ing sleep is consistent with the occurrence of a recuperative process in this group of neurons (*9*), the lack of discharge during sleep seems to result from the behavioral inactivity imposed by sleep, rather than some specific neural inhibitory process.

One might hypothesize that the extended silences resulted from electrode movement that caused loss of unit recording. We can reject this hypothesis. (i) Our recording technique allowed stable recording for extended periods of time in a variety of brainstem areas (*10*). (ii) In every case, the NSA unit spike trains were recorded both before and after silent periods (Fig. 2). In 12 of these neurons, continuous recordings lasting more than 8 hours and including several sleep-waking cycles were obtained. Spike waveshape, signal-to-noise ratio, discharge patterns, and the unique behavioral correlates of discharge were always stable throughout the period of observation. (iii) All of the NSA units had large signal-to-noise ratios and stable spike amplitudes. In no case was a change in spike amplitude observed at the beginning or end of a silent period.

We have encountered these cells in histologically verified sites in midbrain regions (AP 1.0 to 3.0, ML 0.0 to 0.2, DV −0.8 to −3.2) and in the pontine reticular formation (AP 3.0 to 8.0, ML 1.0 to 2.8, DV −3.6 to −7.0). After we were alerted to the existence of NSA cells, about 30 percent of the cells that were encountered were of this type. However, the percentage of these cells in the brain is difficult to estimate accurately. Many of the cells fired only in sporadic bursts and could easily have been overlooked. Cells with more subtle sensory or motor correlates would not have been activated by our simple stimuli. Furthermore, the concentration of NSA units may not be the same in all brain regions, although the frequencies of encounter in the midbrain and pontine regions did not differ.

It was necessary to apply systematic stimulation while exploring for unit activity in order to find NSA neurons. If this were done in other brain areas, other types of NSA units might be found. Adams (*11*) found four otherwise silent cells in the midbrain that were selectively activated during elicited affective behavior, although he did not make sleep recordings.

The existence of NSA cells has been predicted in work by Vladimirova *et al.* (*12*), who calculated, on the basis of histological and electrical field analysis, that fewer than 5 percent of the neurons within range of their cortical microelectrodes showed spontaneous activity. These findings, coupled with the results reported here, suggest that NSA cells constitute a large proportion of neurons in the brain.

The idea that neurons are spontaneously active is incorporated in a wide range of theories of brain function (*2*). The brain's information processing has been conceptualized as a system for extracting signals from the background noise of spontaneous activity (*3*). The existence of large numbers of NSA neurons allows alternative formulations of these theories. The specificity of discharge in these neurons suggests the existence of specialized neural systems operating phasically in relation to specific sensory or motor events. Such systems might have considerably less ambiguity in their output than systems solely employing neurons with high levels of spontaneous activity.

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4 February 1976

Memory: Proline Induces Retrograde Amnesia in Chicks

Abstract. *Intracerebral injection of* L-*proline, 1 minute after one-trial avoidance training of chicks, impaired memory 24 hours later. Chicks injected with proline 1 or 4 hours after training and controls injected with* L-*isoleucine at intervals after training, showed no impairment of memory 24 hours after training. The retrograde impairment of long-term memory induced by proline occurred without the convulsions or electrographic events usually associated with retrograde amnesic treatments.*

The formation of long-term memory has been assumed to include one or more time-dependent steps. Interference with early steps produces a selective loss of recent memory, called retrograde amnesia (*1*). The treatments commonly used to induce experimental retrograde amnesia (*2*) are deleterious agents such as electroconvulsive shock (ECS), chemoconvulsants, anesthetics, and protein synthesis inhibitors, which have multiple and widespread actions. A more physiologically selective chemical treatment would be desirable for tracing the dis-

