post-treatment in physiological saline (37°C) containing uridine-H^3 (25 μc/ml) as well as sodium acetate-H^3 (25 μc/ml). In all cases (chromosome preparations from three pairs of glands) there was extensive chromosomal labeling and heavy labeling in the puffed regions, suggesting that the glands were still able to synthesize RNA after the experimental treatment.

As a result of these observations, it is concluded that gene activation, as displayed by the heat-induced puffing of regions 86F and 87A-B, does not require the acetylation of histones in these same regions. Although the heat-induced puffs might represent a special class of puffs which might not require histone acetylation, the absence of preferential or extensive uptake of sodium acetate-H^3 in any other chromosomal region, puffed or unpuffed, suggests that acetylation of histones does not play a general role in the regulation of RNA synthesis in *D. melanogaster*. It must be added, however, that although extensive acetylation of chromosomes was not detected in these observations, it might serve more specifically to regulate the activity of a very few genetic loci, as appears to be the case for the hormone ecdysone (*5*). It is also entirely possible that gene activation by means of histone acetylation might require the transfer of very few acetyl groups to the histones, which could go undetected by this method. Even if this were the case, the polytene nature of the salivary chromosomes would seem to offer more sites for acetylation, which would thus enhance the possibility of detecting this phenomenon. Finally, there is also the possibility that acetyl groups are lost from the histones under the conditions employed to prepare the chromosome squashes. However, this seems highly unlikely in view of the fact that acetyl groups remain intact during the several extraction and separation procedures, involving the use of various acid solutions, employed by Phillips (*6*) and are only subsequently removed by partial hydrolysis of the histones in 1*N* or 6*N* H_2SO_4 at 100°C for 5 hours.

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Intracellular Olfactory Response of Hippocampal Neurons in Awake, Sitting Squirrel Monkeys

Abstract. *Intracellular recordings of archicortical neurons in a primate have been made and the responses of these neurons in awake, sitting animals have been observed. Electrical stimulation of the olfactory bulbs elicits excitatory postsynaptic potentials in these neurons, which unlike those evoked by septal stimulation, are subliminal for generating spikes. The olfactory and septal pathways may be considered representative of inputs from exteroceptive and interoceptive systems. The findings are discussed in relation to a paradigm for archicortical conditioning.*

The archicortex of the hippocampus represents the simplest type of cerebral cortex. Its predominant nerve cell is pyramidal in shape and is characterized by a long apical dendrite and several basal dendrites. The axon departs from the base of the cell. We are interested in the mode of action of various inputs to the dendrites and cell bodies of these neurons because such knowledge may contribute to an understanding of mechanisms by which cortical cells are conditioned and participate in memory and learning.

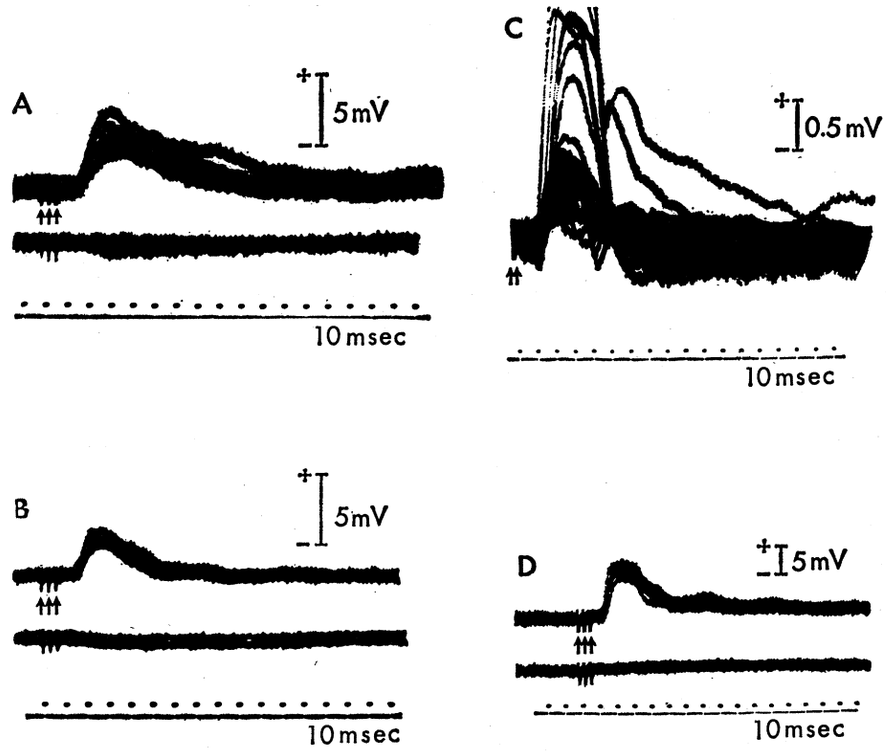
On the basis of extracellular recordings of unit and slow potentials in anesthetized monkeys, Gergen and MacLean (*1*) concluded that impulses from the septum excite hippocampal pyramids more effectively than do those conducted by olfactory pathways. They suggested that the septal input is more effective because it exerts its major excitatory action closer to the trigger zone of the cell. The septal pathway has been shown to be activated by stimulation of the hypothalamus and other structures (*2*) which play an important role in aversive, appetitive, visceral, and humoral reactions. It is probable that olfactory impulses are relayed in part by the perforant pathway from the hippocampal gyrus, which terminates on the apical dendrites (*3*).

