Effects of ethanol on cerebral cortical neurons: Interactions with some putative transmitters

While there is considerable evidence suggesting an interaction of ethanol with putative synaptic transmitter substances in the cns (Kalant, 1970), the techniques of microelectrophoresis have not been utilized to investigate either these phenomena or, indeed, the actions of ethanol on single neurons. Since recent studies in our laboratory (Phillis, Lake & Yarbrough, 1973) have indicated that the depressant actions of biogenic amines on cerebral cortical neurons may be mediated by a calcium-dependent mechanism and since ethanol is known to be a calcium antagonist (Hurwitz, Battle & Weiss, 1962), the present experiments were designed to investigate its effects on cerebral cortical neurons and to probe any interactions between it and presumptive neurohumoral agents in the cortex.

Records were obtained of spontaneous or glutamate-evoked firing of single, somatosensory cortical neurons of 9 male rats (300–400 g, Hooded Sprague-Dawley) maintained under methoxyflurane-N_2O anaesthesia after preliminary induction with intravenously administered thiopentone. Drugs were applied by microiontophoresis or, for ethanol, by electrosmosis (Krnjević, 1971) into the vicinity of the neuron under study.

Extracellular action potentials were recorded through the centre barrel (2M NaCl) of seven-barrelled micropipettes (overall tip diameter 4–8 μm), the outer barrels being filled with a combination of the following drugs: (—)-noradrenaline bitartrate, 0·2M pH 4·5 or 5·0 (Sigma); 5-hydroxytryptamine creatinine sulphate 0·02M, pH 5·5 (May & Baker); L-glutamate, 0·2M, pH 7·0 (Baker); ethanol, 0·2 or 0·4M in 165mM NaCl, pH 6·6 (Standard Chem. Co.); γ-aminobutyric acid (GABA) 0·5M, pH 3·5 (Sigma); NaCl, 2M; acetylcholine chloride, 0·2M, pH 4·0 (Sigma).

Ethanol applying currents produced decreases in the firing rates of 23% of the cortical neurons tested, while the remainder were excited (10%) or unaffected (67%) within the range (30–150nA) of doses utilized (Table 1a). The depressant and excitant effects were weak, immediate in onset, and usually reversible, requiring 1–3 min for recovery. There was no consistent action on spike amplitude except that an increase sometimes preceded the depression of cell firing. Applications of anodal (sodium) currents of equal magnitudes and durations were used to discriminate between genuine responses and current artifacts.

After ethanol application (and recovery to baseline firing rates, if necessary) doses (20–60nA) of noradrenaline, 5-hydroxytryptamine or acetylcholine, which had

Table 1. *Actions of ethanol on cerebral cortical neurons.*

a.

Agent	Total cells[1]	Excited	Depressed	No effect
Ethanol	30	3 (10%)	7 (23%)	20 (67%)

b.

Agonist	Total cells[2]	Antagonism by EtOH
Acetylcholine	7	5 (71%)
Noradrenaline	17	14 (82%)
5-HT	18	12 (67%)
GABA	11	0 (0%)

[1] Figure indicates the number of cortical neurons tested with ethanol (applied with currents ranging from 30–150nA).

[2] Figures indicate the number of cells which were tested for ethanol antagonism of amine- or GABA-induced depressions. The amines were applied with currents ranging from 20–60nA; GABA with currents of 15–50nA.

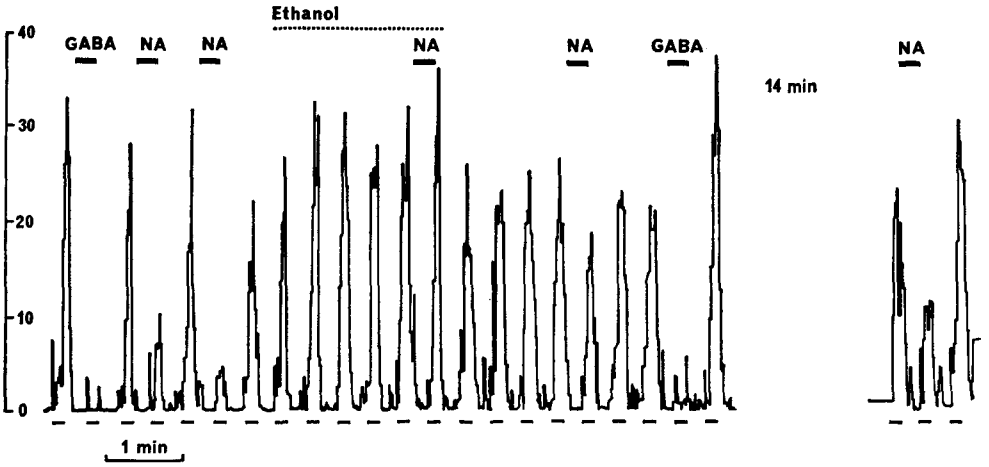

FIG. 1. Effect of ethanol on depression of rat cortical neuron firing produced by noradrenaline (NA) and GABA. Firing rate s^{-1} is shown on the ordinate. Horizontal bars indicate periods of drug application. The bars beneath the ratemeter tracing indicate the brief pulses of glutamate (20nA) used to activate the neuron. NA (50nA) and GABA (50nA) caused depression of firing before ethanol application. Ethanol produced antagonism of NA, but not GABA, depression. Noradrenaline remained antagonized until 14 min later when there was partial recovery (as shown).