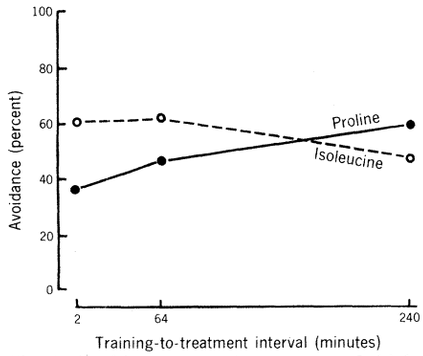

Fig. 1. Avoidance score (percentage of chicks not pecking target within 10 seconds) 24 hours after peck-avoidance training, as a function of training-to-treatment interval. Memory retention of the single avoidance training trial is indicated by a high avoidance score. Reduced avoidance in chicks injected with 300 m*M* L-proline (10 μl per brain hemisphere; closed circles) indicates impaired memory retention. Control chicks were injected with L-isoleucine (open circles).

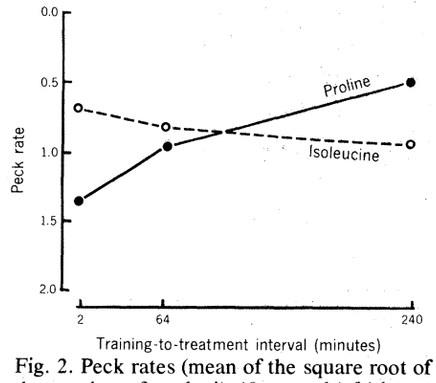

Fig. 2. Peck rates (mean of the square root of the number of pecks in 10 seconds) 24 hours after training, as a function of training-to-treatment interval. Memory retention is indicated by a low peck rate, corresponding to a high avoidance score (Fig. 1). Injection of L-proline (closed circles) shortly after training increases the peck rate; the effect decreases as the training-to-treatment interval increases. Control injection with L-isoleucine (open circles) does not produce this temporal gradient. The standard errors of the adjusted means ranged from 0.114 to 0.140.

ruption of memory to specific brain mechanisms. In a similar approach amnesia is induced with subseizure electrical brain stimulation of small cerebral "hot spots" (*3*). L-Proline disrupts "short-term" memory tested within 4 hours after one-trial avoidance training, at nontoxic doses and without noticeable alterations in brain electrical activity (*4*). We now report that proline has an amnesic effect on long-term memory, tested 24 hours after training.

A total of 505 naive White Leghorn cockerels (strain K-137, Pace/Setter Products, Inc., Alta Loma, Calif.), 44 ± 10 hours old, were housed individually in cartons (8.5 cm in diameter by 16.5 cm deep) throughout the experiment (*5*). The one-trial learning procedure (*6*) consisted of suppressing the chick's spontaneous tendency to peck a target (3-mm stainless steel bead fixed to a thin rod). Suppression resulted from a single 10-second training trial with the target, which had been coated with an aversive liquid (100 percent methyl anthranilate). The extent of peck suppression 24 hours later was used to estimate retention of the training trial. Control groups received identical training but without the aversive coating on the target.

At 1, 63, or 239 minutes after training, each chick received an intracerebral injection in each forebrain hemisphere of 10 μl of 300 m*M* L-proline or of 300 m*M* L-isoleucine as a control, each brought to *p*H 7.2 ± 0.2 with $NaHCO_3$. The effective training-to-treatment intervals (TTI) were designated as 2, 64, or 240 minutes, to allow an arbitrary 1 minute for distribution of the amino acids from the injection sites (*7*). Five hours after training, all chicks were coded to preclude experimenter bias. As the test for retention, the dry target was presented 24 hours later to each chick for 10 seconds.

The first measure of retention was the avoidance score, that is, the percentage of chicks in each group that did not peck during the full 10-second test trial (according to a χ^2 analysis) (Fig. 1). The second measure for each chick was the number of pecks in 10 seconds (with parametric analyses after square-root transformation to normalize distributions) (Fig. 2). Differences between groups were considered significant at the level of $P < .05$ (*8*). We included control chicks trained with a dry target to ascertain whether the administration of proline or isoleucine affected the peck response itself, which was measured 20 to 24 hours later; no performance differences were detected as a function of TTI or of amino acid administered (*9*).

