was five per second, the intensity of the clicks was of the order of 90 db (ref. 0.0002 dyne/cm^2), and the intensity of the photostimulator was rated by the manufacturer at 1.5 million candle power. Twenty-four control animals were reared in boxes of the same size. Half were kept in complete darkness; the other half were exposed for 1 hour to diffuse light and white noise each day (roughly the sum total time of the durations of the individual clicks or flashes to which the experimental animals were subjected). A "split litter" technique was used in assigning rats to these three groups so as to control genetic factors (each litter was divided at random and a third of each litter placed in each group), and the mothers were rotated between groups every 3 days.

Unfortunately, a failure in the air-conditioning system killed all the control animals after we had recorded from only two animals in each control group. Accordingly, a third group of eight rats reared in normal laboratory cages was used. Since no difference in brain activity was apparent between these eight animals and the four remaining from the original control groups, the results from all 12 subjects were pooled.

When the rats were 3 to 6 months old, bipolar electrodes made of 0.25 mm enameled Nichrome wire were implanted in the visual cortex and various subcortical structures; the rats were ultimately killed, and the placements were verified histologically. The animals were then allowed to recover for a week in the boxes in which they had been reared. Each rat in turn was then placed in a restraining sling of the type described by Kimura (*1*), and a light-diffusing dome was placed over its head. After an adaptation period of 1 hour, recordings were made for 52 minutes.

The test procedure was as follows. Control records were taken for 4 minutes. The rat was then photically stimulated at the rate of one flash per second for 2 minutes. This was followed by a 2-minute dark, control period. After this came stimulation by two flashes per second, followed by another 2-minute dark period, and so on until the rat had been exposed to stimulation at frequencies from one to nine flashes per second. Then came a 6-minute dark, control period. During the final 8 minutes of the session, 15 light flashes were presented, one every 30 seconds; only ten of the experimental rats were subjected to this procedure. This somewhat elaborate routine was followed to determine among other things whether the experimental treatment had any effect on photic driving. However, the data we are concerned with at present are based on the 26 minutes without stimulation, and on the last 8 minutes when 15 light flashes were presented.

It is known that light flashes cause a late response in the visual cortex of the unanesthetized rat (*1*). This response consists of spindle-shaped bursts of high-amplitude waves which generally have a wave and spike form; this was seen in all of the experimental and control animals. However, the late response of the experimental animals differed from that of the controls in two ways. First, it could be more easily triggered. For the 15 test flashes given each animal, the mean number of late responses was 11.6 for the experimental rats (range 10 to 13) and 3.2 for the controls (range 1 to 6). Second, measurement of the frequency of each individual wave in each response showed that the modal frequency for eight of the ten experimental rats tested was five waves per second, while for all the controls (and the remaining two experimental rats) it was seven per second. Typical responses are shown in Fig 1*A*.

The most striking difference between the groups, however, was in the spontaneous spindle-shaped bursts of waves, resembling the late response, which were seen in the tracings from the visual cortex of most animals. These bursts occurred, on the average, eight times as often in the experimental group (mean number of occurrences for the 26-minute observation period, 57.4; range, 24 to 28) as they did in