longed incubation periods in mice that had been inoculated intraperitoneally with scrapie when they were born, and we also thought that a developing spleen cell might be needed for replication of the agent.

The hypothesis that after intraperitoneal inoculation the primary site of scrapie replication is strictly limited to the T cells could be readily tested within a year or so by following scrapie replication in T cell–deficient mice, such as the nude strain, both with and without reconstitution of the T cell system. Alternatively, the scrapie permissive spleen cell may be a type other than the T cell, which is susceptible to the same maturation stimulus. In another species, the rhesus monkey, the scrapielike virus of transmissible mink encephalopathy has been found in the brain of a clinically normal animal almost 3 years after it had received oral, intramuscular, and intravenous injections of the virus (*15*). Athymic (nude) mice injected intraperitoneally with hamster-adapted "Chandler" scrapie agent have been found by Marsh (*15*) to exhibit scrapie virus in the spleen for 26 days after injection, the titers being comparable to similarly inoculated normal mice at that time. In any event, careful studies of the existence of this phenomenon in other strains of mice, including the nude, are clearly indicated.

The absence of detectable virus in the animals inoculated at birth, coupled with the normal susceptibility of these animals to challenge, shows that the scrapie virus is present in a nonreplicating latent state for about 1 year. Thereafter, in response to an unknown stimulus, the latent agent becomes active, and detectable scrapie replication begins and appears to follow its usual course to the production of clinical disease. We suggest that latency occurs in the absence of a suitable T cell population when infection is limited to an alternative cell type. During the prolonged latent period, very slow replication occurs until, eventually, there are sufficient virus particles available for the random infection of susceptible T cells. The rate of replication then increases and leads to the neurological disease. This might explain the observed cases of "spontaneous" scrapie in supposedly normal animals (*16*).

These results have powerful implications for man, since scrapie pathogenesis is so similar to that of the human spongiform encephalopathies (SE), including Creutzfeldt-Jakob disease and kuru (*4*). Our results suggest that appropriately timed neonatal or prenatal infection of host species with some strains of SE virus would induce the latent state, with some incubation periods extending as far as old age. In this respect, the parallels which have been drawn between scrapie, Creutzfeldt-Jakob disease, and senility (*5*) imply that variants of the SE group could well cause some of the effects usually regarded as genetically induced old age. If vertical transmission of the agent can occur, as is believed to happen with scrapie in sheep, it would be hard to distinguish the latent SE virus from a gene causing old age. In this view, the earlier conclusion of Parry and others (*17*) that scrapie in sheep is both a hereditary and a transmissible disease seems more conceivable and also may apply to the human situation.

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Virus-Induced Behavioral Alteration of Mice

Abstract. *Neonatal mice were inoculated intracerebrally with lymphocytic choriomeningitis virus (LCM). These mice developed long-term persistent tolerant infections and when tested at 3.5 to 6.0 months of age they showed significant increases in behavioral latency when subjected to open-field tests, and significant decreases in the current level required to elicit a startle response and in locomotor activity in a running wheel. Comparable results were obtained with mice in which persistent infection was induced at 8 weeks of age and which were tested at 3.5 to 6.0 months of age. It was concluded that mice infected with LCM at birth or as adults exhibited long-lasting behavioral abnormalities.*

That virus infections of the nervous system can cause severe behavioral abnormality is well established. The ability of the rabies virus to induce pathological rage, and the more recent knowledge of the mistaken diagnoses of psychosis in some cases of human slow-virus encephalitis (*1*) have strengthened the concept that chronic virus infection of the brain may cause severe behavioral disorders. We have studied long-term persistent tolerant infection in mice with lymphocytic choriomeningitis (LCM) virus, using mice inoculated with LCM virus either at birth or at 8 weeks of age. Mice injected at birth demonstrate immunological tolerance of the virus in spite of high titers in all organs (*2*); those inoculated later exhibit viremia and are similar in most ways (*3*) to those infected at birth. Preliminary behavioral tests with the mice inoculated at birth (neonatal model) (*4*) suggested that mice persistently infected with LCM virus exhibit subtle behavioral differences when compared with uninfected controls. The results described here confirm and extend the early work and appear to establish a clear role for virus infection in the origin of some behavioral abnormalities.

