Pentylenetetrazol: Failure to Improve Memory in Mice

Pentylenetetrazol purportedly improves memory in mice. Irwin and Benuazizi (*1*) suggested that pretrial doses of pentylenetetrazol improve short-term retention, that posttrial doses improve longer-term retention, and that the doses that facilitate retention are much less than those that produce convulsions. We tried to confirm the salient parts of their findings.

Our method was similar to theirs. Male CF1 mice, 9 to 10 weeks old, were placed individually in a small antechamber that led into a larger chamber. When they stepped on the grids of the large chamber, the mice received 0.2 ma of electric shock for 0.5 second. When replaced in the antechamber, these mice took longer to reenter the large chamber than did unshocked mice, a result noted previously (*1*).

Sixteen groups of 14 mice each received oral doses of either pentylenetetrazol or distilled water either 30 minutes before (pretrial) or immediately after the shock (posttrial). Either 8 minutes or 24 hours after the shock, the mice received a second trial, with a cutoff latency of 180 seconds for reentry. Procedure and results are summarized in Table 1.

Pentylenetetrazol had no statistically significant effects on reentry latencies. The two groups receiving posttrial doses of 10 mg/kg had slightly longer reentry latencies than did the two corresponding control groups only because 16 of the drugged mice, as opposed to 11 of the control mice, did not reenter the shock chamber.

As for the pretrial results, none of the drugged groups had significantly longer reentry latencies than did their control groups. Like Irwin and Benuazizi, we did find relatively great variation between some of the mean latencies in attempting to replicate our results, especially under the pretrial conditions. (That the duration of the interval between training and testing affects reentry latencies has already been suggested by Irwin and Benuazizi.)

Table 1. Mean reentry latencies.

Interval between trials (hr)	Dose (mg/kg, free base)				
	Nil	1	3	10	30
			Posttrial		
24	110	98†	108†	124† §	108†
24*	115			134† §	
			Pretrial		
24	76		118‡ §	114‡ §	
24*	110		87† §	110† §	
0.133	71		90†	71†	

* Replication. † P's $> .25$ for comparisons of control and drugged groups within each condition, according to two-tailed rank-sum test (N, 14 per group). ‡ P's $> .10$ (N, 14 per group). § P's $> .30$ when the data for replication are pooled (N, 28 per group).

Observation of additional mice that had been drugged with pentylenetetrazol at 10 or 30 mg/kg indicated that they were more prone to "freeze" than were control mice when replaced in the antechamber after being shocked. Accordingly, any marginal effects that pentylenetetrazol may have had on reentry latencies may have had more to do with emotionality than with cognition. At all events our results do not favor the notion that pentylenetetrazol improves memory in mice (*2*).

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References and Notes

1. S. Irwin and A. Benuazizi, *Science* **152**, 100 (1966).
2. Because slight differences between the procedures of experiments may lead to different results, we describe our method in detail. The antechamber (17.5 by 5 by 12.5 cm high) had plexiglass walls, the rear wall being hinged. The larger chamber, in which the mice were shocked, was a converted Skinner box (Grason-Stadler, model E3125A-100) with the food cup removed. To enter the shock chamber the mice had to cross a 1.5-cm barrier. A Grason-Stadler model-E1064GS shock generator was set at the 0.2-ma calibration. Of 333 mice, 324 entered the shock chamber within 50 seconds on the first trial; the remaining nine were discarded. The mice were fasted for about 4 hours before the start of testing. The volume of medication was 0.1 ml/10 g. Pentylenetetrazol solutions were from the Knoll Pharmaceutical Co. (lot 633). The experimenter was not informed of the nature of the dosage.

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Important in demonstrating the ability of pentylenetetrazol to increase response latency is an experimental arrangement that results in marked reduction of control latencies after 24 hours. This condition was achieved in neither Pearl's study nor (I now note) in my own control study with strychnine, though it was present in the pentylenetetrazol study. In reviewing our data I have found the source of the discrepancy and must confess error in describing the experimental arrangement; it was accurate for the strychnine but not for the pentylenetetrazol study. For the latter, the animals were not equilibrated overnight in their home cages, but were placed in plastic cages (12.5 by 12.5 by 27.5 cm), without access to food or water, on each test day for the period of fasting and for the several hours required for testing all the animals; later they were returned to their home cages.

With this arrangement there appears to be an interaction effect that usually results in markedly reduced control latencies after 24 hours—the condition required for demonstrating drug-induced enhancement. I have been authorized by R. I. Taber of Schering Corporation and J. Gogerty of Sandoz Pharmaceuticals to report that they have been able to confirm my findings with pentylenetetrazol, when these conditions obtained, in about 2 to 3 of every 5 studies undertaken.

We have found "blind" ratings (on a 0-to-8 scale) of the apprehension levels of the animals, during the first 15 seconds in the apparatus, significantly correlated with their subsequent response latencies. I am inclined, therefore, to agree with Pearl's inference that the effect of pentylenetetrazol "may have had more to do with emotionality than with cognition." Furthermore I am convinced it is the apprehension level rather than memory per se that is predominantly measured by the one-trial learning procedure.

