Neuronal Substrate of Classical Conditioning in the Hippocampus

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antagonists. This is confirmed by data showing that D-lysergic acid diethylamide, a mixed agonist-antagonist of the dopamine-sensitive adenylate cyclase (*9*), has similar affinities for both [^{3}H]dopamine and [^{3}H]haloperidol binding sites (*10*). Conceivably the different relative affinities of antischizophrenic drugs for [^{3}H]dopamine and [^{3}H]haloperidol binding sites indicate that these drugs vary in how they affect the dopamine receptor. For instance, some may be more "pure" antagonists than others.

The data reported here demonstrate an extremely close correlation between the clinical and pharmacological potencies of butyrophenones and phenothiazines and their affinities in competing for the binding of [^{3}H]haloperidol to dopamine postsynaptic receptors. This result argues that these drugs do act by blocking postsynaptic dopamine receptors. Reasons for discrepancies between results with the dopamine-sensitive adenylate cyclase and the in vivo and binding data are unclear but may be related to variable degrees of coupling of dopamine receptor sites with the adenylate cyclase (*11*).

Labeling of postsynaptic dopamine receptors by [^{3}H]haloperidol provides a simple, sensitive, and specific means for screening phenothiazines, butyrophenones, and related agents as potential antischizophrenic drugs.

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Neuronal Substrate of Classical Conditioning in the Hippocampus

Abstract. *Neuronal activity in dorsal hippocampus was recorded in rabbits during classical conditioning of nictitating membrane response, with tone as conditioned stimulus and corneal air puff as unconditioned stimulus. Unit activity in hippocampus rapidly forms a temporal neuronal "model" of the behavioral response early in training. This hippocampal response does not develop in control animals given unpaired stimuli.*

The hippocampus has been implicated in learning by many investigators (*1*). Recent studies of hippocampal neurons in the intact, behaving animal have demonstrated clear changes in unit activity during learning (*2*). However, the role of hippocampus in learning remains obscure. We have recently adopted classical conditioning of the nictitating membrane response of the rabbit (*3*) as a model system in which to study neuronal substrates of learning (*4*). The parametric effects of stimulus and training variables and the properties of the response are well established in this system (*5*). Here we report results of an initial study of hippocampal activity during nictitating membrane conditioning.

Animals were anesthetized with halothane, and insulated stainless steel microelectrodes with approximately 5- to 7-μm tip diameters and 40- to 50-μm exposed shafts were permanently implanted (one per animal) in the dorsal hippocampus. Electrodes were localized both with stereotaxic coordinates and physiological recordings during implantation. After 1 week of recovery, animals in the conditioning group were given standard training (*6*): 13 blocks of trials per day, with eight CS-UCS (*7*) paired trials and one CS-alone (1-khz, 85-db, 350-msec tone) test trial per block (117 trials total per day); the intertrial interval was a random sequence of 50, 60, or 70 seconds. The UCS was a 100-msec air puff to the cornea, onset 250 msec after CS onset (CS and UCS overlap). Animals were given one, sometimes two, days of conditioning and then extinguished with at least 13 blocks of CS-alone trials, nine trials per block. Control animals received 13 blocks of unpaired CS and UCS presentations per day, with eight CS-alone trials and eight UCS presentations per day, for 16 unpaired trials per block (204 trials total per day). The sequence was random with a 20-, 30-, or 40-second intertrial interval. To nearly equalize the number of stimulus presentations, the number of unpaired trials was approximately double that of paired trials. All animals were held in a restraining apparatus throughout training. Data from 18 conditioning and 11 control animals are reported here; only acquisition results are given.

Neural activity was recorded on AM-FM tapes and band-pass filtered at 500 to 5000 hertz. Although individual neuron waveforms could be examined if desired, the present analysis was limited to discharges of relatively small groups of units ("multiple unit" discharges) as defined by a pulse-height discriminator set to pass only larger unit spikes. The level of the discriminator was set to maintain a spontaneous mean count of approximately 2 to 6 counts per second. Records were used only where the signal-to-noise

ratio was 3 : 1 or higher and where unit spikes were clearly distinguishable from background. The counts were cumulated in successive 3-msec time bins over the eight paired trials (acquisition) per block, or the eight UCS-alone unpaired trials (controls) per block, with separate totals for the CS-alone trials. Data were collected for 250 msec of the pre-CS period, for the CS period (250 msec), and for 250 msec of the post-UCS-onset period (the UCS period). Analysis consisted of computation of post-stimulus histograms for those time periods and computation of the mean number of counts in the pre-CS period, the CS period, and the UCS period, and the standard deviation (SD) of the pre-CS period. A standard score, $(\overline{CS} - \overline{\text{pre-CS}})/(\text{SD pre-CS})$, or $(\overline{UCS} - \overline{\text{pre-CS}})/(\text{SD } \overline{\text{pre-CS}})$, was computed for each block of trials for the CS period and the UCS period. The individual nictitating membrane responses were recorded with a minitorque potentiometer mounted on headgear worn by the subject throughout training. The nictitating membrane responses were averaged over eight-trial blocks (*6*)