The mice used were from a colony of the outbred Nya : NYLAR albino strain maintained at this laboratory. All animals used for the neonatal model received within 24 hours of birth an intracerebral inoculation (0.03 ml) of a suspension of brain tissue infected with the LM_4 (*5*) strain of LCM (0.03 ml containing 10^5 ID_{50}, where ID_{50} is the infective dose for 50 percent of the population); controls received 0.03 ml of a comparable dilution of normal mouse

brain suspension in 0.04 percent gelatin in tris-buffered Hanks solution (0.01*M*). Each litter was culled to eight pups and left undisturbed until weaning at day 21. At this time, they were segregated by sex and housed in groups until they were tested between 3.5 and 6 months of age. Animals used for the adult model received at 8 weeks of age an intracerebral inoculation of the MB_7 strain of LCM (0.03 ml containing 10^5 ID_{50}); 3 days later each mouse received an intraperitoneal injection of cyclophosphamide (150 mg/kg) (to convert the acute infection to a persistent one). Controls received normal mouse brain diluent intracerebrally and cyclophosphamide intraperitoneally as described above.

For the behavioral tests we used an open-field procedure, startle thresholds in response to electric shock, and measurements of locomotor activity in running wheels.

Open-field testing was carried out in a unit consisting of a black floor divided by thin white lines into a matrix of 49 4-inch (10 cm) squares. The unit was surrounded by walls that were 12 inches (30 cm) high. The test animal was placed in an enclosed 4-inch square formed by two corner walls and a removable L-shaped bracket, 12 inches high. After 30 seconds the barrier was removed, and the subject was observed for 3 minutes; the test was performed once daily for 4 days. The variables measured included latency to first ambulation, the number of squares crossed, and the number of fecal pellets dropped. The test field was washed down with 1 percent aqueous sodium hypochlorite and dried between trials in order to minimize distracting olfactory cues. Analysis of variance was performed on all results; those which were significant are summarized in Tables 1 and 2.

Table 1. Significant statistical comparisons of behavioral measures between mice given persistent LCM infection at birth (neonatal model) and controls, tested at 3.5 to 6 months of age.

Statistical comparison	*F* ratio	d.f.	*P*
	*Open-field latency**		
Virus	9.18	1/101	< .001
Sex	9.72	1/101	< .001
Day of test	3.62	3/303	< .01
Virus and sex interaction	9.52	1/101	< .001
Virus and day interaction	4.42	3/303	< .01
Sex and day interaction	5.00	3/303	< .01
Virus, sex, and day of test	5.35	3/303	< .01
	*Open-field ambulation**		
Sex	4.95	1/101	< .05
Day of test	57.99	3/303	< .001
Virus and day of test interaction	3.65	3/303	< .05
	Startle threshold (female)†		
Virus	16.37	1/18	< .001
Day of test	9.08	3/54	< .001
	Startle threshold (male)‡		
Day of test	10.94	3/84	< .001
Virus and day of test interaction	4.20	3/84	< .001
	Running wheel§		
Virus	4.16	1/64	< .05
Day of test	11.00	3/192	< .001
Virus, day of test, and sex interaction	3.41	3/192	< .05
Sex, age, and day of test interaction	7.00	3/192	< .01
Virus, age, and day of test interaction	5.55	3/192	< .01

*N = 107. †N = 20. ‡N = 30. §N = 72.

Table 2. Significant statistical comparisons of behavioral measures between mice given persistent LCM infection at 8 weeks (adult model) and controls, tested at 3 to 6 months of age.

Statistical comparison	*F* ratio	d.f.	*P*
	*Open-field latency**		
Day of test	9.01	3/108	< .001
	*Open-field ambulation**		
Virus	4.63	1/36	< .05
Day of test	66.50	3/108	< .001
	Startle threshold (male)†		
Virus	5.21	1/28	< .05
Day of test	12.33	1/84	< .001
	Running wheel‡		
Virus	4.82	1/52	< .05
Day of test	12.90	3/156	< .001

*N = 36. †N = 30. ‡N = 54.

Data obtained during the open-field tests were analyzed for latency and ambulation scores. The mice that had been inoculated with LCM within 24 hours of birth took significantly longer to move than did control mice (Fig. 1a). This effect was due to an increase in latency in the infected males, and it demonstrated an interaction between the effects of the virus and the sex of the animal. This effect was shown to be significant by analysis of variance (Table 1). There was also an interaction between virus and day of testing, as evidenced by the changing male latency scores when the test was repeated on four successive days. Similar but less well-marked effects were observed for ambulation scores (Fig. 1b). Males were less active than females and controls, with ambulation decreasing with repeated tests. No significant differences were noted for defecation scores.