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Tetrodotoxin: Comments on Effects on Squid Axons

We would like to clarify an apparent misunderstanding by Watanabe, Tasaki, Singer, and Lerman (*1*) of our report on the blocking of squid axons by tetrodotoxin (TTX) (*2*). Our experimental findings are compatible, as they noted, and agreement should be complete when semantic difficulties are removed.

Watanabe *et al.* (*1*) cited our report, saying, "It has been argued that TTX may be specific for the 'sodium channel,' rather than for the sodium ion." Although this term has been used loosely and frequently to designate that path through which sodium ions normally flow when they are present either inside or outside the nerve membrane, in our paper (*2*) we used the expression early "transiently open conductance channel" to distinguish effects on the early conductance increase (early channel) from those on the late conductance increase (late channel). Alkali cations other than sodium have been shown

to pass through this channel in the squid axon membrane to an equal or lesser extent (*3*). Whenever TTX has been applied externally, there has been a reduction in the ionic current during the early transient phase independent of the alkali cation used or the direction of the current flow (*4*). Furthermore, Tasaki and Singer (*5*) have also shown that a nerve bathed in a sodium-free solution of hydrazinium chloride may have an action potential and an early, transient inward current that is also selectively blocked by TTX. These observations seem to be in complete agreement and have led us to the idea that TTX really blocks the early conductance channel and, thereby, the flow of any ion using this pathway through the membrane.

Watanabe *et al.* (*1*) also question the concept of "separate channels." The idea of two separate channels arose from the observation that TTX blocks only one conductance phase, leaving the other intact. This led us to point out that there were two operationally distinct channels. It is not yet clear how to devise an experiment that would conclusively determine whether the two distinct functions are separated spatially as well.

It seems to us that their results (see *1*) neither support nor deny the hypothesis of separate channels because it is not possible to distinguish effects on the early conductance channel from those on the late conductance channel in observations of action potentials alone. Only data from voltage clamp experiments can give a satisfactory answer to this particular question. In fact, the results of such experiments by Tasaki and Singer (*5*) did show a reduction of the early, transient inward current only in the presence of TTX.

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5. I. Tasaki and I. Singer, *Ann. N.Y. Acad. Sci.* **137**, 792 (1966).

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The main points in our report (*1*) were that tetrodotoxin (TTX) could not be used to determine whether or not the excitation process was sodium-dependent, and that its effects could not be interpreted as evidence for the existence of two spatially separate channels. The concept of a channel specific for Na^+ has become meaningless in view of the fact that a large number of univalent cations can substitute for sodium, and that excitability can even be maintained in external media free of univalent cations (*2*). We would certainly agree that under voltage clamp conditions, TTX eliminates the inward current in a variety of external media and that no distinct effect on the later outward current is observable.

Semantics are indeed a problem. It is unfortunate that many investigators think that the term "channel" refers to an anatomical structure rather than to a conceptual pathway. In the original interpretation of the voltage-clamp data (*3*), the early inward current was attributed to a specific increase in the membrane permeability to Na^+. Within the framework of this interpretation, it was logical to interpret the effect of TTX as specific for the "sodium-carrying system" (*4*). However, since it has become clear that excitability of the squid giant axon can be maintained in various media free of sodium (*2*), it has become necessary to modify the original interpretation. During the period following initiation of a clamping voltage, the membrane becomes more permeable to various univalent and divalent cations. The effects of TTX are particularly apparent during this early period (which may be designated as the "early conductance phase"). However, we feel that it is very misleading to investigators in allied fields for axonologists (*5*) to continue to speak of the "sodium channel." The reasons are:

1) The "sodium channel" is not specific for the Na^+, and TTX effectively blocks excitation in a variety of sodium-free external media.

2) "Sodium channel," as distinguished from "potassium channel," implies that there are two, spatially separate pathways through the membrane. The assumption of two separate pathways is debatable (*6*), and it is not necessary to explain the experimental data (*7*). Indeed, this assumption did not exist in the original interpretation of the voltage-clamp experiments (*3*).

3) The late, strong outward current observed in voltage clamp experiments is the result of the large potential difference maintained by the clamping device. Since the term "potassium channel" is used operationally to represent the pathway for this artificially created portion of the membrane current, the observed ineffectiveness of TTX on the "potassium channel" has only limited implications for the normal action potential.

Experimental evidence indicates that the squid giant axon has the properties of a cation exchanger (as suggested by the large number of cations capable of maintaining excitability) and that significant interdiffusion of cations is present during both the resting and excited states (*8*). These findings suggest that the exchange of cations at negative sites of the membrane macromolecules is the primary physicochemical event leading to excitation (*7*, *9*).

The comment by Moore *et al.* is appreciated and goes a long way toward separating semantic and conceptual differences. Certainly there should not, and can not, be any irreconcilable differences between a primary electrical and primarily physicochemical analysis of excitation. The two approaches are complementary, and continued dialogue will lead to a better understanding of excitation phenomena.

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