and suggests that in a virtual pairing frenzy it could just as easily send out L–C fibre loops in six different directions to form the hexagonal polycomplexes. The polycomplexes may not be so aberrant after all.

It has been suggested that the synaptonemal complex holds certain genes (master genes) in register so that crossing over may occur, and excludes other genes (slave genes) from such recombination[17–19,7]. If the genes potentially able to participate in recombination are all fixed to a specific portion of the synaptonemal complex, such as the lateral element[7], the type of triple pairing shown here should place an absolute restriction on recombination between chromosomes 1 and 3 in the absence of intermediary recombination between 1 and 2 and 2 and 3. On the other hand, if recombination takes place in the region around the synaptonemal complex[9] there should be no such absolute restriction. Investigation of this is somewhat complicated by the fact that homologous chromosomes 1, 2 and 3 probably do not always pair in the same order. Such a problem would be bypassed if a triple crossover event going from 1 to 2, then 2 to 3, and then back to 1 were found. Such crossovers have been described in triploid *Drosophila*[20]. To vindicate this line of reasoning it will be necessary to demonstrate triple pairing in *Drosophila*.

Finally, although triple pairing has been observed in the triploid chicken this does not prove that it exists in other triploids or that quadruple pairing exists in tetraploids. It does, however, suggest a need for a similar examination of other polyploid organisms.

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DAVID E. COMINGS
TADASHI A. OKADA

Department of Medical Genetics,
City of Hope National Medical Center,
Duarte,
California

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1 Newton, W. C. F., and Darlington, C. D., *J. Genet.*, **21**, 1 (1929).
2 Darlington, C. D., *J. Genet.*, **24**, 65 (1931).
3 Darlington, C. D., *J. Genet.*, **31**, 185 (1935)
4 Darlington, C. D., *Cytology*, 119 (Churchill, London, 1965).
5 Moens, P. B., *Canad. J. Genet. Cytol.*, **10**, 799 (1968).
6 Moens, P. B., *J. Cell Biol.*, **40**, 273 (1969).
7 Moens, P. B., *J. Cell Sci.*, **7**, 53 (1970).
8 Ohno, S., Kittrell, W. A., Christian, L. C., Stenius, C., and Witt, G. A., *Cytogenetics*, **2**, 42 (1963).
9 Comings, D. E., and Okada, T. A., *Nature*, **227**, 451 (1970).
10 Comings, D. E., and Okada, T. A., *Chromosoma*, **30**, 269 (1970).
11 Comings, D. E., and Okada, T. A., *Exp. Cell Res.*, **65**, 104 (1971).
12 Moses, M. J., *Ann. Rev. Genet.*, **2**, 363 (1968).
13 Moens, P. B., *Chromosoma*, **28**, 1 (1969).
14 Brinkley, B. R., and Bryan, J. H. D., *J. Cell Biol.*, **23**, 14A (1964).
15 Coleman, J. R., and Moses, M. J., *J. Cell Biol.*, **23**, 63 (1964).
16 Nebel, B. R., and Coulon, E. M., *Chromosoma*, **13**, 292 (1962).
17 Callan, H. G., *J. Cell Sci.*, **2**, 1 (1967).
18 Whitehouse, H. L. K., *J. Cell Sci.*, **2**, 9 (1967).
19 Moens, P. B., *Chromosoma*, **23**, 418 (1968).
20 Redfield, H., *Genetics*, **15**, 205 (1930).

Persistence of Dose Related Behaviour in Mice

IF naive rats are injected with an amphetamine–barbiturate mixture similar to 'Drinamyl' and are tested in an unfamiliar environment—a Y-shaped runway—their spontaneous activity is about double that of controls given only saline[1]. If, after testing, the rats are returned to their home cages and retested in the runway without drugs, about half of the stimulant effect of the drug mixture can still be detected as much as 3 months later[2,3]. This is surprising, for significant amounts of the individual drugs or of any known metabolites are unlikely to be present in the animals' brains for longer than at most 48 h[4,5], though as far as we know information of this kind is not available for mixtures of the drugs. In any case, no such behavioural after-effects can be detected after one or a series of "passive" administrations of the drugs, whereby the rats are merely injected and replaced in the home cage without being tested[3] (R. D. Porsolt, unpublished results and refs. 6 and 7). Only when drug administration is combined with an actual test experience do these long term after-effects seem to occur. In the conditions described they are consistent and reliable and are presumably due to some more or less permanent changes in the organism, which are induced by the first drug/environment experience and which affect subsequent behaviour.