No significant differences were noted in latencies during open-field tests between mice of the adult model and control mice (Fig. 2a). However, the control mice entered significantly more squares than did the experimental animals; infected mice thus showed a significant change in behavior, and their ambulatory scores decreased on repeated testing (Fig. 2b and Table 1).

For the startle threshold tests, animals were placed in an electrified-grid shock box and stimulated by means of a constant low-current shock generator (*6*). The animals' startle thresholds were determined by the method of limits, the current being increased in 5-μa steps. Each animal received four trials (each trial defined as a response to shock) on each of four successive days. A blind technique was used in which neither the experimenter delivering the shock (from a separate room) nor the observer knew whether the animal being tested was infected with LCM virus or was a normal control. The correlation between observer scores was very high (r = 0.96), and the number of false positives (the observer noting a response when no shock was delivered) was extremely low (0.02 to 0.05 percent).

Male and female mice that had been inoculated at birth (neonatal model) were tested separately, and the infected female mice responded at significantly low-

er levels of current than did control mice. In addition, the current level required to elicit a startle response changed significantly with time after the first test. Similar effects were noted for the male test animals, although the main effect of the virus infection was not significant at the 0.05 level ($F = 3.73$, 1/28 d.f., $P < .10$), and all the male animals were more sensitive than females.

A similar testing and analysis procedure was conducted on the male mice of the adult model. The results (Table 2 and Fig. 2c) show that infected mice responded at a significantly lower current threshold than controls, and that the current level required to elicit a startle (for all groups) increased as a function of repeated tests.

Measurements of locomotor activity in Wahmann running wheels were made in order to determine whether virus infection produced behavioral changes in the absence of stress. Male and female mice that had been inoculated with LCM virus at birth were tested at either 15 or 22 weeks of age. Individual animals were allowed continuous access to a running wheel from an attached holding cage for 4 days. The primary variable analyzed was the total number of wheel revolutions per day. A square-root transformation to normalize the data (7) was carried out prior to analysis.

Table 3. Ratio of scores for behavior of LCM-infected mice to control mice.

Measure	Model	
	Neonatal	Adult
Body weight	0.82	0.84
Running-wheel activity	0.89	0.91
Electric shock	0.81	0.79
Open-field latency	2.72	1.48
Open-field ambulation	0.88	0.70

Virus-infected mice were significantly less active than controls (Fig. 1d and Table 1) with locomotor activity changing over time. No differences in levels of activity were noted between male and female or between 15- and 22-week-old animals, although significant three-way interactions among virus, sex, and day of test, and virus, age, and day of test were noted. The same test procedure was used for mice inoculated as adults, and similar results were obtained (Fig. 2d and Table 2). However, when corrections were made for the lower body weight of infected mice (the ratios of infected to control body weights were 0.82 for mice of the neonatal model and 0.84 for those of the adult model), the infected mice ran, respectively, 8.68 and 7.95 percent more than control mice.

