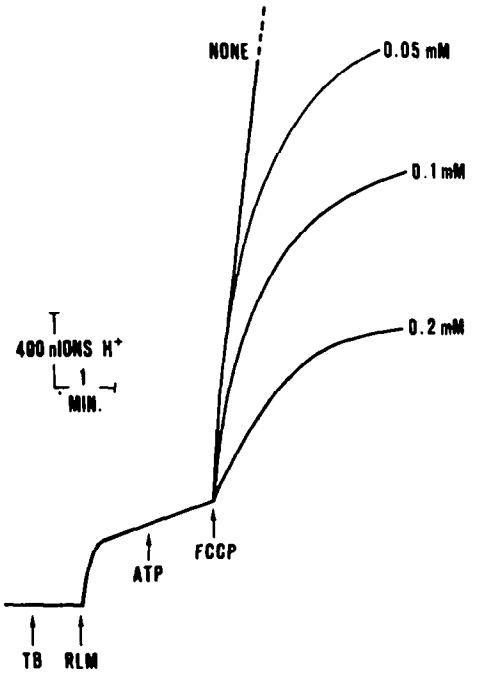

Fig. 4. Effect of trimebutine on mitochondrial ATPase activity stimulated by FCCP. 5 mg of mitochondrial protein (RLM) were added to the following medium: 80mM-KCl, 5mM-Tris–HCl ph 7.4. Final volume 2 ml, temperature 20°. Additions: 0.5mM-ATP, 0.8μM-FCCP.

This oligomycin-like action of trimebutine by inhibiting ATP formation in favour of other energy-dependent processes, like Ca^{2+} transport, can offer a basis for a further insight into the mechanism by which this kind of drug exhibits its pharmacological effects [1, 2].

In the light of current knowledges it is difficult to correlate the rotenone-like action of trimebutine with its oligomycin-like effect. The two actions seem to be quite independent. To our knowledge no other compound shares this peculiar feature.

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Effect of ethanol and stress on gamma-aminobutyric acid and guanosine 3′, 5′-monophosphate levels in the rat brain

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The acute effect of ethanol on gamma-aminobutyric acid (GABA) levels in the brain has been studied by a number of investigators [1–18]. However, the published results are contradictory because increases [1–9], no change [7–16], and decreases [6, 16–18] of GABA levels in the brain were reported. Several factors have been investigated as possible causes of these divergent results. These include differences in the methods used for tissue fixation and GABA determination [1, 8], strain and species differences [7, 10], and different nutritional states [8, 11, 16]. However, none of these factors explains satisfactorily why the observed effects of ethanol on GABA levels have been so variable.

Discovery of a reciprocal relationship between levels of guanosine 3′, 5′-monophosphate (cyclic GMP) and GABA [19] resulted in a renewed interest in the role of GABA as a neurotransmitter. Since acute ethanol administration causes a pronounced decrease in cyclic GMP levels [20–22] and GABA levels are decreased during ethanol withdrawal [23, 24], we decided to re-investigate the effect of acute ethanol administration on GABA levels. We found in one experiment that ethanol produced a dose-dependent decrease of GABA in the cerebellum and pons-medulla oblongata [20]. However, although experimental results were consistent when a single investigator repeated an experiment, they varied when experiments were repeated in our laboratory by various investigators even though the same animal strain, dose of ethanol, and method of GABA determination were used. This variability might be caused by subtle differences in handling of animals before they are killed, thus inducing variable degrees of stress. Therefore, we investigated the interaction between stress and the effects of ethanol on GABA and cyclic GMP levels.