depressed firing before the application of ethanol, were found to be ineffective (Table 1b). Recovery of amine sensitivity occurred within 4–15 min. This antagonism by ethanol of monoamine- and acetylcholine-induced inhibitions of neuronal firing did not extend to the depressions of the same neurons produced by various doses (15–50nA) of GABA (Fig. 1). Doses of ethanol which were without direct action on neuronal firing were often sufficient to produce similar antagonisms of amine-induced depression.

The data presented here are the first reported observations of a direct depressant effect of ethanol on the spontaneous or glutamate-evoked firing of cns neurons. Such an action is consistent with the finding that ethanol decreases spike amplitude and peak sodium conductance in the squid giant axon (Armstrong & Binstock, 1964). Moreover, ethanol appears to have another action of a more prolonged time course, which is in evidence after recovery to control values of spike amplitude and firing from direct depression by ethanol. This is revealed in the antagonism of biogenic amine-induced depressions, but not GABA-induced depression of neuronal firing, by prior applications of ethanol. It is conceivable that this antagonism arises from an effect of ethanol on neuronal calcium, as it is similar to phenomena which we have observed during studies with specific calcium antagonists (Phillis & others, 1973). Hurwitz & others (1962) have previously proposed that ethanol is a calcium antagonist since calcium was able to reverse the inhibition by ethanol of K^+-induced contractures of the guinea-pig ileum. In addition, Seeman, Chau & others (1971) have demonstrated that ethanol dramatically increases the binding of calcium to erythrocyte membranes, and this increased affinity of the membrane for calcium might be expected to restrict calcium movements and to modify the response to agents whose mode of action involves calcium. The direct excitant actions of ethanol which we observed may have arisen due to a blockage or removal of inhibitory input (c.f. Ishido, 1962).

In summary, we have demonstrated that ethanol, when applied directly onto cerebral cortical neurons, has a weak depressant or excitant action on neuronal activity. Moreover, it is an effective antagonist of biogenic amine-induced depressions of neuronal firing in the cerebral cortex. In the light of its known interactions with calcium, we consider these findings to provide further support for our hypothesis that

the depressant actions of amines on cerebral cortical neurons involve a calcium-dependent mechanism.

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Restoration of morphine analgesia in morphine-tolerant rats after the intraventricular administration of 6-hydroxydopamine

A definitive relation between morphine tolerance and the central adrenergic neurons has not been established. Chronic administration of morphine may affect the intraneuronal catecholamine storage mechanisms, since repeated administration of morphine interferes with the depletion of brain noradrenaline and dopamine levels by reserpine (Gunne, Jonsson & Fuxe, 1969) and also with the decrease in brain dopamine levels after administration of 6-hydroxydopamine (6-OHDA) into the lateral ventricles (Nakamura, Kuntzman, & others, 1972). In morphine-tolerant animals, the ability of morphine to decrease brainstem noradrenaline levels was lost (Maynert, 1968). These results suggest that there may be a correlation between morphine analgesia and release of brain noradrenaline. In addition, morphine-induced depletion of noradrenaline has been observed only in the hypothalamus (Vogt, 1954; Moore, McCarthy & Borison, 1965) mesencephalon, diencephalon and spinal region (Reis, Rifkin & Corvelli, 1969), indicating differences in regional sensitivity. Further, the electrolytic destruction of the dorsomedial (DMH) or ventromedial hypothalamic nuclei (VMH) is known to abolish an established tolerance to morphine (Kerr & Pozuelo, 1971a, b). These areas are rich in nerve endings of the ventral noradrenaline neuron pathway (Ungerstedt, 1971). The effects of morphine on the central adrenergic neurons may also be related to behavioural changes elicited by morphine such as motor excitation and catalepsy as well as to the analgesic action itself.

We have examined the effect on morphine analgesia in morphine tolerant rats of administration of 6-OHDA into lateral ventricle or into the VMH or DMH areas of the brain.

For measuring analgesic activity, the tail-flick method described by Nakamura, Kuntzman & others (1973) was used.

To obtain rats tolerant to morphine, male Wistar rats (Royal Hart strain), 250 g, were injected intraperitoneally 4 times daily (7 and 11 a.m. and 3 and 7 p.m.) with