

Potentiation of neuronal responses to natural visual input paired with postsynaptic activation in the hippocampus of the awake monkey

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Extracellular recordings were made in the hippocampal formation while the monkey performed a simple visual discrimination task in which the stimuli consisted of coloured patterns presented serially. By using this natural visual input to single hippocampal neurones, and experimentally pairing one of the stimuli with postsynaptic excitation induced by iontophoretically applied L-glutamate, we demonstrate that selective synaptic potentiation can result. Seven of 29 neurones tested showed potentiation. The potentiation was selective in that enhanced responses only occurred to the previously paired stimulus, and not to other visual stimuli. The potentiation was observed in CA3 neurones and lasted approximately 15 min. In each case the latency of the potentiated response occurred at 160 ms following the onset time of the stimulus.

Potentiation of synaptic transmission in the hippocampus [1, 20] provides a neurophysiological model for analysing the mechanisms of synaptic modification which underlie some forms of learning and memory [8, 13]. Typically in studies of synaptic plasticity the presynaptic neurones are induced to fire synchronously and at high frequency through electrical stimulation. When this produces or is paired with strong depolarization of the postsynaptic neurone, test pulses applied later to the presynaptic pathway produce a potentiated response in the postsynaptic neurone [7]. However it is not known whether synapses activated by normal afferent firing that is associated with postsynaptic excitation can lead to potentiation of those synapses. Nor is it known whether single neurones within the hippocampus are able to code specific associations related to arbitrary and complex stimulus input patterns.

Under thiopentone sodium anaesthesia the monkey (*Macaca fascicularis*, weight 3.0–3.8 kg) was implanted with a stainless-steel holder on which an adaptor could be fitted for later recording sessions [16]. During recording sessions the conscious monkey was seated in a primate chair while extracellular recordings were made from single neurones in the hippocampal formation. Six

pairs of coloured fractal patterns [15] were used as visual stimuli in a discrimination task [16]. One pair of patterns was used for a given experiment. Each pattern was serially presented in randomized order for 1–2 s onto a video screen 30–100 cm in front of the monkey. The monkey was required to make a behavioural response (lick at a tube or touch at a screen) following the presentation of one of the visual patterns (either S1 or S2) in order to receive approximately 0.6 ml of fruit juice. Following the presentation of the other pattern the monkey withheld making a response in order to avoid the delivery of 0.2 ml of aversive saline solution. Each trial was signalled to the monkey by a 500 ms, 400 Hz cue tone whose offset coincided with visual stimulus onset. There was an intertrial period of 5–7 s. Electrical activity of single neurones was recorded (100 Hz to 5 kHz bandwidth) using the tungsten-filled centre barrel of 4–7 barrelled pipettes. Neuronal responses were defined as the number of spikes that were counted in a 500 ms time window starting 100 ms after visual stimulus onset [16]. At least one drug barrel contained Na L-glutamate solution (0.5 M, pH 8.5). Another barrel contained 150 mM NaCl solution for automatic current balancing. Iontophoretic applications were switched on up to 500 ms (median = 200 ms) before visual stimulus onset, and lasted for between 450–850 ms (median = 600 ms). Iontophoretic currents ranged from 20–200 nA (median = 100 nA) which typically caused postsynaptic excitations of 500 ms duration (range 250–2000 ms). During the performance of the dis-

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crimination task the presentation of one coloured pattern stimulus (S1) was paired with the excitatory effect of iontophoretic application of glutamate. The presentation of a second coloured pattern (S2) was randomly interspersed with S1 presentations in a series of trials, but was not paired with iontophoretic glutamate. Between 30 and 70 pairings were made with S1 presentations in each experiment before neuronal responses were tested by presenting the visual stimuli without iontophoretic pairing. Glutamate was used in the experiments since there is evidence that it is the excitatory neurotransmitter within the hippocampus [19], and has a high affinity for *N*-methyl-D-aspartate, AMPA and kainate receptors [14, 18]. Local application of glutamate would ensure that postsynaptic activity alone was induced without influencing presynaptic afferent inputs [4]. Onset time, duration, and current for glutamate applications were adjusted for a given neurone, so that the maximal excitatory effect occurred approximately 100 ms after visual stimulus onset, and lasted for typically 500 ms while S1 was being displayed.