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 200–250 g, were used for all experiments, and 4 g/kg of ethanol (16.7% v/v) (USP) was administered in a volume of 3 ml/100 g *per os* by intubation. It had been found in previous experiments that this dose produces an inability to stay on an inclined plane in 65 per

Table 1. Interaction between stress and effect of ethanol on GABA levels in rat brain*

	Control	Hot plate	Ice water
Cerebellum			
Control	0.85 ± 0.06 (10)	0.89 ± 0.05 (9)	0.77 · 0.07 (9)
Ethanol (4 g/kg)	0.89 ± 0.03 (10)	0.72 ± 0.03†‡ (10)	0.66 ± 0.05‡ (10)
Pons-medulla oblongata			
Control	0.72 ± 0.03 (10)	0.75 ± 0.06 (10)	0.63 ± 0.04 (9)
Ethanol (4 g/kg)	0.62 + 0.05 (10)	0.63 ± 0.05 (10)	0.60 ± 0.03 (10)

* Rats were killed by microwave irradiation immediately after exposure to hot plate for 20 sec or after swimming in ice water for 30 sec. GABA levels are expressed in μmoles/g of tissue ± S.E.M., and the number of rats is given in parentheses.

†$P < 0.01$, compared to control animals exposed to the same stress.

‡$P < 0.001$, compared to ethanol–treated animals not exposed to stress. For additional statistical analysis see text.

cent of rats [25] and that the maximal blood ethanol concentration (3.10 ± 0.27 mg/ml) occurs 1 hr after ethanol administration (L. Volicer and B. I. Gold, unpublished results). All control rats were given an equivalent volume of water in the same manner.

Stress was induced 1 hr after ethanol or water administration by immersion of rats in an ice–water bath (4°) for 30 sec or by placing rats on a hot plate (55°) for 20 sec. The experiments were performed between 10:00 a.m. and noon, and immediately following the end of the exposure the rats were killed by irradiation for 9 sec in a modified microwave oven (Litton-Menumaster) with microwaves focused at the head [26]. Brains were immediately removed and dissected into four areas: cerebellum, pons-medulla oblongata, cerebral cortex and subcortex. Tissues were frozen on dry ice and stored at −70° until assayed.

Each sample was weighed and homogenized with a teflon-glass Tri-R Stir-R homogenizer in 0.5 N perchloric acid containing 1 mM EDTA. For the pons-medulla oblongata 1 ml (and for the other samples 2 ml) of perchloric acid was used. The homogenates were kept on ice, and centrifuged at 5200 g and 4° for 15 min, 100 μl of supernatant fluid was used for the determination of GABA by the method of Okada *et al.* [27]. Cyclic GMP was separated from 1 ml of the supernatant fluid of cerebellar homogenates by column chromatography [28] and was determined after succinylation [29] by a radioimmunoasay [30].

Exposure to the hot plate or ice water did not change GABA levels significantly in any brain area, although the levels in the cerebellum and pons-medulla oblongata of ice-water-exposed animals were slightly lower than those of controls (Table 1). An analysis of variance indicated that stress produced an overall decrease of GABA levels in the cerebellum ($f = 6.29$, $P < 0.01$) but not in the pons-medulla oblongata ($f = 0.22$). Ethanol did not change cerebellar GABA levels in control animals, while it significantly decreased the level in hot plate-exposed animals. Animals exposed to ethanol and ice water had significantly lower cerebellar GABA levels than animals exposed only to ethanol, and there was a significant interaction between ethanol and stress ($f = 4.63$, $P < 0.05$). In the pons-medulla oblongata, ethanol did not change GABA levels significantly in any of the groups of animals. However, analysis of variance indicated that ethanol treatment produced an overall lowering of GABA levels ($f = 5.2$, $P < 0.01$), while there was no ethanol–stress interaction ($f = 0.11$) (Table 1). Ethanol did not change GABA levels in the cerebral cortex and subcortex of either control or stressed rats.

Cerebellar cyclic GMP levels were increased significantly by the ice–water stress (Table 2). This increase is in agreement with results of Dinnendahl [31] and Dinnendahl and Gumulka [32]. Ethanol decreased cyclic GMP levels in all three groups of animals. However, cyclic GMP levels in ethanol-treated rats, exposed to stress before death, were significantly higher than cyclic AMP levels of ethanol-treated controls (table 2). This indicates that the dose of ethanol, used in the present study, did not completely block the effect of stress on cyclic GMP levels or that ethanol was less effective in decreasing cyclic GMP levels in stressed animals than in controls.