Extracellular recording with microelectrodes is useful for detecting the discharge of single nerve cells, but it is inadequate for revealing excitatory or inhibitory postsynaptic potentials developing in an individual cell. To obtain more information about the differential effects of septal and olfactory inputs on pyramidal cells, we have undertaken experiments involving intracellular recordings in awake, sitting squirrel monkeys. Such experiments have been made possible for the first time by adaptation of a technique for intracerebral exploration with microelectrodes (*4*). Heretofore, intracellular recording has commonly involved open operations on the brain, paralysis of the animal, pneumothorax, and other measures to prevent cerebral movement caused by vascular pulsation and respiration. Our technique provides an essentially closed system, and experiments can be conducted without the use of open surgery and the depressing effects of a general anesthetic.

Ten squirrel monkeys were fitted with a special stereotaxic device according to the method described (*4*). Paired Teflon-coated stainless steel stimulating electrodes were permanently implanted in the olfactory bulbs, septum, and fornix. Testing stimuli were one to three shocks (negative constant current 0.5 msec pulses) with an intensity of 0.3 to 1 ma. The shocks to the olfactory bulb were of sufficient intensity to cause sneezing if applied at a frequency of 30 per second. The electrodes in the fornix enabled identification of hippocampal pyramids by means of the antidromic spike. Intracellular and extracellular recordings were obtained with glass micropipettes filled with 2*M* potassium citrate or 3*M* KCl.

Fifty impaled units in the hippocampus responded to one or more of the inputs used. They were tested on the average for a period of 6 minutes. Twelve of 50 units responded to stimulation of the bulb with three shocks with EPSP's (excitatory postsynaptic potentials), the latencies of which

Fig. 1 (right). Responses (EPSP's) of hippocampal and entorhinal neurons to stimulation of olfactory bulb. (A, B, and C) Intracellular recordings from three different hippocampal neurons. (D) An intracellular response of an entorhinal unit, the location of which is illustrated in Fig. 2. Upper tracings are superimposed intracellular responses to olfactory bulb stimulation; lower tracings show corresponding extracellular or so-called outside potentials. (A, B, and D) Direct-coupled recordings with stimulus trains (arrows) applied once every 2 seconds. (C) A capacitor-coupled recording (time constant, 1 second) at higher gain than the direct-coupled channel, shows to better advantage recruitment occurring with stimulus frequency of four per second.



ranged from 15 to 18 msec. The EPSP shown in Fig. 1A was from a unit in area CA3 of the anterior hippocampus [frontal plane AP +6 of brain atlas (*5*)]. It occurred with a 17-msec latency, reached an amplitude of 5 mv, and had a duration of 90 msec. The EPSP shown in Fig. 1B was also from a unit in area CA3 of the anterior hippocampus (AP +6). The response of this unit to stimulation of the olfactory bulb every 2 seconds was an EPSP of 2.7 mv. The latency was 18 msec, and the duration was 70 msec. The EPSP in Fig. 1C was recorded from a unit in area CA2 of the anterior hippocampus (AP +7). There was no response to stimulation of the olfactory bulb applied every 2 seconds, but at a frequency of four per second an EPSP appeared and was followed by recruitment without the generation of spikes. The latency of the EPSP was 15 to 18 msec. The shortest latency was observed with the largest recruited EPSP. With recruitment, a second hump of the EPSP was frequently observed at 50 msec. When this second hump appeared, the duration of the EPSP increased from 50 to 150 msec. Thus far, no unit in the hippocampus has shown an inhibitory postsynaptic potential in response to olfactory bulb stimulation. Of 77 units tested during extracellular recording none was affected by stimulation of the olfactory bulbs.