Our data from the open-field tests show that infected male mice are more

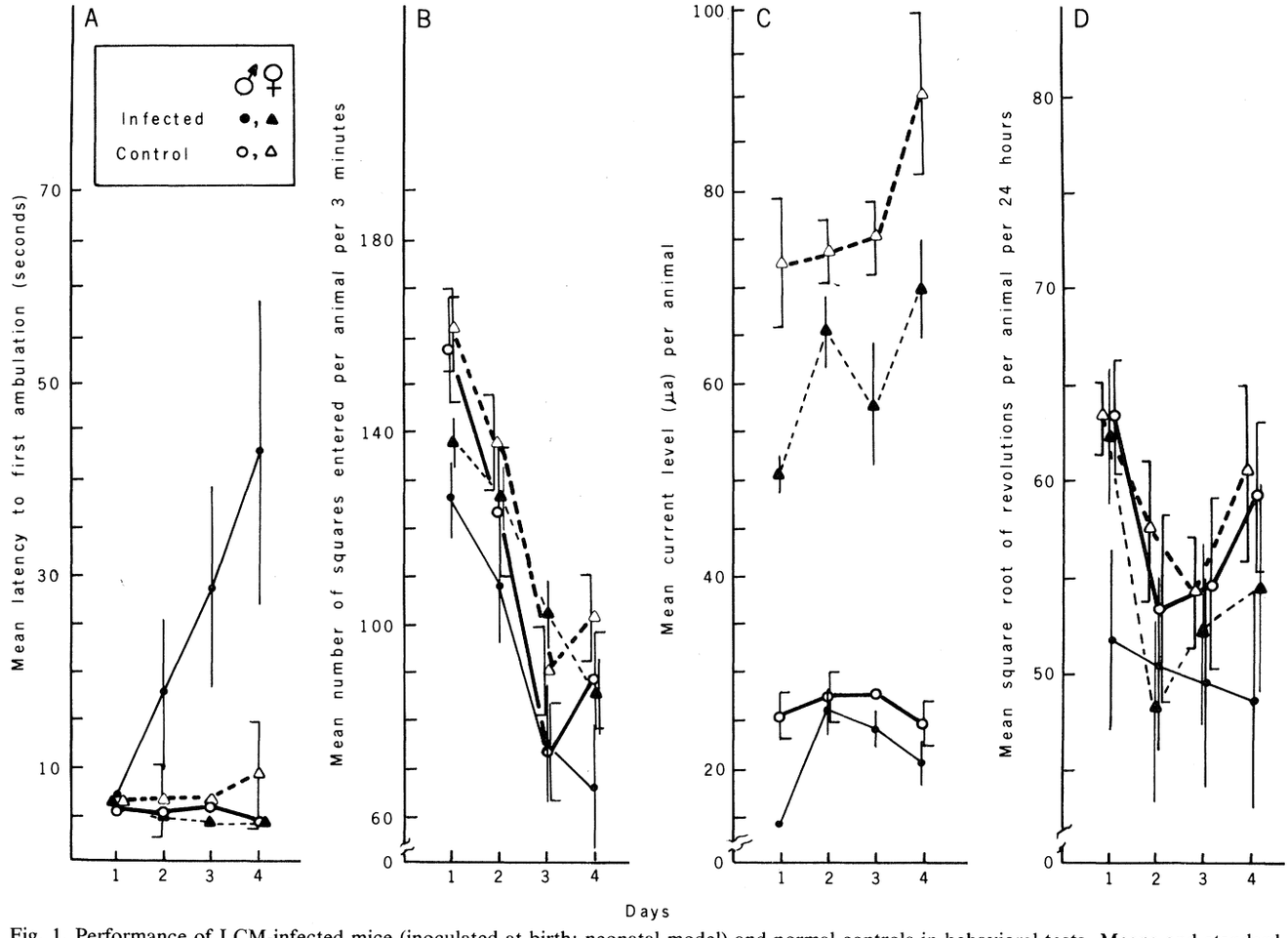

Fig. 1. Performance of LCM-infected mice (inoculated at birth: neonatal model) and normal controls in behavioral tests. Means and standard errors of the means are shown, except that for clarity of presentation, standard errors less than 2.0 are not shown. (A) Open-field tests: latency to first ambulation. Each trial lasted 3 minutes. The test groups were 20 infected males, 19 control males, 35 infected females, and 33 control females. (B) Open-field tests: the number of squares entered in 3 minutes [with the same test groups as in (A)]. (C) The current level required to elicit a startle response. The mean response for each animal was determined from four trials per day. The test groups were 15 infected males, 15 control males, 10 infected females, and 10 control females. (D) Running-wheel revolutions expressed as a square-root transformation. The test groups were 18 infected males, 18 control males, 18 infected females, and 18 control females.