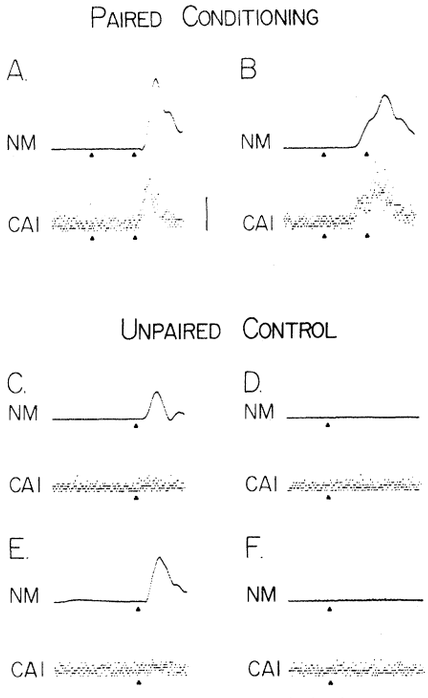

Fig. 1. Upper trace: Average nictitating membrane (*NM*) response for one block of eight trials. Lower trace: Hippocampal unit post-stimulus histogram for one block of eight trials. (A) First block of eight paired conditioning trials, day 1. (B) Last block of eight paired conditioning trials, day 1, after conditioning has occurred. First cursor indicates tone onset; second cursor indicates air puff onset. (C) First block of eight unpaired UCS-alone trials, day 1. (E) Last block of eight unpaired UCS-alone trials, day 2. Cursor indicates air puff onset. (D) First block of eight unpaired CS-alone trials, day 1. (F) Last block of eight unpaired CS-alone trials, day 2. Cursor indicates tone onset. Total trace length is 750 msec. Height of vertical bar to right of CA1 unit post-stimulus histogram in (A) is equivalent to 13 neural spike events.

All conditioning animals rapidly developed a characteristic neural response in the hippocampus early in training. A typical example is shown in Fig. 1. The hippocampal unit poststimulus histogram and averaged nictitating membrane response for the first block of eight paired trials are given in Fig. 1A. Note that unit activity in the hippocampus exhibits a pronounced increase which temporally precedes and parallels the behavioral nictitating membrane response. A single-trial analysis was completed for conditioning animals showing this marked hippocampal response in the first eight-trial block (for example, Fig. 1A). The response is not present in the first few trials; it then develops rapidly (see Fig. 2). As behavioral conditioning develops, the hippocampal response moves forward in time, always preceding in latency (typically 25 to 35 msec) and paralleling the behavioral nictitating membrane response. An example after conditioning has developed is shown in Fig. 1B. This same pattern occurred in all conditioning animals.

Standard score analysis indicated that the relative amount of hippocampal unit activity increases progressively over training for the conditioning animals. For the first block of eight trials, the mean standard score for the 18 conditioning animals was 5.43 for the UCS period and 1.39 for the CS period. For the last block of training, after conditioning had developed, the mean standard score was 18.61 for the UCS period and 5.69 for the CS period. These results were obtained consistently for all hippocampal electrodes in CA1, CA3, and CA4 that were seen in histology to have recording tips in the pyramidal cell layer, and for all dentate electrode tips in the granule cell layer. Electrode tips not clearly in these layers yielded negative or inconsistent results.

Data from the control group of animals given unpaired CS and UCS trials indicate that the rapid development of hippocampal activity in the UCS period in the conditioning animals (Fig. 1A) is in fact due to the conditioning training. Examples of data from a control animal are shown in Fig. 1, C to F. Note first that there is no nictitating membrane response and no hippocampal response to CS alone. There is, of course, a substantial behavioral nictitating membrane reflex response to the UCS alone. However, there is virtually no increase in hippocampal unit activity. For the first and last blocks of unpaired trials, the mean standard scores of the 11 control animals were 2.41 and 2.05, respectively, for the UCS period (UCS-alone trials), and 0.54 and 0.30, respectively, for the CS period (CS-alone trials). Wilcoxon tests showed significant differences (paired versus unpaired groups) in neural activity scores for both CS and UCS periods at the $P < .001$ level. Additionally, for several paired animals analyzed, individual spontaneous nictitating membrane responses given in the pre-CS period were accompanied by only small increases in hippocampal activity (comparable to that seen in unpaired controls), while nictitating membrane responses to the tone–air puff complex later in the same trial were correlated with large unit increases. Thus, hippocampal activity is not evoked by the tone CS per se, nor by the air puff UCS per se, nor is it a necessary concomitant of the behavioral nictitating membrane response.