In each of the 29 neurones studied, a statistically significant excitatory effect of glutamate (mean induced firing rate = 70 spikes/s) was observed during the pairing procedure, as indicated by a significant interaction term ($\alpha=0.05$) in a two-way factorial analysis of variance (ANOVA). Pre-pairing control responses to visual stimuli S1 and S2 had a mean of 32 spikes/s (mean intertrial

spontaneous activity = 27 spikes/s). When tested, seven of the 29 neurones displayed significant potentiation of their responses (mean = 54.5 spikes/s) to the visual pattern that had previously been paired with iontophoretic glutamate (Figs. 1 and 2). The relevant data for these seven neurones only is presented below. In each case the ANOVA interaction term for the potentiation effect had $P < 0.0017$ (critical significance level from Bonferroni's inequality). Potentiated responses were between 44–239% (mean = 159%) of pre-pairing control firing rates, and lasted for between 8–27 min (mean = 14.7 min). Regression analysis of the temporal profile indicated that decay occurred with respect to time, rather than with respect to the number of trials completed. The test responses to S2 (mean = 28.6 spikes/s) were usually unchanged compared with pre-pairing control responses, although in 2 cases responses were reduced, and in 1 they were increased. In two experiments spontaneous firing increased following the pairing procedure. However, in each experiment the significant interaction was due primarily to selective increases in firing to the previously paired pattern. On no occasion was selective depression of responses observed.

The location of recording sites is shown in Fig. 3 as filled circles and crosses. Neurones that displayed potentiation (crosses) were found in subfield CA3 (6 sites) and in the dentate gyrus (one site).

Earliest potentiated response latencies were deter-

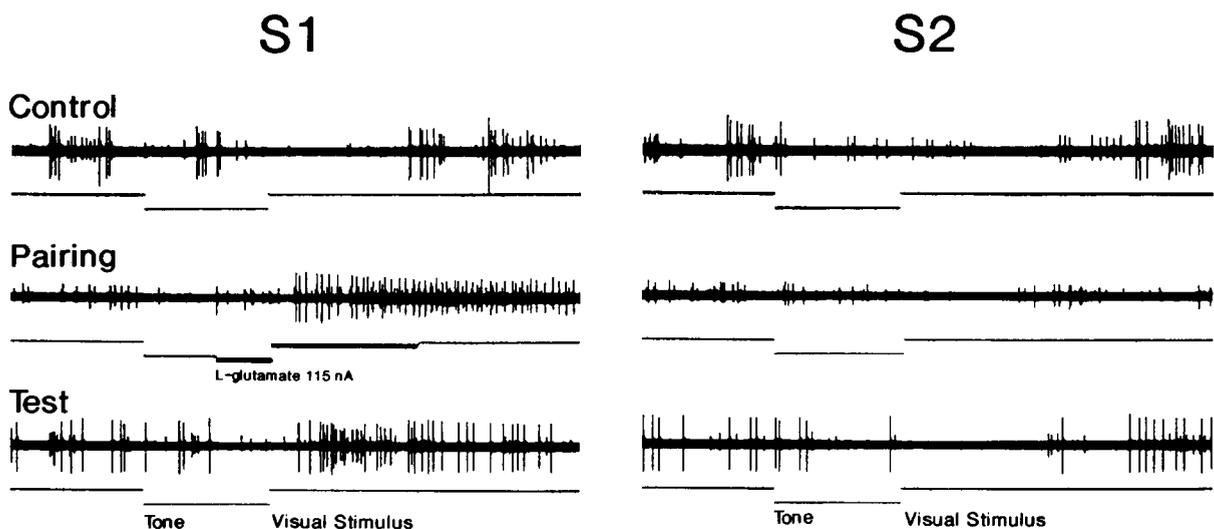


Fig. 1. Typical oscilloscope photographic records from an experiment where a neurone displayed potentiated responses to one coloured pattern stimulus (S1) after glutamate pairing. The activity of the neurone is represented by the larger amplitude vertical deflections from a horizontal baseline (of noise). The sets of records on the left and right show firing activity during single S1 and S2 trials respectively. In the pre-pairing control stage (top) there was little activity during the presentation of either S1 or S2. During pairing (middle), S1 presentation was paired with an iontophoretic pulse of glutamate (115 nA for 800 ms, represented by the thickened line of the trace below); S2 was not paired with iontophoretic applications. An excitatory response was evoked approximately 95 ms after S1 onset. There was no activity during the corresponding S2 trial. The trials taken from the test stage (bottom) show a potentiated response to S1. The visual patterns were presented at the offset of the auditory tone (represented by a 500 ms downward deflection of trace below each record).