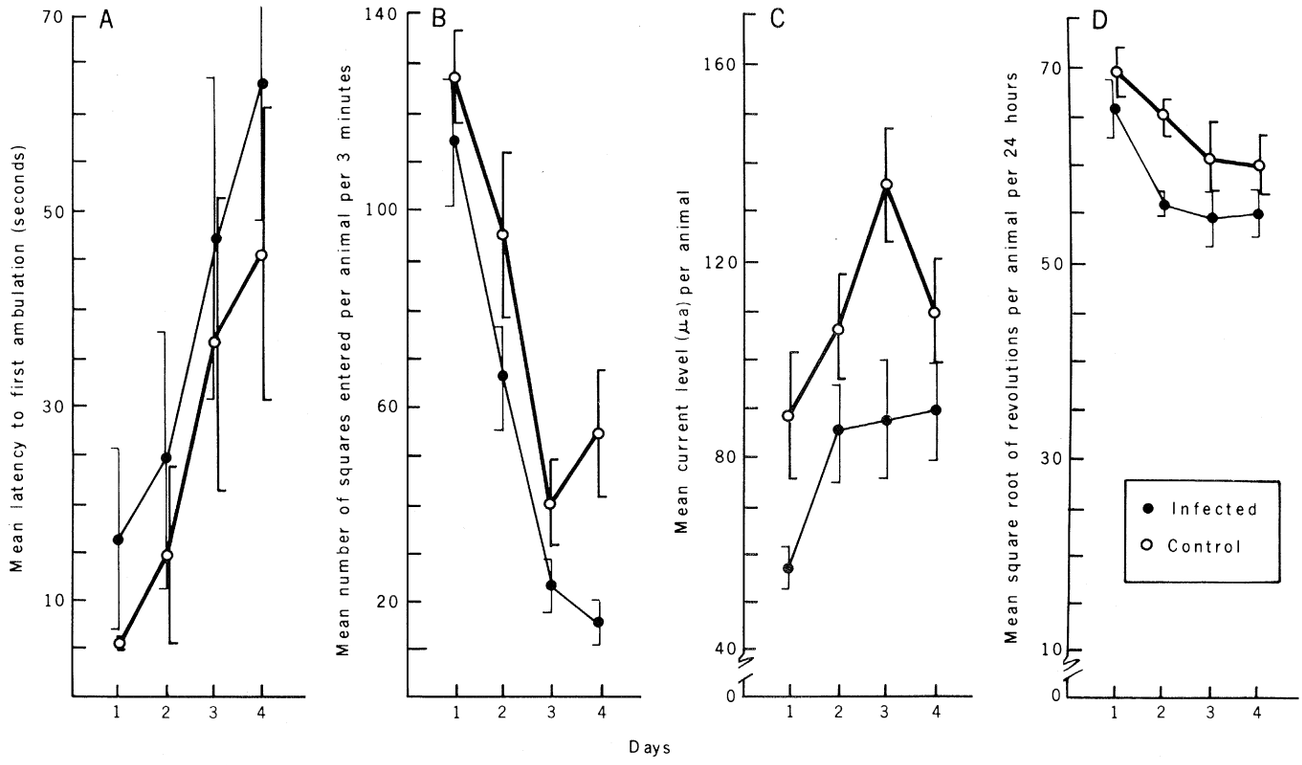

Fig. 2. Performance of LCM-infected mice (inoculated at 8 weeks: adult model) and normal controls in behavioral tests. Data expressed as in Fig. 1. (A) Open-field tests: latency to first ambulation. Each trial lasted 3 minutes. The test groups were 18 infected males and 18 control males. (B) Open-field tests: the number of squares entered in 3 minutes. Same test groups as in (A). (C) The current level required to elicit a startle response. The mean response for each animal was determined from four trials per day. The test groups were 16 infected males and 16 control males. (D) Running-wheel revolutions expressed as a square-root transformation. The test groups were 27 infected males and 27 control males.

reluctant than controls to explore a novel environment. Both male and female infected mice gave startle responses at lower current levels than controls, and the effect was evidently potentiated by the stress induced by the novelty of the test environment, as shown by their sensitivity to shock decreasing with repeated tests. The running-wheel results provide evidence that, although the infected test animals ran significantly less than controls, they were not debilitated. Indeed, when all infected animals were compared with all controls, they ran an average of 3.7 as opposed to 4.4 km/day, respectively.

Although earlier studies have shown that some strains of mice, including Nya : NYLAR, develop glomerulonephritis beginning 7 to 10 months after neonatal inoculation with LCM virus (*8*), this factor does not appear to be relevant to our studies, which were completed prior to this age. We are impressed by the similarity of the behavioral results shown by the neonatal and adult models (Table 3). Taken together, these results strongly suggest that the observed differences between experimental and control mice are not a function of either neonatal illness or lack of maternal care, even though the mothers invariably suffer temporary sickness caused by cross-infection from their young inoculated at birth (*9*); instead, the results indicate that the differences are caused by a direct action of the virus on the brain.

Immunofluorescence study (*10*) of the brain tissue from persistently infected mice has shown that many neurons are infected with the virus; they contain viral antigen and exhibit maturing virus at their surface. It seems likely that this process would alter the neurophysiology of conduction and excitation in the affected cells. Further evidence of a direct effect of virus on the brain is the report (*11*) that the level of choline acetyltransferase, an enzyme believed to be involved in brain neurotransmitter metabolism, is higher than normal in both mouse brain and cultures of neuroblastoma cells when these are persistently infected with LCM virus.

We draw two conclusions from these data. First, both neonatal and adult infection of laboratory mice with LCM virus produces long-term changes in the behavior of animals which appear normal in other respects. This finding accords with the possibility that infectious agents may exist which cause behavioral disorders in the absence of neurological symptoms in man. Second, since inapparent LCM infection of rodent colonies is not an uncommon event (*12*), it is clear that this infection could cause altered patterns of behavior which might be mistakenly ascribed to genetic or other factors.

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