Retropulsion, GABA, and Possible Hallucinatory Behavior in Mice

Several hypotheses[1,2] have appeared which associate CNS levels of various aromatic amines with hallucinatory behaviors. Psychopharmacological tests of these hypotheses often use abnormal postures, twitches[2], or gaits[3] in rodents as indices of hallucinations. The present paper describes one such index – retropulsion in mice – which was observed after administration of amino oxyacetic acid (AOAA), an agent which increases[4] endogenous CNS levels of GABA by competitively inhibiting – α – ketogluterate transaminase[5].

Experimental. One hundred fifteen 16-day-old C57BL/6J mice were s.c. injected with either saline (control) or AOAA (experimental). The dose of 20 mg/kg of AOAA (dissolved in unbuffered saline solution) has repeatedly[6] been demonstrated to increase brain GABA in juvenile C57BL/6J mice. After injections, subjects were placed in individual clear plastic cages and observed for 5 h. Although the experimenter was aware of which mice received AOAA, a subsequent double blind procedure has verified the results described below.

Results. All 92 experimental subjects exhibited hypothermia and immobility within 15 min of AOAA injections. Approximately half the mice also showed mild tremors and/or a complete extension of the tail in an anterior-posterior direction. The 23 control animals typically assumed a sleeping posture with their tails partially wrapped around their bodies.

After 15–30 min, 56 of the 92 experimental subjects expressed clonic or clonic-tonic convulsions, and 39 experimental subjects showed definite retropulsion (walking backwards) which began approximately 90 min after injections. The Table gives a more detailed breakdown of these data. Although it was not necessary for a mouse to convulse before exhibiting retropulsion, the two behaviors were correlated ($C^7 = 0.31$; $\chi^2 = 9.96$; $df = 3$; $p < 0.02$). Although the convulsive phase of the syndrome was stereotyped, the retropulsions were highly individualistic; some mice crawled backwards, while others moved with hopping-like motions. In most cases, only the forelimbs were involved, although some animals also used the hind limbs. None of the 23 control subjects exhibited convulsions or retropulsions.

Discussion. Previous studies[8] have described the convulsive effects of AOAA but, to my knowledge, the present paper is unique in its observation of retropulsion in response to this agent. Insofar as could be ascertained, retropulsion in mice has only been reported twice in the psychopharmacology literature, and both reports associated it with hallucinogenic behavior. WOOLEY[3] described retropulsion in mice as resulting from an apparent 'hallucination of sliding down an inclined plane' due to their LSD treatment. He also referenced[3] a study in which LSD induced Siamese fighting fish to swim backwards.

The time course of retropulsion corresponds rather well with several other behavioral effects ofAOAA. Increased threshold for the auditory startle reflex[9], elevation of the electroconvulsive threshold[8], and protection from certain behavioral aftereffects of juvenile acoustic trauma[10] are all maximal approximately 90 min after injection of a comparable dose of AOAA. Although it requires approximately 5 h[8] for AOAA to produce maximal elevations of brain GABA, the rate of GABA increase is highest 90 min after injection.

Present theories concerning the mechanisms of psychotomimetic agents are often couched in terms of their effects on indole amines and catecholamines. If retropulsion is a valid index of hallucinatory activity, and is causally elated to the effects on brain GABA, altered states of consciousness may be more dependent upon relative levels of all the biologically active central amines, and not just of the aromatic amines.

Zusammenfassung. Amino-oxyacetsäure, eine Substanz welche das endogene Gehirnniveau anGABA erhöht, führte in 16 Tage alten Mäusen zu LSD-ähnlichen Verhaltensweisen wie Hypothermie: zu Krämpfen und Rückwärtslaufen.

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Relationship of convulsion and retropulsion in 92 mice treated with AOAA

Maximum severity of convulsions	No mice showing associated retropulsion	No mice showing no retropulsion
Clonic-tonic	20	15
Clonic	11	10
No convulsion	8	28

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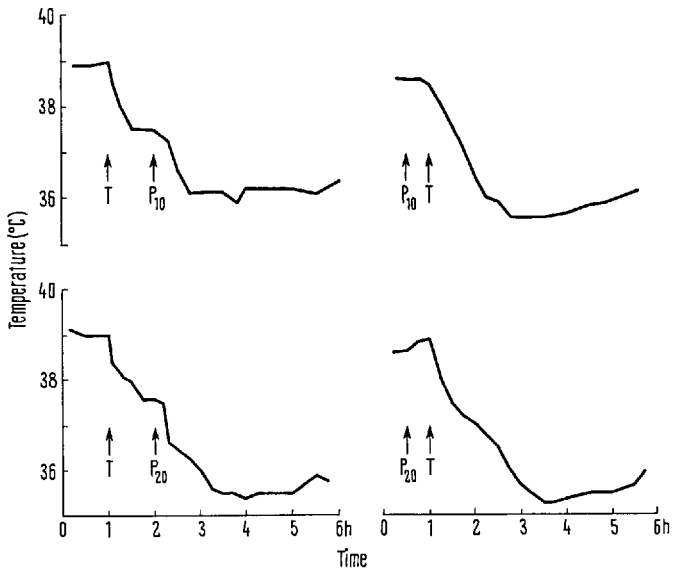
Phenitrone and Marihuana Induced Hypothermia