In a separate experiment, we studied the effect of ethanol on cyclic GMP and GABA levels in the cerebellum of animals which were handled daily for 8 weeks before death. We found that the cyclic GMP level in these animals was much lower (112 ± 12 pmoles/g, n = 10) than in rats which were not accustomed to handling (Table 2, $P < 0.001$). Ethanol administration decreased this level by 85 per cent (to 17 ± 6 moles/g, n = 9), significantly more than in the other groups of animals (Table 2, $P < 0.001$). In contrast, GABA levels of regularly handled animals were not affected by ethanol administration.

This indicates that handling of naive rats induced a certain amount of stress, as evidenced by the increase of cyclic GMP levels, but that this degree of stress was not sufficient to result in a decrease of GABA levels in ethanol-treated rats. However, the degree of stress induced by handling and inserting the animal into a holder in the microwave oven is difficult to

Table 2. Effects of stress and ethanol on cyclic GMP levels in the rat cerebellum*

	Cyclic GMP levels		
	Control	Ethanol	% Decrease
Control	404 ± 87 (10)	127 ± 16+ (10)	68.8
Hot plate	483 ± 104 (9)	205 ± 30†‡ (10)	57.6
Ice water	730 ± 129‡ (9)	264 ± 42†‡ (9)	63.8

* Rats were killed by microwave irradiation immediately after exposure to a hot plate for 20 sec or after swimming in ice water for 30 sec. Cyclic GMP levels are expressed in pmoles/g of tissue ± S.E.M., and the number of rats is given in parentheses.

+ $P < 0.02$, compared to control animals exposed to the same stress.

‡ $P < 0.05$, compared to animals not exposed to stress.

quantify, and the results of our previous experiments [20] indicate that handling may induce stress comparable to exposure to hot plate or cold water immersion used in this study. The degree of stress induced by animal handling might also be responsible for the differential effect of ethanol in normal and aggressive mice [33].

Our results agree with reports of the lack of ethanol effect on GABA levels [7–16]. In addition, they might explain why in studies in which GABA levels in several brain areas were measured, the decreased GABA levels were observed mostly in the cerebellum [6, 16]. On the other hand, we do not have any explanation for increases of GABA levels after ethanol administration observed by some investigators [1–9]. In some experiments, we observed an increase in GABA levels in the cerebral cortex and subcortex after 0.67 and 1.35 g/kg of ethanol (R. Williams and L. Volicer, unpublished data), but in other experiments we were unable to confirm this finding.

Levels of cyclic GMP and GABA were not changed reciprocally by stress procedures. This is in agreement with the results of Mao *et al.* [19], who reported an increase of both cyclic GMP and GABA levels in the cerebellum of rats exposed to 4°. Cyclic GMP levels are affected directly or indirectly by several neuro-transmitters [34–36], and the effect of stress and ethanol on these systems might mask the reciprocal relationship between cyclic GMP and GABA levels described by Mao *et al.* [19].

There is some evidence that stress modifies behavioral effects of ethanol. Vigorous exercise as well as other forms of stress decrease the degree of intoxication in rats and this effect is not prevented by adrenalectomy [37, 38]. Individual differences in the degree of psychomotor disturbances of subjects receiving the same dose of ethanol were attributed to variations in arousal or stress [39, 40]. Kawi [41] described a positive correlation between anxiety and the minimum amount of alcohol necessary to cause slurred speech and interpreted this to mean that resistance to ethanol intoxication may depend on the level of anxiety of an individual. It is possible that stress-dependent effects of ethanol on GABA and cyclic GMP levels play a role in the stress-induced attenuation of the behavioral effects of ethanol.

In conclusion, results of this study indicate that ethanol affects brain cyclic GMP and GABA levels differentially according to the degree of stress to which the animal was exposed before death. The stress increased the effect of ethanol on GABA levels, but it decreased its effect on cyclic GMP levels. The interaction between ethanol and stress might explain some controversy concerning the effect of ethanol on GABA levels.

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