

at trial 2 the animals were more hesitant, sniffed more and walked less deliberately.

These experiments show that if the circumstances in which drugs are first encountered are appropriate, clear and dose-dependent effects on behaviour can persist for a surprisingly long time. A possible explanation could be that memory of the previous behaviour is reactivated by exposure to the original test situation.

We thank Mr R. Pollard, Mr R. Peto, Professor H. McIlwain, Dr J. O'Keefe and students at University College and Birkbeck College for help. This work was supported by a grant from the National Institutes of Health, US Public Health Service.

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Received November 26, 1970.

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## Identification of a Volatile Constituent formed by Homogenates of *Acacia georginae* exposed to Fluoride

We reported<sup>1</sup> that some homogenates of *Acacia georginae* and of other plants can convert added inorganic fluoride to a volatile compound, which is lost after alkaline combustion. We found examples in which the total fluoride estimated by acid diffusion after combustion was less than the fluoride estimated by acid diffusion without combustion. The first hypothesis which we examined was that vinyl fluoride ( $\text{CH}_2=\text{CH}_2\text{F}$ ) was formed. Some evidence (although not decisive) was obtained for this by gas chromatography using a column of 'Poropak S'. We then explored the possibility that a volatile fluoroketone might be formed. Monofluoroacetone has been found in the

livers of rats perfused with fluoroacetate<sup>2</sup>, and we record here evidence which suggests its presence as one of the volatile fluorine-containing constituents of the *A. georginae* homogenates.

Where possible, AnalaR reagents were used. Fluoroacetone was supplied by Aldrich Chemical Co. and AnalaR 2,4-dinitrophenyl hydrazine was recrystallized. Inorganic fluoride (and any fluoride freed by acid) and organically combined  $\text{F}^-$  were estimated by Hall's method<sup>3</sup>.

The gases from the homogenate, prepared and reinforced as described previously<sup>1</sup> during 1 h of incubation at 30° C, were drawn through a solution of 2,4-dinitrophenyl hydrazine (0.1% in 2 N HCl). The mixed hydrazones were extracted into ethyl acetate and treated as in the protocols.

For gas chromatography, compounds were prepared by drawing the gases from the homogenate through a tube (30 cm × 2.5 cm) containing  $\text{CaCl}_2$  for approximately 2 h. An evacuated flask was then attached to the  $\text{CaCl}_2$  tube, and gently warmed, so that the collected gases entered the flask, which was cooled in  $\text{C}_2\text{H}_5\text{OH}/\text{CO}_2$ . Controls showed that 20 µg amounts of both monofluoroacetone and acetone could be detected on the polyethylene glycol 600 column at 82° C after this treatment.

The "hydrazones" were spotted on to Whatman No. 52 paper, previously dipped in 30% phenoxyethanol/acetone, and blotted. The chromatogram was run for 48 h, in an atmosphere saturated with ligroin (boiling point, 80–100° C), the liquid phase being phenoxyethanol saturated with ligroin<sup>2</sup>.

Controls showed that fluoroacetone can be recovered as 2,4-dinitrophenyl hydrazone from combustion mixtures and from plant homogenates. In our first experiment a homogenate, made by grinding in a mortar 2.7 g of leaves from a young plant of *Acacia georginae*, was strained through muslin to make a total volume of 7 ml. The plant had been watered weekly for more than 40 weeks with 200 ml. of a solution containing 200 µg of NaF. The homogenate was divided into two halves, each reinforced with adenosine triphosphate, pyruvate and phosphate<sup>1</sup>, making a total volume of 3.6 ml., pH 7.4. Inorganic NaF, 50 µg/g, was added to one half. During incubation for 1 h at 30° C air was passed over the homogenates into an acid solution of 2,4-dinitrophenyl hydrazine ( $\alpha$ ). After the incubation, the hydrazones present were shaken six times with approximately 1/5 volume of ethyl acetate. A portion of the combined ethyl acetate extracts was taken to a low volume *in vacuo*, treated with LiOH and Mg succinate and ashed at 400° C. The fluoride in this fraction ( $\alpha$ ) was estimated by acid diffusion<sup>3</sup>;  $\beta$ , the residual aqueous extract from  $\alpha$ , was neutralized and evaporated to dryness; the gases produced were drawn through acid 2,4-dinitrophenyl hydrazine and, using the same procedure as in  $\alpha$ , fraction  $\beta$  was obtained.

**Table 1** Fluoride Analyses on the 2,4-Dinitrophenyl Hydrazones obtained from the Vapours arising from Homogenates of *Acacia georginae*—Additions of NaF

Fraction	nmol $\text{F}^-$	
	No addition	+ NaF
$\alpha$	58	630
$\beta$	157	157

For details, see the description of our first experiment.

Table 1 shows that the amount of a volatile ketone, behaving as fluoroacetone, is much increased by the addition of  $\text{F}^-$  to the homogenate. A control containing everything except the homogenate showed no volatile  $\text{F}^-$ .

In our second experiment a similar treatment was given to another homogenate from *A. georginae*. After extracting the "hydrazones" with ethyl acetate and their reduction to a suitable volume, they were run on paper chromatograms, as

described. Spots were found running in the same position as the 2,4-dinitrophenyl hydrazone of fluoroacetone, although acetone ran differently. As stated by the Japanese workers, acetaldehyde hydrazone ran in the same position as fluoroacetone hydrazone, but we failed to separate the two hydrazones of acetaldehyde and fluoroacetone on the column, as recommended<sup>2</sup>. The presence of fluoroacetaldehyde could not be ruled out, although it seems improbable in view of its instability<sup>4</sup>.