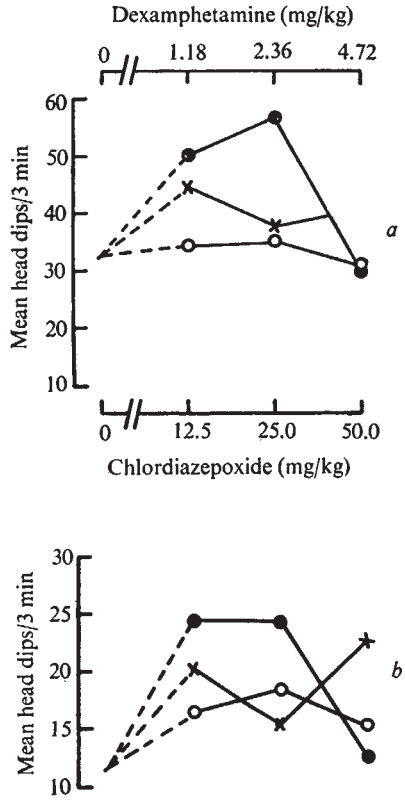

Fig. 1 Head dipping by mice under the influence of three doses of dexamphetamine (×) or chlordiazepoxide (○) given alone and in combination (●) in a constant ratio of approximately 1 : 10 by weight at trial 1 (*a*). The mice were tested on a hole board 20 min after intraperitoneal injections, and the number of times they dipped their heads into holes during a 3 min trial is shown on the ordinate. Each point represents the mean for eight mice and there were twenty-four controls. With one dose of the mixture, the amount of head dipping was greatly increased but the separate drugs had little effect. At trial 2 (*b*) 1 week later, curves of similar shape, though at a uniformly lower absolute level, emerged, even though the mice were now tested without any drug or injections. The standard errors ranged from 2.39 (saline) to 4.48 at trial 1 and 1.64 (saline) to 4.97 at trial 2. The scale of the ordinate for trial 2 has been doubled for easier comparisons of shapes.

To analyse long term effects of single drug experiences further, we have now used several doses of a different drug mixture, a different species of rodent—the mouse—and a different test environment—a hole board instead of a Y-maze. The drug mixture consisted of dexamphetamine combined with chlordiazepoxide ('Librium'—a drug which, like amylobarbitone, is widely used to allay anxiety) instead of amylobarbitone, for previous experiments with rats had shown

mixtures of these two drugs to be even more effective in stimulating activity than were amphetamine–barbiturate mixtures[8]. We used mixtures of drugs because they induce much greater increases in activity than any dose of the separate ingredient drugs[9], and they therefore provide a favourable baseline from which to study after-effects.

Groups of eight naive female adult mice, Porton strain, and housed four per cage, were injected intraperitoneally with one of three doses of a dexamphetamine–chlordiazepoxide mixture in a ratio of 1:10 by weight, or with the separate constituents, or with saline. The four mice in a cage all received the same treatment. The particular ratio of the two drugs was chosen because previous Y-maze experiments with rats[9] and hole board experiments with mice[10], using amphetamine–barbiturate mixtures, had shown that dose ratios which were optimal in rats were also effective in mice. Twenty minutes after injection the mice were placed singly on square horizontal wooden boards with sixteen holes evenly spaced in four rows[11]. Behaviour was scored during 3 min as follows: (1) total number of head dips: a dip was counted whenever the mouse put its head into a hole far enough for both its eyes to disappear below the surface of the board—to explore holes is very natural for mice; and (2) amount of walking about on the board, determined by drawing on specially prepared paper grids the path which the mouse followed on the board: the number of times the animal's path crossed the grid lines was taken as a quantitative index of the amount of walking. Other forms of behaviour such as grooming, defaecation and urination were also recorded.

