Table 3. Detoxication of 217AO in the presence and absence of squid axon homogenate. Incubation mixture contained 90 to 100 mg of axonal material per milliliter and 10^{-5} mole of 217AO per liter at *p*H 7 to 7.5, room temperature, and aerobic conditions; the incubation time was 24 hours. Results are shown as mean ± standard error of the results of the four experiments in each group.

Concentration of 217AO after 24-hr incubation (mole/liter)	Detoxication (%)
In presence of axonal homogenate	
1.02×10^{-5}	20 ± 7
0.74×10^{-5}	
0.68×10^{-5}	
0.74×10^{-5}	
No axonal material; aqueous medium only	
0.95×10^{-5}	10 ± 5
1.02×10^{-5}	
0.81×10^{-5}	
0.83×10^{-5}	

the possible enzymatic detoxication of 217AO by incubating the agent ($10^{-5}M$) with a homogenate of whole squid axon for 24 hours. The results indicate that in the 1 to 2 hour period of the penetration and electrical studies, when 10^{-2} to $10^{-3}M$ 217AO and single axons (15 to 20 mg) were used, the detoxication of 217AO either enzymatically or nonenzymatically was negligible (Table 3).

These results appear to indicate that the propagation of an action potential is not blocked in the presence of an inhibitor of AChE capable of crossing permeability barriers. The fact that the inhibitor is applied externally and is found internally in its active form at a concentration some 10^4 times greater than that required to produce 100 percent inhibition of a solution of AChE may seem to raise the question of the essentiality of AChE for the permeability changes associated with electrical activity (*11*). Similarly, at the time at which conduction was blocked, concentrations of physostigmine were found in the axoplasm of the squid giant axon which were about 10^3 times greater than those required to inhibit AChE in solution (*8*). Even more relevant is the question of the amount of uninhibited AChE in the axon under these conditions. Attempts to determine AChE activity in squid axons after their treatment with high concentrations of 217AO are made difficult by the small fractions remaining to be measured of an initially low amount of enzyme activity, by the low substrate penetration if intact material is used, and by the release of small amounts of entrapped inhibitor if homogenization is attempted. Recent work (*12*) indicates that treatment with detergent or venom may be combined with the use of acetylthiocholine and electron microscopy, although even in a thin frozen section 1000 Å thick, or less, AChE does not react with the substrate except after treatment of the section with detergent. Whether or not the question is now answerable, AChE does seem to be intimately associated with the conducting membranes of many excitable tissues (*12, 13*). Furthermore, there is biochemical evidence that AChE is essential for bioelectric activity (*8, 11, 14*). The organization of the conducting membranes may be as complex as that of other membranes, such as those of mitochondria (*15*). Individual permeability barriers may exist for these subcellular organized elements. There is evidence that only a fraction of the area of the axonal membrane is involved in the actual events of conduction (*16*); the 217AO may have penetrated elsewhere. On the basis of recent studies (*17*) one may suppose that charges surrounding the subunits containing the AChE system prevent compounds from reaching and reacting with the enzyme. In addition, the rather low solubility of 217AO in lipids (oil-water partition coefficient ≈ 0.1) (*3*), while obviously not preventing the penetration of this compound into the axon, may prevent its reaching all parts of the membrane components. Thus, in axons first treated with venom (Table 2), the 217AO reaches the subcellular apparatus with the resultant block of conduction.

The ability of 217AO to penetrate into the interior of an axon without blocking electrical activity appears at present to be an unexplained observation. The use of homologs of 217AO may prove to be a new and interesting approach to a chemical exploration of membrane substructure.

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Synaptic Vesicles of Inhibitory and Excitatory Terminals in the Cerebellum

Abstract. *Populations of synaptic vesicles within cerebellar terminals considered excitatory or inhibitory on the basis of physiological evidence differ with respect to size and shape. Size rather than shape appears to be the main morphological difference between these populations. Elongation of vesicles is dependent on fixation with aldehyde fixatives, and both size and elongation change with age mainly during maturation.*

Several investigators (*1*) have reported nerve terminals containing elongated synaptic vesicles in nervous tissue fixed in aldehydes. Uchizono (*2*) has suggested that excitatory and inhibitory terminals contain synaptic vesicles that differ in shape. A similar suggestion has been made recently by Bodian (*3*). We have analyzed the shape and size of vesicles within terminals of basket, Golgi, and Purkinje cells

Table 1. At 14, 35, 90, or 420 days the differences among the mean diameters of excitatory versus inhibitory population are significant ($P \leq .001$) for either the major or the minor axis. The only exception is the difference between the major diameters of basket and parallel populations at day 14. One millimeter is approximately equal to 58 Å; the mean vesicle area is given in square millimeters as the area of an ellipsoid where 1 mm^2 is equal to 3.36×10^3 Å. Abbreviations: Major, M; minor, m; standard deviation, S.D.