This experiment shows that a volatile fluoro-ketone is present in the vapours above the homogenates. Further evidence was sought by gas chromatography.

In our third experiment, using the technique described, the vapours from a homogenate of *A. georginae*, treated with LiOH and Mg, were passed through the CaCl<sub>2</sub> tube while being gradually evaporated to dryness. After they had been transferred to the evacuated flask, the vapours were passed through the gas chromatograph and showed a peak corresponding in position to that of monofluoroacetone.

We have thus proved chemically that F<sup>-</sup> leaves the homogenates of *A. georginae* in a gaseous form, and that some can be isolated as a 2,4-dinitrophenyl hydrazone, behaving at least in part as monofluoroacetone. The same fluoroacetone has been found in the vapours of homogenates treated as for combustion. It has therefore been proved that the apparent loss of F<sup>-</sup> in our previous experiments<sup>1</sup> is attributable to the escape of fluoride in a volatile form. At the same time, calculation shows that the F<sup>-</sup> collected in this experiment does not represent more than 13% of the total amount of F<sup>-</sup> disappearing, so that there are probably other volatile F<sup>-</sup> compounds still to be identified. It is interesting to note that fluoroacetone is relatively non-toxic, and that it could arise from fluoroacetic acid<sup>5</sup>.

We thank the Wellcome Trust for grants and Professor F. G. Young for facilities.

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Received January 16; revised March 1, 1971.

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## Visual Contour Masking

WE wish to report an effect we noticed accidentally while observing the parallel grid figure of Gregory's book *Eye and Brain*<sup>1</sup> (Fig. 1). When the page was stationary only the vertical grid would be seen; but during rapid to and fro, left to right movement the fins of the Müller-Lyer figure became visible. The latter happened to be on the reverse side of the page (136). Holding the page so that the grid was horizontal did not make possible the observation of the Müller-Lyer figure either when stationary or with left to right movement—although it could be seen during up and down movement.

This effect was confirmed on a group of twenty-two naive university students. The page with the parallel grid was removed from the book and affixed to a white card, so that the legend to the figure was not visible. The subjects were told that there was a figure behind the bars, and that they had to describe,

draw or guess what it was. Initially, the grid was horizontal and stationary, then it was moved to and fro from left to right. The procedure was repeated with the bars vertical and stationary, and then moving from left to right. Only in this last condition did the subjects report the presence of lines inclined to the grid, and drew the fins of the Müller-Lyer figure; sometimes the vertical stems were added or the outer ends were joined.

A similar effect can be observed with a ray figure of the type described by Mackay<sup>2</sup>. If a circle is drawn on the back of a sheet of paper of suitable thickness with a ray figure on the front it cannot be seen when the ray pattern is stationary, but it is visible during rotation. It is unlikely that the complementary images produced by the patterning prevent perception of the circle, as blacking out a large central circle abolishes the complementary images but does not result in the appearance of the circle (when the pattern is stationary).

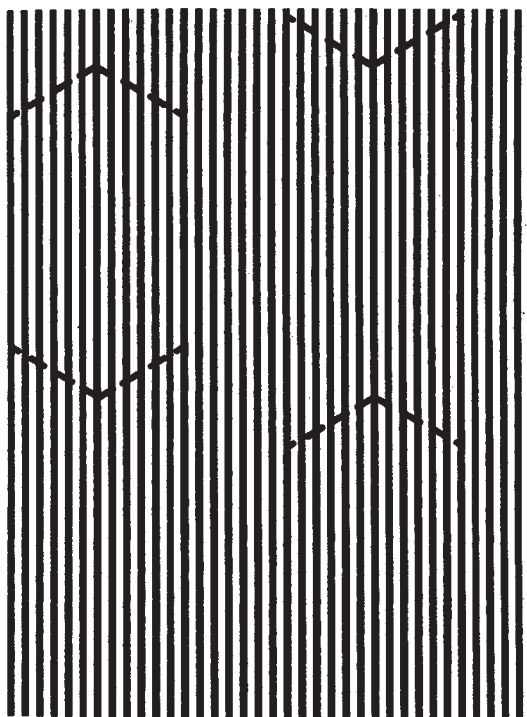


Fig. 1 The grid figure from Gregory's *Eye and Brain* (135). On the reverse page to this grid was a Müller-Lyer figure (in the position indicated by the broken lines which we have added). The Müller-Lyer figure could be seen during left to right movement, but not during up and down movements of the page.

These results are pertinent to recent work concerned with visual contour masking. A figure presented for some time masks another figure subsequently presented, if it is in or near the same orientation<sup>3</sup>. The point of interest in this communication is that the masking occurs simultaneously; that is, a figure with repetitive contours of high contrast can mask a figure of very low contrast inclined relative to it. Successive visual masking has proved amenable to neurophysiological interpretation in terms of orientation detectors<sup>4,5</sup>, and the same approach can be applied to this effect. In this case the orientation-specific analysers stimulated by the parallel grid would inhibit the firing of analysers for other orientations (probably in a manner similar to that in binocular contour rivalry). During left to right movement of the vertical grid acuity would be reduced<sup>6</sup>. Presumably, this is accompanied by reduced stimulation of the orientation-specific analysers, with consequent reduction in the inhibition of other orientation analysers; it is in these conditions that the inclined figures are seen. When the grid is horizontal and moved from left to right, acuity is not