The EPSP in Fig. 1D was recorded from a unit in the entorhinal cortex. The locus is indicated by the arrow in Fig. 2. This unit responded with an EPSP to stimulation of the olfactory bulb every 2 seconds. The duration was 120 msec, and the amplitude was 9 mv. The latency of 12.5 msec, which was shorter than those of the EPSP's of hippocampal neurons, supports other evidence the entorhinal cortex relays olfactory impulses to the hippocampus (*3*).

Unlike stimulation of the olfactory bulb, the application of three shocks to the septum was effective in eliciting EPSP's that reached sufficient amplitude to bring about a spike discharge of cells in the same location (*6*). This finding of a greater excitatory effect of the septal pathway is in agreement with

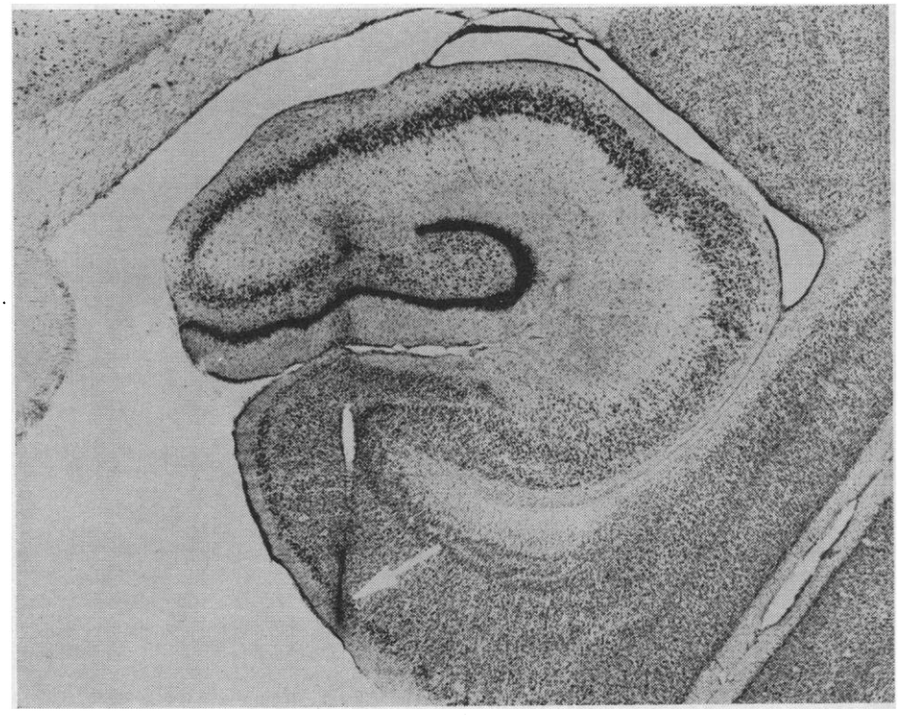


Fig. 2 (right). Arrow points to locus of unit in entorhinal cortex from which intracellular recording shown in Fig. 1D was obtained. Microelectrode was fixed at this point. Gliosis outlining the tip developed during the intervening days before the animal was sacrificed.

Gergen and MacLean's observations with extracellular recording (*1*). The fact that olfactory is less effective than septal stimulation could be explained if there were fewer impluses reaching the hippocampal cells in the former case or if there was a temporal dispersion of impulses. Or, compatible with the hypothesis stated in the introduction, it is possible that the major excitatory effect of olfactory impulses occurs at a greater distance from the trigger zone than that caused by the septal input.

The olfactory and septal pathways may be considered as representative inputs to the hippocampus from exteroceptive and interoceptive systems, respectively. Our experiments have revealed that stimulation of each input is effective in causing graded excitation of hippocampal neurons, with the notable difference that only the septal EPSP's are associated with cellular discharge. The duration of the EPSP's in each case may greatly exceed that reported for neocortical neurons (*7*). The hypothalamus, which has reciprocal connections with the septum (*8*), plays an important role in aversive, appetitive, visceral, and humoral reactions of an unconditional nature. In their paradigm for archicortical conditioning, Gergen and MacLean likened septal impulses to unconditional stimuli of classical conditioning, as these impulses are capable by themselves of discharging units (*1*). Olfactory and other stimuli of external origin, on the contrary, were pictured as analogous to conditioning stimuli, lacking the capacity, when at first acting alone, to cause discharge.