Our study demonstrates that an amnesia gradient is induced by proline, as judged by a retention test given 24 hours later. In the chicks injected with proline, amnesia is evidenced by the gradient of increased retention as a function of increasing TTI (Figs. 1 and 2). The interpretation of these data as a demonstration of retrograde amnesia is supported further by the fact that control injections of isoleucine resulted in no performance gradient as a function of TTI. Comparisons between chicks injected with proline and with isoleucine at the same TTI revealed that (i) at 2 minutes, the proline group showed less avoidance and more pecking; (ii) at 64 minutes, there were no differences between groups; and (iii) at 240 minutes, the avoidance scores did not differ, although the chicks treated with proline had an unexplainably lower peck rate.

The effectiveness of proline as an amnesic agent in chicks is surprising. Behaviorally, the dose used caused only a transitory drowsiness with recovery of normal behavior within 15 minutes and with no indication of motor convulsions. Electrophysiologically, we did not observe abnormal activities known to be associated with other treatments that produce amnesia (*10, 11*). In contrast, amnesic agents such as ECS, chemoconvulsants, anesthetics, and protein synthesis inhibitors ordinarily require doses that cause deleterious effects, including brain seizures, motor convulsions, isoelectric electroencephalographic (EEG) activity, unconsciousness, illness, and death (*2*). Furthermore, the chick is refractory to the amnesic effects of potent agents effective in rodents, for example, injection of pentylenetetrazol at doses up to the median lethal dose (*12*). Similarly, retention in the chick is resistant to electrical brain stimulation treatments delayed more than 30 seconds after training, unless they produce prolonged EEG spiking associated with brain seizures (*13*). Therefore, proline is a useful neurobiological treatment in memory research.

Extension of the hypothesis of short-term memory formation proposed by Van Harreveld and Fifkova (*4*) offers a plausible neurobiological interpretation of the amnesia reported here. Their hypothesis postulates a central role for glutamate—independent of its role as a putative neurotransmitter—that is blocked by proline in a reversible and apparently competitive manner. Neural activity associated with a learning experience is proposed to lead to a patterned release of intracellular glutamate ion into the extracellular fluid, where it increases the permeability of the dendritic and somatic plasma membrane to sodium. Extracellular electrolytes diffuse into the affected neurons, and water enters to maintain the osmotic equilibrium. The resultant swelling of neuronal processes, including dendritic spines, causes a decrease in the longitudinal electrical resistance of the spines (*14*), thus increasing the effectiveness of their synaptic excitation (*15*). This hypothesis offers a specific morphological change as the basis for the "facilitated neuronal pathways" often said to underlie memory formation (*1*). The demonstration that proline acts to block the release of intracellular glutamate (*16*) was used to explain the loss of short-

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.
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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-

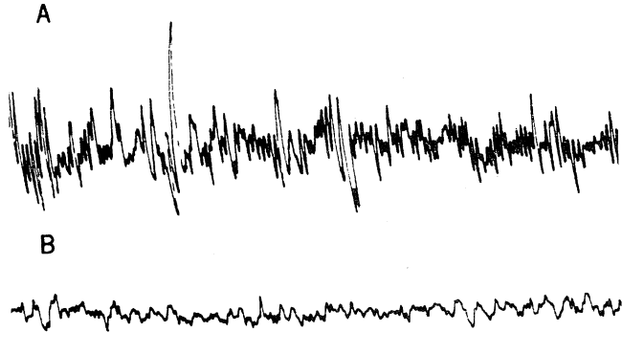

Fig. 1. Polygraph recordings of tremors from 27-day-old quaking mice over a 10-second period. (A) Quaking mouse from the control purified diet group (6 ppm of Cu). (B) Quaking mouse from the copper supplemented diet group (250 ppm of Cu) showing a reduction in tremor frequency.