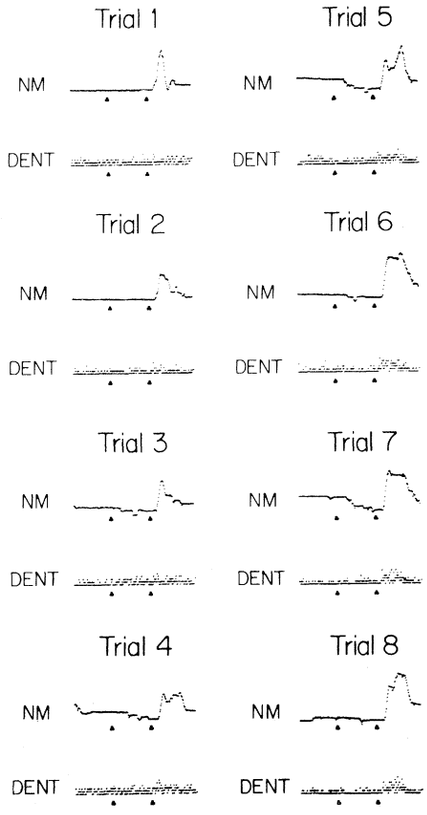

Fig. 2. Single-trial analysis of first block of eight paired conditioning trials. Upper trace: Individual nictitating membrane (*NM*) response for a paired conditioning trial. Lower trace: Hippocampal unit post-stimulus histogram for a paired conditioning trial. First cursor indicates tone onset; second cursor indicates air puff onset. Total trace length is 750 msec; *DENT*, dentate.

Consequently, the marked increase in hippocampal activity that develops early in training is dependent only upon the paired CS-UCS conditioning procedure. Since it develops within a very few trials of training, it is likely to be the earliest, or certainly one of the earliest, neuronal indications that learning is occurring. In this sense, it might be considered an initial process in the formation of the "engram." This rapidly developing hippocampal activity is reminiscent of short-term or "primary" memory in human information processing theories (*8*), and is suggestive of various mnemonic functions hypothesized for the hippocampal formation (*9*).

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Rapid Discrimination of Rewarding Nutrient by the Upper Gastrointestinal Tract

Abstract. *When certain nutrients are injected into the stomachs of rats that are drinking one of two samples of nonnutrient, flavored water, the rats will (within a 10-minute session) choose the flavor paired with the nutrient. Such rewarding effects are obtained with predigested milk but not with similarly treated glucose or fresh milk. The results suggest the presence of rapidly acting, specialized, nutrient receptors in the upper gastrointestinal tract.*

It is not yet known how animals recognize some substances as food. Some investigators have suggested, by analogy with the work of Garcia *et al.* (*1*) on conditioned aversion, that the long-term beneficial aftereffects of a substance become conditioned to its taste (*2*). However, others, such as Gibbs *et al.* (*3*) and Snowdon (*4*), have postulated the existence of physiological mechanisms in the upper gastrointestinal tract, which signal the presence of food, presumably without an intervening process of learning. With the exception of an early experiment by Miller and Kessen (*5*), on the interpretation of which Holman (*6*) has cast doubt, support for the second view has been confined to work which shows reduction of intake after the injection of some nutrient into the upper gastrointestinal tract. However, we have shown that the injection of a palatable nutrient into the stomach of rats (1 ml of sesame oil) leads to a strong conditioned aversion to the fluid being drunk before the injection (*7*). Similarly, the injection of 1*M* glucose into the duodenum (0.6 ml/min, 3 ml total volume) also leads to a conditioned aversion to fluid drunk by rats just before the injection (*7*). The reduced intake after gastric injection may not be due to detection of nutrient by the gut as has been believed, but to some other cause. In Holman's own work (*6*) drinking of a flavored liquid was followed by an injection of nutrient into the stomach, and a preference for the flavored fluid developed. However, as trials were spaced 24 hours apart, a taste preference based on the long-term beneficial aftereffects of the nutrient may have accounted for the results; Holman did, in fact, interpret his findings in terms of such a hypothesis.

To provide a more stringent test of the hypothesis that the upper gastrointestinal tract immediately recognizes food, we gave rats a choice between two nonnutrient flavors. As the animal drank one of the flavored liquids, nutrient was pumped into its stomach through an implanted tube at the same rate as it drank. When the rat drank the other flavor, no nutrient was injected. Each daily session lasted 10 minutes. A successful choice of the liquid paired with the nutrient could then be made on only fairly immediate consequences of the arrival of nutrient in the upper gastrointestinal tract, especially because the rats almost invariably sample both flavors during the initial sessions. Such a choice does occur, but only when the injected nutrient has been digestively modified.

In the first experiment we implanted a Silastic tube in the stomachs of eight albino rats (300 to 350 g, male, Sprague-Dawley). They were given 2 weeks to recover and 1 week to become accustomed to a 22½-hour food and water deprivation schedule. They were then given a choice between two nozzles containing flavored water, one banana (0.5 percent Schilling banana flavoring) and the other almond (0.5 percent Schilling almond flavoring). When four of the rats drank the almond-flavored water, whole milk was injected through a long plastic connector into the stomach at the same rate and volume as they drank. When they drank banana-flavored water, nothing was injected. For the other four rats, the pairing between flavor and milk was reversed. There were nine daily 10-minute experimental sessions. In a second experiment, another eight rats had two Silastic tubes implanted into the stomach. The identical experiment was then