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ACTIONS OF HALLUCINOGENS ON ANTS (*FORMICA PRATENSIS*)—I. BRAIN LEVELS OF LSD AND THC FOLLOWING ORAL ADMINISTRATION

H. R. FRISCHKNECHT and P. G. WASER
Institute of Pharmacology, University of Zurich, Gloriastrasse 32 CH-8006 Zurich, Switzerland.

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Abstract—1. Ants having free access for 2 days to sugar-water containing 500 μg/ml LSD showed (by fluorescence spectrophotometry) drug levels of about 7 ng in their brain.

2. The kinetics of drug uptake into the head-ganglia were determined by liquid scintillation counting after a single oral administration of tritiated LSD or THC.

3. Peak values were found at 12–24 hr (LSD) and at 24–48 hr (THC) respectively after drug application. Related to drug concentrations of 100 μg/ml in the food, the maxima corresponded to 150 pg LSD and 800 pg THC respectively per brain (or about 0.04% and 0.22% of the ingested drug amount).

4. Drug elimination from the nervous system was faster for LSD (half-time about 12 hr) than for THC (half-time 3 days).

5. The results were connected with attributes of the blood-brain barrier and peculiarities of the alimentary tract in ants.

INTRODUCTION

The present experiments were carried out in order to compare the behavioral effects of hallucinogenic substances with their uptake into the central nervous system. The accumulation of LSD and THC in the brain of ants was measured at different times after single oral application of the drugs with the food. The mechanism for the efficacy of centrally acting drugs was examined for the first time in ants. Similar investigations in other species, e.g. small laboratory mammals, are not comparable because of the morphological differences in the anatomy of the alimentary tract and of the blood-brain barrier.

MATERIALS AND METHODS

All investigations were done with worker ants of the species *Formica pratensis* Retz. They were taken out of a colony on the "Pfannenstiel" (13 km S.E. from Zurich) and kept in a formicar with their genuine nesting material. During this period in our laboratory we made sugar-water (250 mg/ml sucrose) available to them.

LSD: fluorimetric method

In the first series of experiments D-lysergic acid diethylamide (LSD, Sandoz Basle) was used as the tartaric salt. The drug was dissolved at concentrations of 50 or 500 μg/ml in water with 250 mg/ml sucrose and given to the hungry ants for two days in unlimited amounts. Afterwards the head-ganglia (brain) of the ants were removed and homogenized. The drug content was determined by the fluorimetric method of Axelrod *et al.* (1957).

The brains of 20 or 25 LSD-fed ants were dissected and ground up in an all glass homogenizer (Kontes AA) with 0.3 ml water. The homogenizer was washed twice with 0.7 ml water making up a sample of 1 ml. This was added to 8 ml heptane + 0.18 ml isoamylalcohol + 0.2 ml 1 M NaOH + 1.2 g NaCl. The mixture was shaken for 30 min to concentrate the LSD into the heptane phase at a basic pH. Thereafter the sample was centrifuged for 10 min at 18,800 *g* in a Sorvall RC-2 at 0°C. 7.4 ml of the heptane phase was added to 1 ml 0.004 N HCl and the LSD extracted at low pH into the water phase by shaking and centrifugation for every 10 min. Then the overlaying heptane was sucked off and the acidic phase was measured in a Farrand spectrofluorimeter. The intensity of the fluorescence was compared to those of LSD-solutions with known drug concentrations to evaluate the drug content of the homogenized brains.

The sensitivity of this method however turned out to be not sufficient for tracing the drug uptake after a single feeding with sugar-water containing non-toxic amounts of LSD. Therefore all succeeding experiments were done with tritiated LSD, just as the investigations with THC.

[^{3}H]LSD, [^{3}H]THC: liquid scintillation counting

The [^{3}H]LSD was produced by Dr A. Chang Sin-Ren in our laboratories by reductive dehalogenization of 2-bromo-lysergic acid diethylamide by means of tritium gas. We had the drug at our disposal in cristalline form (long needles) with a specific radioactivity of 2 mCi/mg. To increase the solubility in water, we added D-tartaric acid (Fluka Buchs) in an adequate quantity.

The Research Triangle Institute made the tritiated delta-9-tetrahydrocannabinol ([^{3}H]THC) available to us. It was a clear oil of varnish like consistency (purity 97%) and had a specific radioactivity of 24 μCi/mg. The THC was always fed as an emulsion in sugar-water containing 10 vol% of Tween-80.

Starved ants were fed on sugar-water containing 100 μg/ml [^{3}H]LSD or 570–750 μg/ml [^{3}H]THC (plus 10 vol% Tween-80). Immediately after termination of food intake, ants with extended hindquarters were captured and cleansed from contaminations by dipping in water. From these, insects were taken at increasing time intervals for analysis of incorporated radioactivity. The brains were dissected and their radioactive content evaluated by means of a liquid scintillation counter (Packard TriCarb, Model 3375).