Age of nerve endings (days)	Vesicles (No.)	Diameters (mm)				Vesicle elongation index (M/m)	Mean vesicle area ($A = \pi$ Mm/4) (mm^2)
		Major		Minor			
		Mean	S.D.	Mean	S.D.		
				Basket			
7	187	7.86	1.04	6.46	0.94	1.21	39.84
14	336	7.04	1.19	5.75	.94	1.22	31.71
> 90	253	6.51	1.02	4.55	.83	1.43	23.75
420	309	6.22	1.15	3.68	.94	1.69	17.96
				Golgi			
> 90	280	5.49	0.81	4.14	0.68	1.32	17.71
				*Purkinje**			
7	209	8.16	0.96	6.74	1.15	1.21	42.14
14	277	6.60	1.09	4.87	0.72	1.35	25.17
35	290	7.01	1.48	5.22	1.07	1.34	28.68
> 90	283	6.33	1.05	4.52	0.80	1.40	22.42
330	305	7.59	1.40	4.44	.96	1.70	26.11
				Mossy			
14	316	8.02	1.08	6.95	0.83	1.15	43.67
35	289	7.60	1.08	6.33	.88	1.20	37.68
> 90	295	7.16	0.93	5.80	.67	1.23	32.86
420	429	7.23	1.14	5.43	1.01	1.33	30.71
				Climbing			
14	167	7.73	1.16	6.51	1.07	1.18	39.37
420	80	6.84	0.83	5.72	0.66	1.19	30.71
				Parallel			
14	175	7.19	0.73	6.06	0.80	1.18	33.13
> 90	282	7.04	.92	5.89	.81	1.19	32.56
420	296	6.79	.98	5.51	.85	1.23	29.27

* A small fraction of the populations classified as Purkinje terminals probably is not inhibitory and may contaminate the sample.

and of mossy, climbing, and parallel fibers in the mouse cerebellum, and have correlated the results of this analysis with the excitatory or inhibitory nature of these terminals, as indicated by neurophysiological evidence of Eccles *et al.* (*4*) and Ito *et al.* (*5*).

Mice 7, 14, 35, 90, 330, and 420 days old were perfused with glutaraldehyde, and small blocks of cerebellar cortex or cerebellar nuclei were then fixed in osmium according to Karlsson and Schultz's technique (*6*). For the identification of terminals the following criteria were used: profiles of terminals synapsing with branchlet spines were selected as parallel fibers; profiles containing very densely packed vesicles and synapsing with spines of the soma or dendrites of the Purkinje cell other than branchlets were classified as climbing terminals; profiles synapsing with the soma of the Purkinje cells were identified as basket terminals; and small profiles, in the periphery of the glomerulus of the granular layer, synapsing with dendrites of granule cells, and clearly different from small evaginations of the large terminals of mossy fibers, were considered Golgi terminals. These and other criteria for the identification of terminals within the cerebellar cortex of the mouse perfused with glutaraldehyde have been reported elsewhere (*7*). Except for the identification of climbing fibers, which differs, at least partially, from that of Hamori and Szentagothai for the cat (*8*), the remaining identifications agree basically with those of other investigators (*9*). Their validity rests mainly on the correlation found between the distribution of terminals in the cerebellar cortex as observed with the electron microscope and that shown by Golgi studies (*7, 10*). Our identification of the climbing fibers is also supported by developmental evidence (*7*). We classified nerve terminals on the soma of the lateral cerebellar nuclei as Purkinje terminals, as we assumed that the great majority of these terminals originated in the Purkinje cells (*11*). The great similarity with respect to morphological characteristics found to exist between recurrent collateral terminals in the molecular layer (*7*) and most of the terminals seen on the soma of the lateral cerebellar nuclei cells strongly supports this assumption.

For the study of the morphological characteristics of the synaptic vesicles, we measured the major and minor diameters of several thousand synaptic vesicles with clear cores from several hundred identified nerve endings. In order to be able to compare vesicle shape and vesicle size among the populations of vesicles analyzed, we derived an "elongation index" and a "mean vesicle area" from the mean values of the major and minor diameters of the vesicle profiles. In this report, excitatory or inhibitory synaptic populations refer to populations of synaptic vesicles within terminals that, as determined by neurophysiological studies (*4, 5*), respectively fire (excite) or suppress the firing (inhibit) of the cells with which they made synaptic contacts. According to this evidence, terminals of basket, Golgi, and Purkinje cells are inhibitory; and mossy, parallel, and climbing fibers are excitatory.

Table 1 summarizes the measurements of synaptic vesicles of six types of terminals; the top three are excitatory; the bottom three, inhibitory. For comparative purposes we shall refer mainly to the values that define the shape ("vesicle elongation index") and the size ("area of mean vesicle") of synaptic vesicle populations. Let us first compare the five samples from a 90-day-old animal; these represent the populations of synaptic vesicles in mature animals. All were from the same animal, tissue block, and tissue section, except for the Purkinje sample. The synaptic vesicles within basket, Golgi, and Purkinje terminals (inhibitory) were significantly smaller ($P < .001$) and more elongated than those in mossy and parallel fibers (excitatory). Compare, for instance, the size and elongation of vesicles in the terminals of Golgi cells and mossy fibers. There were no significant differences with respect to vesicle size among the two excitatory populations at this age. This was also true for Purkinje and basket populations. Golgi terminals, however, contained synaptic vesicles with a mean area that is much smaller than that of vesicles in terminals of either Purkinje or basket cells. These differences are highly significant ($P<.001$). Next, compare the changes in shape or size of synaptic vesicles in each type of terminal as a function of age. There is a consistent decrease in size accompanied by an increase in elongation as the animal ages. These

changes were very pronounced among populations of basket and Purkinje cells (inhibitory) and much less marked among the other three excitatory populations. Figure 1 illustrates some of these changes in three types of terminals.