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Somatosensory Thalamic Neurons: Effects of Cortical Depression

Abstract. *Deafferented somatosensory thalamic neurons showed hyperactivity, followed by greatly reduced activity, after initiation of cortical spreading depression; local cooling of sensorimotor cortex was followed only by the inactive phase. Stimulation of contralateral midbrain reticular formation during the inactive phase failed to induce the typical increase in discharge rate of somatosensory thalamic neurons, but produced desynchronization in unaffected cortex. These results indicate that corticothalamic discharge is necessary for sustaining the ongoing activity of deafferented somatosensory thalamic neurons and for maintaining their responsiveness to stimulation of the reticular formation.*

Many sensory neurons within the central nervous system show an ongoing "spontaneous" discharge after elimination of specific sensory inflow (*1*, *2*). Fluctuations in excitability and in rate and pattern of discharge may occur, even when stimulus conditions are constant (*3*). Such observations provide functional evidence for convergence upon central sensory neurons of influences other than those originating within their receptive fields. The changes in single neuron activity associated with sleep and arousal or with desynchronization of the electrocorticogram (ECoG) are generally assumed to reflect activation by the brain stem reticular formation. However, convergence at subcortical relays must be investigated before effects at higher levels can be interpreted. Repetitive electrical stimulation of the midbrain reticular formation of the cat increases the rate of discharge of lateral geniculate neurons (*4*) and of neurons of the somatosensory relay nucleus ventralis posterior (VP)

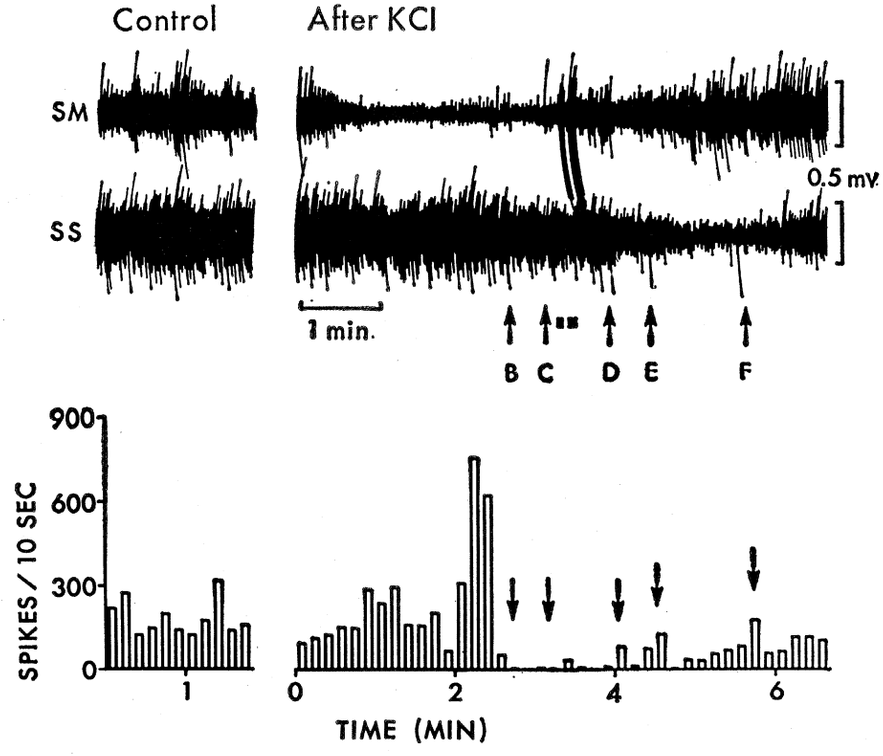

Fig. 1. Effect of cortical spreading depression on activity of a single VP neuron. Electrocorticographic recordings of sensorimotor (SM) and anterior suprasylvian (SS) cortex shown above. Histogram shows concurrent counts of neuron discharges in consecutive 10-second periods. Potassium chloride (2.5*M*) was applied to sensorimotor cortex after control run (left), a few seconds before start of experimental run (right). Arrows mark onsets of stimulus trains applied to contralateral reticular formation. Stimuli were 100 bidirectional pulses per second; each phase was 0.3 ma and lasted 0.1 msec. Rectangles mark periods of stimulation of afferent tracts in midbrain at 1.2-second intervals.