term memory (*4*). In essence, proline prevented the transformation of the glutamate impulse pattern associated with conditioning into the corresponding swelling of the dendritic spines, which otherwise would have endured about 4 hours after conditioning.

Our finding that proline impairs retention after 24 hours suggests that the patterned swelling of dendritic spines which serves as the basis of short-term memory may also, upon stabilization, serve as the basis for long-term memory. The mechanism by which swelling of dendritic spines would be stabilized is not known, but it might involve formation of structural proteins (*17*). The conceptualization, however, that, in the model of memory formation described, short-term memory depends on a simple morphological expansion of dendritic spines, and that long-term memory depends on stabilization of that morphological change, appears to be plausible, parsimonious, and heuristic.

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7. Twenty chicks given similar injections of India ink, and killed immediately, revealed ventricular distribution of ink bilaterally in 14, and unilaterally in 4, and with localized distribution in 2.
8. The experiment was conducted as a modified block design. Some replications contained no control groups trained with the untreated target and other replications did not have both of the longer (64- or 240-minute) training-to-treatment intervals but every replication contained aversively trained groups treated at a TTI of 2 minutes with proline or isoleucine. The 2-minute groups had approximately 100 subjects each, whereas the longer TTI groups had approximately 75; the groups trained with the untreated target had 30 to 60 subjects. In order to adjust for shipment differences in performance, an analysis of covariance was conducted with the covariates representing block membership. The resulting adjusted mean values for each group were then compared by *t*-tests.
9. The baseline 24-hour avoidance score of 2-day-old chicks trained with the aversive bead target was 70 percent, which does not differ significantly from the corresponding avoidance score of 79 percent in 1-day-old chicks [E. Lee-Teng and S. M. Sherman, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 926 (1966)]. The 24-hour avoidance score observed by Van Harreveld and Fifkova (*4*) in 8-hour-old chicks, with intervening tests applied 45 minutes and 4 hours after training, was only 36 percent. This low baseline score makes it technically difficult to detect any reduction in 24-hour avoidance induced by proline and may explain the apparent absence of the proline amnesic effect 24 hours after training 8-hour-old chicks (*4*).
10. For details of the methods and results, including electrographic and computer analysis of multiple unit and EEG events in 25 neonatal chicks, see L. K. Gerbrandt, M. J. Eckardt, M. I. Simon, A. Cherkin, in preparation.
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15. Indirect evidence from other types of studies is compatible with the Van Harreveld and Fifkova hypothesis. For example, electrical stimulation of the perforant fibers that terminate on dendrites of the granular cells in the fascia dentata resulted in a potentiation of the dentate response lasting 1 to 10 hours [T. V. P. Bliss and T. Lømo, *J. Physiol. (London)* **232**, 331 (1973)]. Such stimulation also swelled the dendritic spines on the stimulated cells, as determined by electron microscopy; the swelling appeared within 2 minutes after stimulation and was still evident 1 hour later (*17*).
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Copper Supplementation in Quaking Mutant Mice: Reduced Tremors and Increased Brain Copper

Abstract. *Mice homozygous for the mutant gene* quaking (qk) *with a high frequency of axial tremors had a low concentration of copper in the brain. Supplementation during pregnancy and lactation with a high level of dietary copper greatly reduced the frequency of tremors and brought brain copper level to normal in the offspring. It is suggested that* qk *affects copper metabolism.*

Prevention of the deleterious effects of a mutant gene by supplementing trace elements in the maternal diet has been demonstrated in the mutant mouse *pallid* (*1*) and in "screw neck" mink (*2*) with manganese. More recently, we showed that copper supplementation ameliorated the effects of the mutant gene *crinkled* (*cr*) on postnatal survival and on the thickness and pigmentation of the skin (*3*). We now report that some of the effects of the mutant gene *quaking* (*qk*) in mice are likewise alleviated by copper supplementation and suggest that the gene affects copper metabolism.

