

The role of glutamate in pigeon optic tectum

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It is well known that superficial layers of the pigeon optic tectum contain high levels of endogenous glutamate² and that in the tectum a high-affinity uptake system exists for this excitant amino acid¹⁰. Subcellular and autoradiographic studies indicate that up to 30% of the exogenously accumulated glutamate is localized within synaptosomes². After retinal ablation the high-affinity uptake for glutamate is markedly reduced in the pigeon¹¹ and in the frog optic tectum¹⁶. However, other studies in the rabbit¹⁵ and also in the pigeon² do not support this conclusion. Microiontophoretic application of glutamate excites a large proportion of tectal neurons¹. These data suggest that glutamate may be a transmitter in the pigeon optic tectum.

In a preceding electrophysiological investigation we studied the interaction of the alkaloid L-5,6-dimethoxyaporphine (L-nuciferine)^{3,7,12} with excitant amino acids on neurons of the pigeon optic tectum. This compound antagonizes the action of L-glutamate and, to a weaker extent, that of L-aspartate⁸. In order to provide further evidence for glutamate as an excitatory transmitter in the present study, the effects of known glutamate antagonists L-nuciferine and glutamic acid diethylester (GDEE)⁹ and of non-glutamate antagonists on synaptically induced activity in the pigeon optic tectum were investigated with the aid of microiontophoretic techniques.

The experiments were performed on 59 pigeons (*Columba livia*). The animals were anesthetized with Equithesin (i.m., 0.3 ml/100 g body weight), paralyzed by injecting Pavulon (pancuronii bromidum, Organon Oss, Holland, initial dose 0.4 ml), artificially ventilated by unidirectional airflow of a O₂-CO₂ mixture⁴ and body temperature maintained between 40 and 41 °C using a heating pad. The left optic tectum was exposed on its dorsolateral side, and the dura opened. Eyelid, cornea, lens and vitreous humour of the contralateral eye were removed. A bipolar steel electrode was placed on the optic nerve. For the electrical stimulation of the optic nerve, single rectangular pulses of 0.2–1.5 mA intensity and 0.1–0.3 msec duration were delivered. Extracellular recordings of action potentials were obtained from tectal neurons with the 4 M NaCl-filled barrel (5–15 MΩ resistance, 4.5 μm diameter) of a 5-barreled

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micropipette. The other channels contained the following compounds to be ejected microiontophoretically: L-glutamate (1 M, pH 7.5), L-nuciferine (10 mM, pH 3.0), GDEE (0.5 M, pH 4.0), atropine sulphate (10 mM, pH 5.7) or dihydro- β -erythroidine (0.01 M in 0.165 M NaCl). The majority of neurons were sampled at a radial distance between 900 and 1200 μ m from the tectal surface, which corresponds to the thirteenth layer in Cajal's terminology⁵, the stratum griseum centrale⁶ and to the 'P-zone' of Holden^{13,14}.

Recordings were made from a total of 135 tectal cells. The majority of these cells were discharging spontaneously and could be further excited by iontophoretic applications of glutamate (Fig. 1A). Following single pulse electrical stimulation of the contralateral optic nerve the average latency to the first spike was 6.8 msec and the duration of the response was 6 msec (see Fig. 1E, control). When the stimulus strength