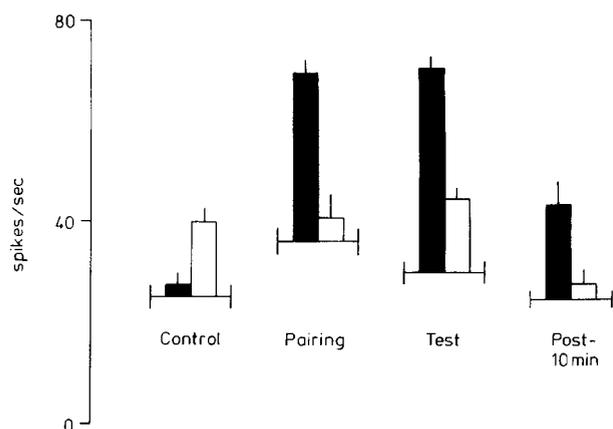


Fig. 2. Another example of a neurone that showed potentiation to a visual pattern that had been paired with glutamate iontophoretic applications. The mean responses ($n=20$ at each stage) are plotted in spikes/second relative to the mean intertrial spontaneous level of firing (\pm S.E. bars). The two different patterns used, S1 and S2, are represented by filled and open bars respectively. From left to right are shown: the mean responses before pairing (labelled Control); responses during pairing (Pairing) in which S1 was paired 40 times with iontophoretic glutamate at 130 nA, 700 ms duration; responses after pairing (Test); responses after 10 min from the end of the pairing procedure (Post-10 min). During the pairing stage a clear excitatory response to glutamate was evoked in conjunction with S1 presentations; firing during S2 remained unchanged. At test a clear potentiated response was observed to S1 (interaction $P<0.0001$), S2 responses again remained constant. Ten min later the mean response to S1 was still enhanced compared with control, responses to S2 had decreased although this alone did not account for the continued significant interaction.

mined for each neurone through the use of a cumulative sum of the difference between the test phase histograms for S1 and S2 [3, 16]. In each of the seven cells the mean latency was 160 ms (± 10 ms accuracy) after visual stimulus onset, representing remarkable between-neurone consistency of responses. This 'fixed' latency for potentiated responses suggests that synaptic modification may be dependent on the oscillatory phase of activity among afferents (e.g. theta rhythm [6]), possibly synchronized by the pre-stimulus auditory cue.

In the primary visual cortex of the anaesthetized cat it has been demonstrated that pairing a visual stimulus with extracellular current pulses can result in long-lasting (>15 min) enhancement of neuronal responses to that stimulus [5]. By using the awake behaving monkey in the present study we not only avoided any direct pharmacological effects of anaesthesia [2], but also allowed normal attentional processes to occur and therefore to provide the hippocampus with natural visual information.

The potentiated responses were related to the sensory properties of S1, or else to a unique mapping of S1 to a particular behavioural response. They were not due to