Phenitrone [3-(Hexahydro-1H-azepin-1-yl)-3'-nitropropiophenone hydrochloride], at a dose of 10–15 mg/kg i.p., has been reported to prevent the development of catalepsy in dogs exposed to the smoke of burning hashish. Animals first rendered cataleptic with hashish reverted to a normal behavioural state 6–10 min after administration of phenitrone (20 mg/kg i.p.). The concentration of tetrahydrocannabinols in the hashish was not determined but the dose administered was equivalent to 2–3 times the threshold dose[1].

GRUNFELD and EDERY[2] have reported profound behavioural changes in dogs and monkeys following i.v. ad-



Temperature records from 4 rats. Phenitrone in 2 doses (P_{10}, 10 mg/kg; P_{20}, 20 mg/kg i.p.) given before or after Δ^9-THC (T, 20 mg/kg i.p.) failed to prevent the fall in temperature.

ministration of Δ^9-tetrahydrocannabinol (Δ^9-THC) in doses of 500 µg/kg and 100 µg/kg respectively. These were 2–3 times the minimal effective doses. Comparable cataleptic doses in the rat were in the range 10–20 mg/kg i.p.[2]. The Rhesus monkey has been suggested as more suitable model than the dog for assessing the pyschopharmacological activity of marihuana extracts[3]. In both these species phenitrone was without effect on the behavioural responses to Δ^9-THC[4].

Marihuana extract causes marked hypothermia in the rat[5] at a dose level (20 mg/kg i.p.) similar to that producing catalepsy. Since changes in body temperature can be easily measured this response should be useful for gauging any antagonistic effect of phenitrone.

Method. Temperature recordings were made from female Sprague Dawley rats, weighing 200–250 g, using a rectal thermistor probe with the animals placed in a temperature controlled cabinet maintained at 22 ± 0.5 °C. Marihuana extract containing 17.1% Δ^9-THC (supplied by NIMH) was dissolved in olive oil to a concentration of 20 mg/ml Δ^9-THC. Phenitrone hydrochloride (Aldrich Chemical Co., Inc.) was dissolved in sterile water to a concentration of 10 mg/ml.

Results. Administration of Δ^9-THC (20 mg/kg i.p.) caused an immediate fall in temperature in 6 rats. 1 h after Δ9-THC, phenitrone (10 mg/kg i.p. in 3; 20 mg/kg i.p. in the remaining 3) was injected. Not only did the phenitrone fail to reverse the falling temperature but in every case there appeared to be potentiation of the hypothermic effect of Δ^9-THC. As seen in the Figure, the temperature had levelled off prior to injection of phenitrone in 2 animals; after phenitrone the sharp fall was resumed. The potentiation was more pronounced with the higher dose. Injection of phenitrone alone, at both doses, was without significant effect on the animals' core temperature; sometimes a slight fall, sometimes a rise of up to 0.5 °C was seen. In a further group of 6 rats injection of phenitrone (10 or 20 mg/kg i.p.) 30 min before Δ^9-THC (20 mg/kg i.p.) failed to prevent the development of hypothermia (Figure) and in 4 animals the temperature reached lower levels than those recorded in earlier studies[5].

Discussion. On the basis of these data phenitrone does not appear to be a specific antagonist of Δ^9-THC as claimed, indeed it appears to potentiate the effect of the latter. These findings are in contrast to those reported for cholin ergic blocking agents[6] and narcotic analgesic antagonists[7] which completely arrest the hypothermic effect of the corresponding agonists. WAHLQVIST et al.[8] have recently reported that Δ^9-THC is bound to the plasma proteins, as much as 95% being so associated in man. It is possible that phenitrone is displacing Δ^9-THC from protein binding sites thus accounting for the increased response in the present experiments. Such potentiation could possibly constitute a serious hazard should clinical use of phenitrone be considered.

Résumé. Le phénitrone, souvent considéré comme antagoniste spécifique de la marihuana, n'a non seulement pas empêché la baisse de température corporelle causé par la tétrahydrocannabinol (THC), mais potentialisé l'effet hypothermique de cette dernière. Il est possible que cette potentialisation est par suite du déplacement de la THC localisée dans les protéines plasmiques.

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