The rate of change of vesicle size and shape appears to be greater in younger animals, as if maturational rather than aging processes were responsible for these changes (Table 1). Factors intrinsic to the vesicles, such as the average age of the vesicle populations, or extrinsic ones such as the completion of the glial environment of cells and terminals, could very well effect the changes in younger animals as they age. The vesicles decrease in size with age, and it seems that they elongate progressively upon reaching a critical mean area of about 10^5 Å^2. The low elongation index and the reduced rate of decrease of size among excitatory vesicle populations may perhaps be accounted for by the fact that the vesicle populations hardly ever reach this critical size. Since vesicle elongation appears to be dependent upon aldehyde fixatives (*1, 2, 3*), some tissue fixed in osmium has been analyzed. In mice, excitatory (mossy) and inhibitory (Golgi) terminals seem to differ with respect to vesicle size but not with respect to elongation. In agreement with these observations are those of Lenn and Reese (*12*), who have found in ventral cochlear nucleus perfused with osmium boutons containing small synaptic vesicles and calyceal terminals filled with larger vesicles. Accepting the physiological data by Pfalz (*13*), these investigators have concluded that the boutons containing small vesicles may be inhibitory. The terminals of normal and degenerating centrifugal cochlear nerves, considered to be inhibitory, when fixed in osmium contain circular synaptic vesicle profiles, but during degeneration they have elongated vesicles (*14*). Elongated vesicles in degenerating terminals fixed in aldehyde or osmium have also been described by Walberg (*1*). These observations, plus the fact that excitatory terminals improperly fixed in glutaraldehyde may show elongate vesicles, suggest that more experiments similar to those initiated by Walberg (*15*) are needed to determine under which experimental conditions elongation and vesicle size may change. It is possible, although there is no evidence available yet, that vesicle populations of different sizes have dissimilar molecular organizations that are responsible for their differential elongation in certain mediums. Actually, as pointed out by Whittaker (*16*), the form of the native synaptic vesicles is not yet established.

Our data on material perfused with aldehyde statistically demonstrate that in the mouse cerebellum excitatory and inhibitory synaptic vesicle populations differ in size. They also suggest that the basic morphological difference between excitatory and inhibitory populations may be size rather than shape. Furthermore, our data on adult material suggest that all three types of excitatory terminals analyzed may contain a common type of vesicle population in terms of size, whereas among the inhibitory terminals there appear to be at least two types of vesicle populations, one in Golgi terminals and another common to basket and Purkinje terminals.

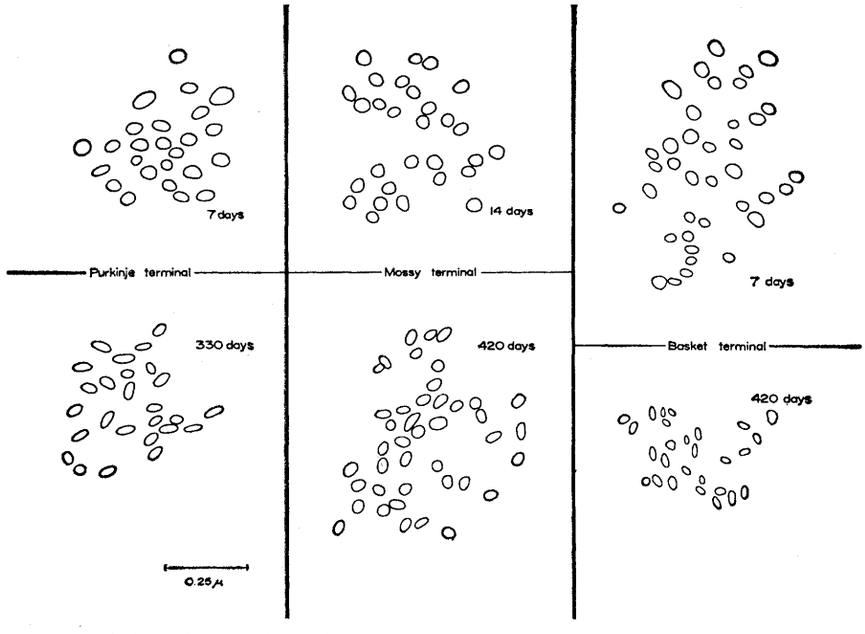

Fig. 1. Samples of synaptic vesicles from excitatory (mossy) and inhibitory (basket and Purkinje) terminals at several ages. Measurements in Table 1 were made from tracings like the above at a magnification of 178,000. The original magnification in the negative was 14,800.

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