Maternal copper deficiency has been known to affect the developing central nervous system of mammals since the work of Bennetts (*4*) on the etiology of enzootic ataxia in lambs. Studies in lambs (*5*), guinea pigs (*6*), pigs (*7*), and rats (*8*) show that the offspring of females having a copper deficiency exhibit ataxia, tremor, clonic seizures, myelin aplasia or demyelination, and reduced levels of brain cerebrosides and sulfatides. The mutant gene *quaking* in mice of the C57 Bl/6J strain produces in homozygotes an intermittent axial body tremor that is first seen about day 10 and per-

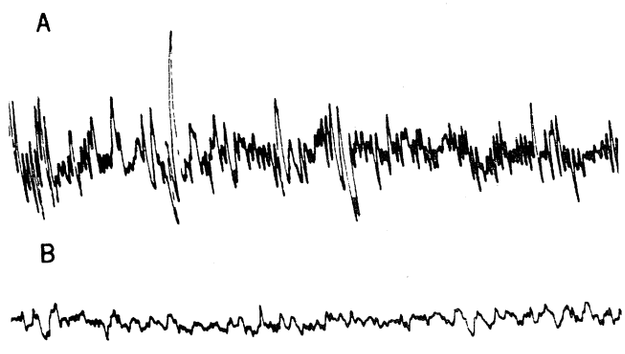

Fig. 1. Polygraph recordings of tremors from 27-day-old quaking mice over a 10-second period. (A) Quaking mouse from the control purified diet group (6 ppm of Cu). (B) Quaking mouse from the copper supplemented diet group (250 ppm of Cu) showing a reduction in tremor frequency.

sists throughout life. Such animals also show abnormalities of myelin and reduced levels of brain cerebrosides and sulfatides (*9*). The similarity of the phenotypic manifestations of the gene *qk* and those of offspring deficient in copper led us to investigate the relation of the gene to copper metabolism by measuring the copper content of the brain in quaking mice and by determining the influence of copper supplementation on the tremor activity characteristic of these mutants.

Females heterozygous for *qk* were fed throughout gestation and lactation one of three diets: complete purified (control) diet, containing 6 parts per million (ppm) of copper; a copper-supplemented purified diet containing 250 ppm of copper; or a commercial laboratory feed (*10*) (12 ppm of copper), referred to as stock diet. The purified diets were sufficient in all other nutrients as previously reported (*11*). The litters were left with their mothers throughout the experiment, which was terminated by 29 days after birth. The pups had free access to their mother's diet at all times.

Copper concentration in the brain was measured at 21 days of age in mice homozygous for *qk* and their littermate controls. Animals were decapitated under ether anesthesia, and their brains were quickly excised and weighed. The brains were wet ashed with 16*N* nitric acid, concentrated by evaporation, and diluted with deionized water. Copper concentration was determined by atomic absorption spectrophotometry (*12*).

In one experiment we studied the effect of copper supplementation on the frequency of the axial tremors characteristic of *qk/qk* mice. Female mice homozygous for the gene *qk* from each of the three dietary treatment groups were tested for frequency of tremors at 21, 23, 25, 27, and 29 days of age. A modification of the method of Henderson and Woolley (*13*) was used to monitor and record the tremor activity of the animals. A piece of magnetic tape, 1 cm^2, was fixed by adhesives to the mouse along the spinal axis directly above the hind limbs. The mouse was placed in a beaker around which was wrapped copper wire. The movement of the magnetic tape induced a current which was amplified by a Grass p511R preamplifier and recorded on a Grass model 7 polygraph (*14*). Animals were given a 30-minute acclimatization period in the beaker before testing began. Background noise was kept at a minimum. Tremor frequency was determined by counting needle deflections over an average of six representative 5-second periods on each day of testing. A representative photograph of a polygraph recording is shown in Fig. 1, in both supplemented and nonsupplemented animals.

Table 1. Brain weight and copper concentration in 21-day-old quaking (*qk/qk*) and nonquaking (+/?) mice.