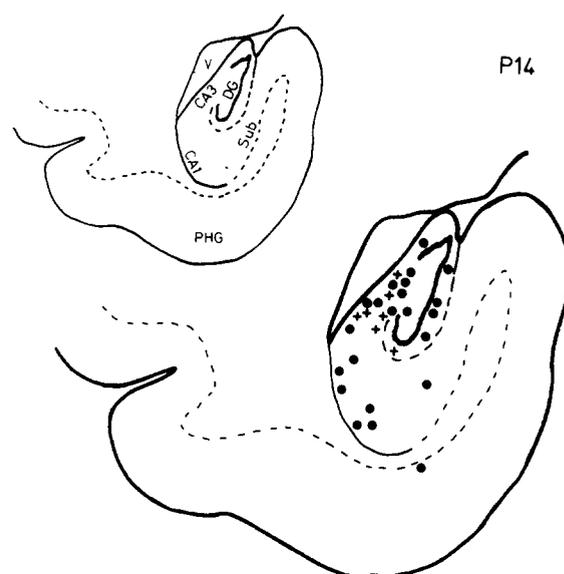


Fig. 3. Histological localization of recording sites in the hippocampal formation. Crosses represent sites where a neurone displayed significant potentiation to S1, filled circles are sites where a negative result was obtained. Six positive sites were in the CA3 subfield, and one in the dentate gyrus. Recording sites were reconstructed posterior (P) to sphenoid following a regression analysis of brain microlesion positions on electrode tip locations (indicated on X-ray photographs taken at the end of each experiment). Recordings were made between P12 mm and P20 mm from sphenoid, the sites illustrated are at P14 mm. Inset shows the different areas of the hippocampal formation: CA1 and CA3, subfields of hippocampus; DG, dentate gyrus; PHG, parahippocampal gyrus; Sub, subiculum; V, ventricle.

an association with reward/non-reward or to the movement requirements of the task. This was determined by testing the responses to an alternative pair of patterns that were equally familiar to that used in the pairing procedure. There were no enhanced responses to either of the alternative stimuli (data not shown), even though the reward contingencies and behavioural responses were identical to those for the experimental pair of stimuli.

It is conceivable that the observed potentiation did not occur within the hippocampus, but was due to synaptic modifications at an earlier stage of the visual processing. This possibility appears unlikely for at least two reasons. Firstly, the iontophoretically applied glutamate had a limited locus of influence due to the short (850 ms or less) applications used, and due to the active uptake mechanisms that exist in the brain. Movement of the electrode tip by more than 30 μ m resulted in a dramatically reduced excitatory effect of applications [12]. Therefore only one or a very few neurones would be sufficiently activated during the iontophoretic pairing procedure. Secondly, photographic records, obtained for 4 of the 7 potentiated cells, showed that glutamate applications during the pairing stage evoked the same distinc-



Fig. 4. Photographic records (retraced) of single action potentials evoked during presentation of S1 (below) and S2 (above) during the test stage. The action potentials evoked during S1 presentation (previously paired with glutamate) were broader compared with those evoked during S2 presentation (previously unpaired). Vertical bar = 50 μ V; horizontal bar = 0.5 ms.

tive broad spikes that occurred to S1 during the test stage (see Fig. 4). Such broadening of the action potential shape is indicative of dendritic spikes [17]. This observation suggests that, for at least four of the cells, the increase in synaptic efficacy occurred only at specific synapses on the recorded neurone.

Six of the 7 neurones that showed potentiation were found in subfield CA3 (Fig. 3). A previous study showed that neurones within CA3 had a higher probability of coding single sensorimotor associations than many other subareas of the primate hippocampal formation [3]. In addition, their latency for potentiated activation (160 ms) was similar to that previously found for learned associations between visual stimuli and spatial motor responses (which had a mean latency of 152 ms [3]). The similarity between experimentally induced potentiations using glutamate pairing and the potentiated responses that normally occur to specific learned associations, strongly suggest that the present data is of relevance to the study of neural mechanisms of normal associative learning.

An anatomical feature of CA3 is the existence of extensive collateral fibres [9], which within CA3 tend to act as an auto-associative network [10]. It is likely that these dense reciprocal interconnections between pyrami-

dal cells account for the unusual associative capabilities of neurones in this region.

The presence of neuronal responses to the visual stimuli during the control period (before the pairing with glutamate) would indicate the existence of functional synapses which, during the glutamate pairing, could be enhanced to produce potentiation. However, in our experiments only 2 of the 7 potentiated neurones showed significant responses to S1 (firing greater than spontaneous baseline) in the control period. One possibility is that, in addition to increased efficacy at existing synapses, increases in the number of functional synapses is an important mechanism underlying potentiation in the hippocampus. It is of interest that, under certain *in vitro* conditions, latent synaptic pathways are revealed in CA3 neurones following tetanic stimulation of their mossy fibre input [11].

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