explains the principal characteristics of the population of synaptic vesicles: localization to the most peripheral regions of the neurone where hormone release occurs; the frequent clusters of vesicles so close to the plasma membrane that they often seem to be adherent to it (Fig. 2*f*); and the effect of stimulation in increasing the number of these vesicles while reducing the number of neurosecretory granules. Indeed, this last phenomenon prompted conjecture a decade ago that the vesicles might somehow be derived from the granules (Holmes and Knowles[9]).

Although there are no other reports of exocytosis in vertebrate neurones, it has been observed in several invertebrate neurosecretory systems[10–12]. And in these systems too there is evidence for the formation of synaptic vesicles by micropinocytotic activity[13]. The existence of exocytosis in such diverse material suggests that it may be a general mechanism of secretion in neurones.

A more extensive account of our findings and interpretations was given at the recent meeting of the Society for Endocrinology, held in Bristol, and will be published elsewhere[14].

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Auditory and Somatic Sensory Responses evoked in the Cerebral Cortex of the Isolated Dog Brain

When a dog brain is isolated from all structures except the skull and perfused with blood from a compatible donor animal, it has properties which make it especially useful for metabolic studies of neural tissue[1,2]. In all studies conducted with the isolated dog brain, the EEG has been used as a measure of the viability of the isolated and extracorporeally maintained brain. There remains the question of whether the isolated brain is functioning sufficiently well to respond to normal external stimuli.

To determine whether sensory responses can be evoked in the isolated dog brain, four dogs anaesthetized with methoxyfluorine were prepared in the usual manner[1,2]. In two of the dogs a small amount of tissue was left on the snout to permit mechanical stimulation of the skin. The structures of the external, middle and internal ear are intact in all isolated brain preparations. It was thus possible to present a tactile stimulus to the skin of the snout and an auditory stimulus to the ear of the dog. The cutaneous stimulator consisted of a lever attached to an electromagnetic device energized with a 3 ms pulse. The fast forward and backward movement of the lever

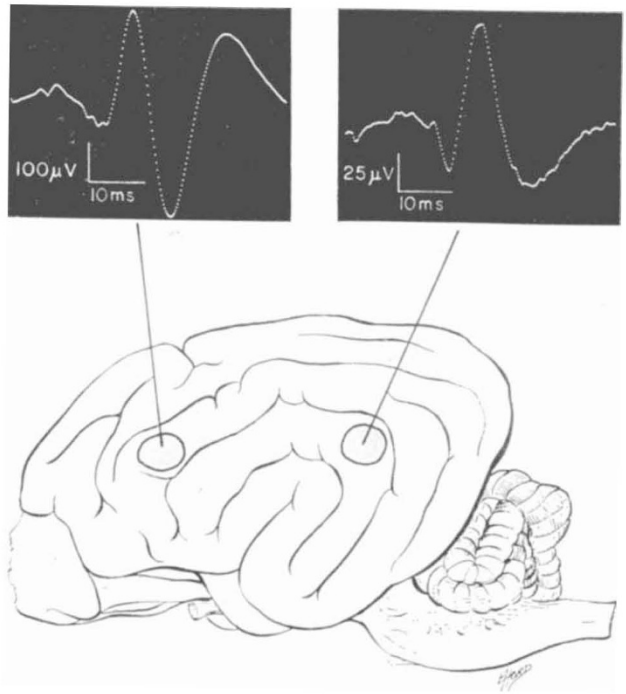

Fig. 1. Average of 100 somatic sensory responses (left) and 100 auditory responses (right) recorded from cerebral cortex of the isolated dog brain.

produced a discrete tap on the skin. The auditory stimulus was a moderately loud click presented through a loudspeaker situated about 1 m from the dog's ear. A craniectomy was performed to remove the skull over the auditory and somatic sensory areas of the left hemisphere. An electrode carrier with the active lead was clamped to the head holder and the indifferent lead was clamped to the skull. The responses were amplified, observed with an oscilloscope and recorded on magnetic tape along with pulses marking the onset of each stimulus. In four successive experiments, responses to auditory stimuli were consistently observed with the oscilloscope. In the two experiments in which the skin on the dog's snout was left intact, responses to tactile stimuli were clearly observed with the oscilloscope in the somatic sensory area contralateral to the side stimulated. At the conclusion of the experiments, 100 taped responses from each point on the somatic sensory cortex and 100 taped responses from each point on the auditory cortex were averaged separately using a Fabri-Tek Instrument Computer. Examples of the averaged auditory and the averaged somatic sensory responses are shown in Fig. 1.

These experiments demonstrate that it is possible to isolate and artificially to maintain the brain of the dog for several hours without seriously impairing the sensory systems of the brain. This provides further evidence that the isolated brain is a reasonably physiological preparation which can be safely used for physiological and biochemical studies.

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