Group	Dietary copper (ppm)	Genotype	Animals (No.)	Brain weight* (g)	Copper concentration (ppm)
Purified control	6	+/?	5	0.358 ± 0.019†‡	1.735 ± 0.197
	6	*qk/qk*	5	0.374 ± 0.197‡	1.097 ± 0.048§
Copper supplemented	250	+/?	5	0.398 ± 0.008‡	2.025 ± 0.040
	250	*qk/qk*	5	0.398 ± 0.006‡	1.998 ± 0.097

*Wet weight. †Mean ± standard error of the mean. ‡No significant differences (by Student's *t*-test). §Significantly lower (by Student's *t*-test) than nonquaking animals on control diet ($P < .02$) and quaking and nonquaking mice fed the copper supplemented diet ($P < .001$). There were no significant differences between the other three groups.

Table 2. Frequency of tremors in quaking (*qk/qk*) mice.

Diet group	Mice (No.)	Number of tremors per 5 seconds at days postpartum				
		21	23	25	27	29
Stock control (12 ppm of Cu)	3	48.4 ± 6.6	54.7 ± 3.8	52.0 ± 3.5	48.9 ± 5.6	57.3 ± 5.5
Purified control (6 ppm of Cu)	7	49.9 ± 2.6	58.4 ± 1.8	55.9 ± 1.7	55.1 ± 0.8	52.7 ± 2.9
Copper supplemented (250 ppm of Cu)	7	30.8 ± 1.4*†	36.8 ± 2.6†‡	35.6 ± 2.2†‡	32.0 ± 1.4*†	33.6 ± 1.2†‡

*Significantly lower than stock fed controls ($P < .05$). †Significantly lower than purified diet control ($P < .001$). ‡Significantly lower than stock fed controls ($P < .01$).

The copper concentration of the brain in mice of the control diet group was significantly lower than it was in their heterozygous littermates (Table 1). However, when copper supplementation of the diet was provided during the prenatal and suckling periods, the brain copper concentration was normal in the homozygous mutants. In the nonmutant heterozygous mice, copper supplementation did not raise the brain copper concentration significantly above the normal level. Supplementing copper during the prenatal and suckling periods significantly ($P < .001$) reduced the frequency of tremors in *qk/qk* mice on all 5 days tested, from 21 to 29 days of age (Table 2). There were no differences, however, in tremor frequency between animals from purified control diet and stock diet groups, indicating that the purified diet per se was not responsible for the alteration in tremor activity. There were no observable differences between the three groups in amplitude or duration of tremors. In nonquaking (+/?) animals there were no measurable tremors.

The *qk/qk* mice that were fed the diet supplemented with copper also seemed to have more normal coats, with less sparse hairs, than either of the control groups. Litter size, birth weight, and gain in body weight, however, were the same in all three groups.

These results indicate that at least some of the phenotypic characteristics of the gene *qk* are related to copper metabolism. The expression of the gene appears to be related at least in part to the copper status of the animal; however, tremor was not completely prevented by copper supplementation, even though the copper concentration of the brain was normal. The question of when copper supplementation is required for amelioration of the genetic effects of this mutant gene cannot be answered from this experiment, since the high copper diet was fed during both the gestation and suckling periods and was available to the offspring when they could eat dry food. Thus, the effect of supplementing copper may occur either during the prenatal or the postnatal period, or both. Nevertheless, the results suggest that *quaking* mice have an abnormality of copper metabolism, and this mutant may therefore provide a neurological model for study of the role of copper in early development of the nervous system.

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Neuroelectric Correlates of Conditioning

Gabriel *et al.* (*1*) reported changes in multiple-unit responses in the rabbit's medial geniculate body associated with the acquisition and reversal of a discriminative conditioned avoidance response. In both stages of the experiment the positive conditional stimulus (CS^+), which was followed by shock, purportedly evoked larger neural responses than the CS^-, which was not followed by shock. The conditioned discrimination and its reversal were regarded as adequate conditions for "producing unambiguous associative effects."