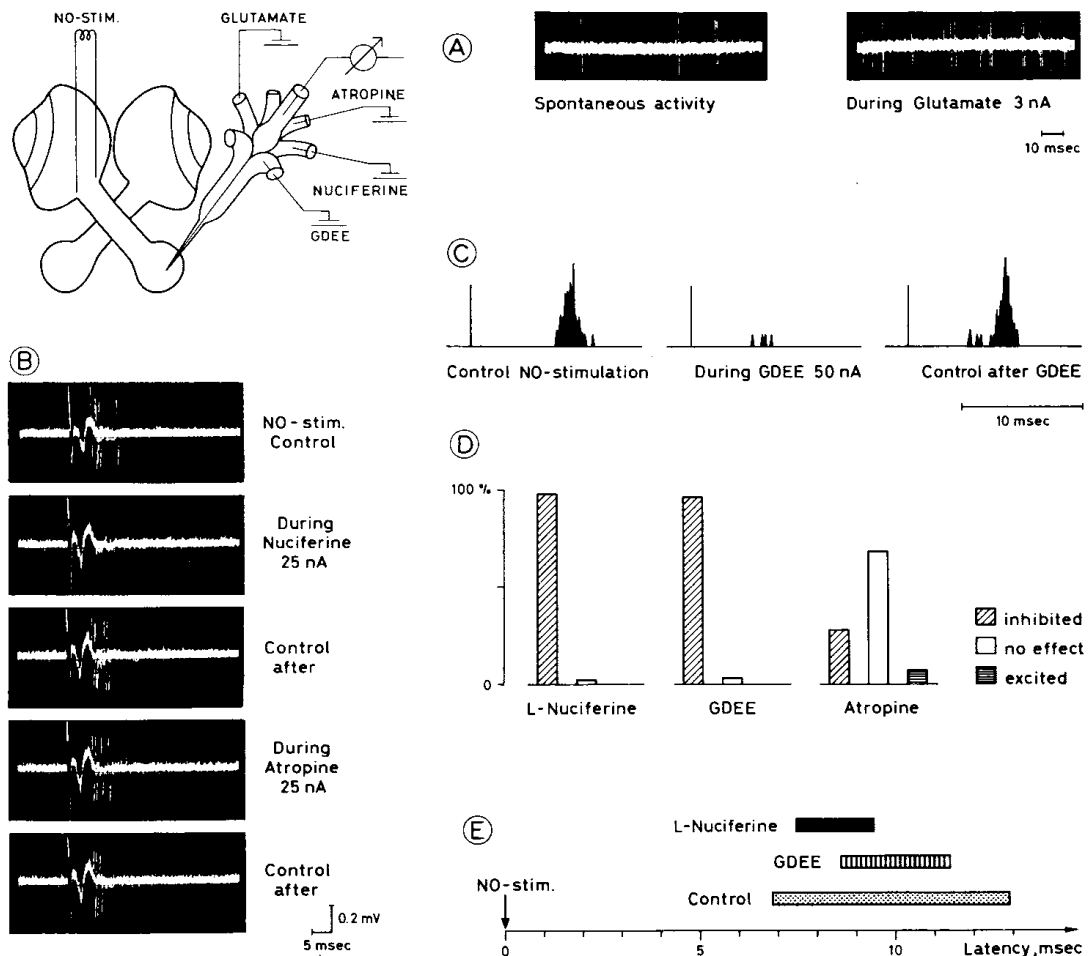


Fig. 1. Differential effects of nuciferine, GDEE and atropine on the synaptic excitation elicited by electrical stimulation of the contralateral optic nerve (NO-stimulation). The experimental procedure is illustrated on the top left figure. A: spontaneous and glutamate-induced activity of a tectal cell. B: effect of L-nuciferine (25 nA) and atropine (25 nA) on synaptic activation (5 superimposed sweeps). C: effect of GDEE (50 nA) on synaptic activation illustrated on a PST-histogram (50 sweeps). D: percentage histograms of the cells during nuciferine ($n = 85$), GDEE ($n = 59$) and atropine ($n = 46$). Note that during atropine a few cells excited. E: differential effects of nuciferine and GDEE in comparison with control discharge shown by shortest latencies and time ranges during spike display.

was kept at threshold (average discharge rate 0.9 spikes/sweep), an antagonistic effect on synaptic excitation could be demonstrated in all but 3 out of 73 cells tested with nuciferine and all but 3 out of 55 cells tested with GDEE (Fig. 1D). The responses were either abolished (Fig. 1B) or, as shown in a poststimulus time histogram, reduced (Fig. 1C). More detailed investigations showed that the average number of spikes evoked per impulse was reduced to slightly greater degree by nuciferine than by GDEE, and the latency of the first evoked spike was less prolonged by nuciferine (0.6 msec) than by GDEE (1.8 msec). In addition, the duration of the evoked response was reduced to a greater degree in the nuciferine-blocked cells than in those affected by GDEE. In contrast to the clear-cut antagonistic action of glutamate antagonists, the action of similarly applied atropine was less pronounced. A total of 65% of tested cells were not influenced by this drug (Fig. 1D). An example of such a cell is illustrated in Fig. 1B. However, on 28% of cells a depressive effect was observed. Preliminary investigations with dihydro- β -erythroidine also seemed without specific effect on the synaptic-evoked activity.

These findings confirm that glutamate strongly excites a large population of cells within superficial tectal layers by potentiating their synaptically induced activity. The fact that glutamate antagonists, such as nuciferine and GDEE, depress the synaptic excitation in a specific way, lends considerable support to the hypothesis that glutamate acts as an excitatory transmitter in this region.

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