After the original 3 min test (trial 1) the mice were replaced in their home cages and left for 1 week; they were then given their second test (trial 2) on the same boards but without any drug or other injections. Otherwise the procedure was identical with the procedure followed at trial 1.

The results show that at the first trial the separate drugs in any of the three doses used hardly affected the number of times that the mice dipped their heads into the holes, as compared with saline controls (Fig. 1, trial 1); with chlordiazepoxide most of the head dips were, however, characteristically concentrated in the first minute of the 3 min trial[8]. With the two smaller doses of the mixture, on the other hand, head dipping increased markedly as compared with saline ($P<0.05$ and $P<0.01$ for the lowest and for the middle dose of the mixture respectively, Mann–Whitney U test). With the biggest mixture dose, the amount of head dipping was no different from that of the controls, but the mice were very ataxic. When all the mice were retested, without any drugs, one week later (Fig. 1, trial 2) the shapes of the original dose-response curves seemed to re-emerge (Kruskal–Wallis one way analysis of variance, $H=17.675$, d.f. 3, $P<0.001$) though the absolute scores for all groups were approximately 50% lower than at trial 1. Fig. 1 shows results for all possible kinds of head dip combined; these include first dips into a hole, which accounted for the largest proportion of dips, repeat dips into the same hole, which occurred fairly often with amphetamine alone and with the 2.36 : 25 mixture dose, and dips over the edge of the board, which were rarer. The increases in repeat dips are consistent with the often reported finding that some doses of amphetamine are apt to produce various repetitive or "stereotype" movements. When the three kinds of head dip were plotted separately, a similar re-emergence of dose-response curves from trials 1 and 2 was found. As for the amount of walking on the board, again the separate drugs seemed to have little effect (Fig. 2, trial 1); the mixture, however, increased walking, especially in the smallest dose used ($P<0.05$); with the highest dose, the amount of walking was no different from the saline controls, possibly because of ataxia. At trial 2, without drugs, the shapes of the curves again well resembled those of the dose-response curves at trial 1 (Kruskal–Wallis analysis of variance for the mixtures $H=7,860$, d.f. 3, $P<0.05$) except that mice which had received the biggest dose of dexamphetamine at trial 1 walked as much as naive controls, which may have been due to an intensified amphetamine effect because of grouping. In all other cases the absolute levels at trial 2 were approximately half the trial 1 levels, as was found with the number of head dips. In general the variances in the walking results were larger than in the head dip results. The two kinds of measure were not significantly correlated, but they seemed equally sensitive to the drugs and to their after-effects.

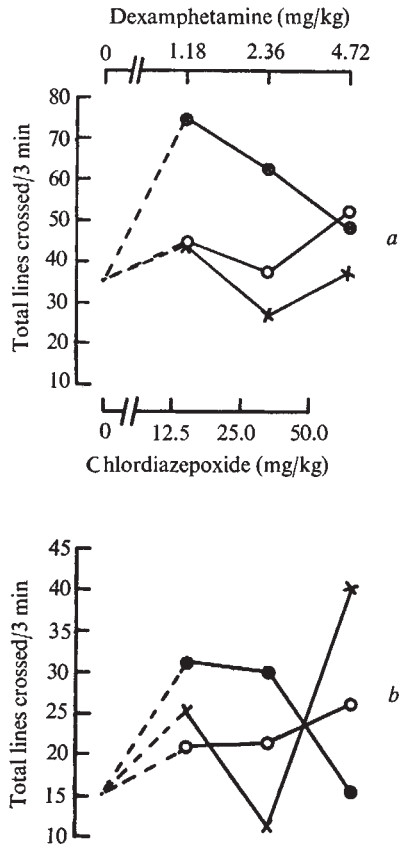

Fig. 2 Amount of walking over the hole board by mice treated as described in Fig. 1. Moderate doses of the drug mixture at trial 1 increased walking but the separate drugs did not have much effect. At trial 2, without drugs, except for mice which had had 4.72 mg/kg of dexamphetamine, the dose-response curves were recreated. The scale of the ordinate for trial 2 has been doubled for easier comparisons. Symbols are as in Fig. 1.