The controls employed by Gabriel *et al.* are appropriate for one kind of nonassociative effect, specifically, effects that are not correlated with conditioned changes in behavior. They are not adequate, however, for nonassociative effects that actually depend on conditioned changes in behavior. Suppose, for example, that an increase in level of arousal leads to an increase in neural activity evoked by a CS. Such an increase in arousal can result from the presentation of a noxious unconditional stimulus (UCS) like electric shock. A general increase in arousal might occur in an aversive conditioning situation and have little or no relationship to the conditioned changes in behavior. It might, nevertheless, enhance the neural activity evoked by the CS as long as the heightened arousal is maintained by the repeated presentation of shock in the conditioning procedure. Such a change in evoked activity would be revealed as nonassociative by the discrimination and reversal controls employed by Gabriel *et al.* If, on the other hand, the increase in arousal were itself conditioned together with, say, an instrumental avoidance response, one might expect the conditioned arousal to lead secondarily to a "conditioned" increase in the CS-evoked response. Is this an associative change in the evoked response? Only in a trivial sense, because it is not unique to the conditioning operation and throws little light on the conditioning process. It is, nevertheless, correlated with conditioned changes in behavior, and one would expect it to remain so throughout discrimination and reversal learning.

It is possible that the effects reported by Gabriel *et al.* are of this kind, although that is not the only possibility. Other behavioral changes, for example, in orientation toward conditional stimuli, may also be confounded with conditioned changes in behavior, and one needs assurances that adequate measures have been taken to eliminate such possibilities. Level of arousal seemed the most appropriate variable to illustrate the argument, however, for we (*2*) and others (*3*) have shown that changes in late components of evoked activity in primary afferent pathways during conditioning can reflect mainly conditioned arousal or fear responses. Such changes in the later components of evoked activity remain a strong possibility in the experiment by Gabriel *et al.* To show that modifications in evoked activity are in some way unique to a conditioning process or are primary changes not dependent upon behavioral modifications has become a demanding task. This is not to argue that such modifications cannot be found; there is some evidence for them (*4*), but very little considering the numerous claims. Ad hoc arguments that the data have not been compromised are encountered more often than adequate controls.

My main intention in this note has been to call attention to conceptual difficulties in the study by Gabriel *et al.* which are not, however, peculiar to this study. There are, however, several technical shortcomings in their report. Consider, for example, the data in the left-hand column of their figure 1, which presumably support the conclusion that in the final stage of acquisition the geniculate responses to the CS^+ were larger during the first 40 msec than the responses to the CS^-. This appears to be the case for subject 44; but the opposite relationship is seen in the data from subject 42, and it is difficult to distinguish any systematic differences between the curves for the other three subjects. There is no statistical evaluation of this difference. Moreover, the arbitrary selection of different points on the curves of individual subjects as illustrative of significant differences is at variance with accepted statistical practices; and the standard deviations used as the measure of those differences have no relevance to statistical decisions about the differences between the curves for the CS^+ and CS^- conditions. The reversal data in the right-hand column are only a little less disturbing, especially in view of a confounding difference between the initial (preconditioning) amplitudes of the responses to the two CS's (which Gabriel *et al.* were preparing to explain in a later publication) and a statistical evaluation based on not just the early (5 to 40 msec) activity, but on the complete response.

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Hall agrees that differential conditioning and reversal of short-latency medial geniculate nucleus (MGN) neuronal activity (*1*) is an associative neuronal effect. However, he argues that the effect may be associative "only in a trivial sense." Hall's judgment of triviality seems to us to be based on his belief that our effect "depend[s] on conditioned changes in behavior." We presume Hall expects a trivial neuronal effect to be "correlated with conditioned changes in behavior . . . and to remain so [correlated] throughout discrimination and reversal learning."

We interpreted Hall's phrase, "depend[s] on conditioned changes in behavior," to mean either (or both) of the following: (i) the associative neuronal responses were mediated by prior conditioned behavioral activity; (ii) the effects were positively correlated with conditioned behavioral activity in all stages of conditioning and reversal. Neither assertion is descriptive of the data. The latencies of the differential neuronal responses (5 to 40 msec) were too brief for those responses to have been mediated by prior conditioned stimulus (CS) related behavioral responses. In fact, it is unlikely that the briefest latencies of neu-