The problem of how much, if anything, of what has been learnt under the influence of drugs can be elicited later in the undrugged state has been much discussed. Various experimenters using drugs similar to ours have reported less retention the greater the original dose, and some have argued that with sufficiently large doses virtually nothing is learnt, and animals behave at the second, non-drug trial as though the first trial had not occurred; this phenomenon is often referred to as "dissociation" or "state-dependent" learning[2,10,12–15].

Our experiments show that not all drugs act in this way; with the highest dose the animals should have behaved more like naive controls than with the smaller. In fact, all animals seem to have reproduced a constant proportion of their behaviour, regardless of the original dose. The 50% reduction in activity at trial 2 with saline is what usually happens when mice are exposed for a second time to this kind of unfamiliar environment and may be regarded as a sign of reduced curiosity, or habituation. It seems that the mice which had been given drugs, similarly, "remembered" their drug-induced abnormal behaviour, and to some extent reproduced it, when the opportunity was given; from gross observation it also appeared that

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.

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MARIAN DORR
HANNAH STEINBERG
M. TOMKIEWICZ

Department of Pharmacology, University College, London

DAPHNÉ JOYCE
R. D. PORSOLT
A. SUMMERFIELD

Department of Psychology, Birkbeck College, London

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1 Rushton, R., Steinberg, H., and Tinson, C., *Nature*, **192**, 533 (1961).
2 Rushton, R., Steinberg, H., and Tinson, C., *Brit. J. Pharmacol.*, **20**, 99 (1963).
3 Rushton, R., Steinberg, H., and Tomkiewicz, M., *Nature*, **220**, 885 (1968).
4 Groppetti, A., and Costa, E., *Intern. J. Neuropharmacol.*, **8**, 209 (1969).
5 Van der Kleijn, E., *Arch. Pharmacodyn. Ther.*, **178**, 193 (1969).
6 Porsolt, R. D., Joyce, D., and Summerfield, A., *Nature*, **223**, 1277 (1969).
7 Porsolt, R. D., Joyce, D., and Summerfield, A., *Nature*, **227**, 285 (1970).
8 Rushton, R., and Steinberg, H., *Nature*, **211**, 1312 (1966).
9 Rushton, R., and Steinberg, H., *Brit. J. Pharmacol.*, **21**, 295 (1963).
10 Bradley, D. W. M., Joyce, D., Murphy, E. H., Nash, B. M., Porsolt, R. D., Summerfield, A., and Twyman, W. A., *Nature*, **220**, 187 (1968).
11 Boissier, J. R., and Simon, P., *Thérapie*, **17**, 1225 (1962).
12 Bindra, D., and Reichert, H., *Psychopharmacologia*, **10**, 330 (1967).
13 Bohdanecky, Z., and Jarvik, M., *Arch. Intern. Pharmacodyn.*, **170**, 58 (1967).
14 Sachs, E., in Zubin, J., and Hunt, H. F., *Comparative Psychopathology*, 249 (Grune and Stratton, New York, 1967).
15 Overton, D. A., in Efron, D. H., Cole, J. O., Levine, J., and Wittenborn, J. R., *Psychopharmacology: A Review of Progress*, 918 (US Government Printing Office, PHS Publication No. 1836, 1968).

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

WE reported[1] 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 ($CH_2=CH_2.F$) 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[2], 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 F^- were estimated by Hall's method[3].

The gases from the homogenate, prepared and reinforced as described previously[1] 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 $CaCl_2$ for approximately 2 h. An evacuated flask was then attached to the $CaCl_2$ tube, and gently warmed, so that the collected gases entered the flask, which was cooled in C_2H_5OH/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[2].

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[1], making a total volume of 3.6 ml., *p*H 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 (α). 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 (α) was estimated by acid diffusion[3]; β, the residual aqueous extract from α, was neutralized and evaporated to dryness; the gases produced were drawn through acid 2,4-dinitrophenyl hydrazine and, using the same procedure as in α, fraction β 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 F^- No addition	+NaF
α	58	630
β	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 F^- to the homogenate. A control containing everything except the homogenate showed no volatile 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