For each sample the brains of two LSD-fed ants proved to be sufficient. To estimate the radioactivity in the

THC-fed ants, five brains per sample had to be used. Samples with two brains of control-fed ants (sugar-water) served for determination of the background. These two or five brains were ground in a homogenizer with 0.1 ml methyl alcohol. The homogenizer was washed twice with 0.4 ml methyl alcohol, making a sample of 0.5 ml. This milky suspension was added to 10 ml scintillation fluid (7 g butyl PBD l toluene) + 3.5 ml methyl alcohol. Each sample was counted 5 times for 5 min in the liquid scintillation counter. The drug content of each sample was computed from net counts per minute, counting efficiency and specific radioactivity of the drug.

Table 1. Relative fluorescence intensities (excitation 330 nm, emission 430 nm) of standard solutions with known LSD-contents and of their extracts (n = 13–41)

LSD content (ng/ml)	Standard solution (mean ± S.E.)	Extract (mean ± S.E.)
0	5 ± 1	12 ± 1
10	15 ± 2	23 ± 2
100	83 ± 5	98 ± 7
1000	775 ± 13	840 ± 80

RESULTS

Fluorescence-spectrophotometric studies with LSD

In preliminary examinations with LSD in 0.004 N HCl we established the maxima of excitation- and emission-spectra to be at 330 nm and 430 nm respectively. Subsequently we measured the fluorescence intensities of standard solutions with known drug concentrations at these wave-lengths (external standardization). Furthermore we carried out the whole extraction procedure with these test solutions to get an internal standardization. These results are summarized in Table 1.

The fluorescence intensities of the extracts showed elevated values compared to the corresponding external standard. On vigorous shaking, heptane and isoamylalcohol formed tiny drops in the acidic water phase. They remained in suspension even after centrifugation and augmented the fluorescence values by scattering the exciting light.

The relative fluorescence intensities of brain extracts from control- and LSD-fed ants are summarized in Table 2.

The brain extract from undrugged ants didn't show any additional fluorescence intensities compared to the background (extract with 0 ng/ml LSD, Table 1). Likewise the measurement of a brain extract from ants which had free access to food with 50 μg/ml LSD for 2 days did not exceed these values. These low-dose drugged ants rarely showed unusual behavior. Their movements exhibited sporadic periods of unsteadiness. In addition they sometimes displayed fumbling movements with their forelegs in the air at each step.

In contrast, ants offered sugar-water with 500 μg/ml LSD showed serious toxic symptoms. They couldn't move after 2 days, lay on their side or back and managed only weak motions of legs and antennae. Half of them would have died within 5–10 days. The fluorescence intensities of brain extracts from these ants attained remarkable values. They were compared to extracts with 100 ng/ml LSD for an estimation of the drug content. The results are entered in Table 2.

In additon, we recorded the complete excitation- and emission-spectra of the last mentioned brain extract. They were compared with those of an extract with 100 ng/ml LSD (Fig. 1).

The spectra showed perfect correspondence in their shape. This is good evidence for a common cause of the fluorescence, namely by LSD. On the other hand, the peak intensities in the spectra were variable, and did not always agree with the values in Tables 1 and 2. After prolonged excitation, as is necessary to scan spectra, the fluorescence intensities decrease (e.g. to 55% after 4 min).

Liquid scintillation studies with [^{3}H]LSD and [^{3}H]THC

In a first series of investigations, ants were fed on sugar-water containing 100 μg/ml [^{3}H]LSD. We determined the radioactivity of 10 homogenates containing two brains each at 11 different periods after food intake (1, 2, 4, 6, 12, 18, 24, 30, 42, 48 and 72 hr). In addition, background-assays were made by measuring homogenates of two brains of non-radioactively fed ants at intervals of five samples.

The radioactivity of these controls didn't exceed the usual background values of 20–25 cpm, and were significantly below all samples containing brains of [^{3}H]LSD-fed ants. These had a minimum of about 70 cpm net, i.e. when background was subtracted. Peak values, averaging 460 cpm net, were found 12–18 hr after food intake.

The drug content in ant brains (Fig. 2) showed a steep increase within 12 hr after feeding and maxi-

Table 2. Relative fluorescence intensities (330, 430 nm) of brain extracts and their drug contents after a 2 days feeding of the ants with sugar-water containing LSD in different amounts.

LSD in sugar-water (μg/ml)	Brains/ sample	Fluorescence intensity	Drug content per sample (ng)	Drug content per brain (ng)
0	10	17.3	—	—
0	15	7.3	—	—
0	15	7.5	—	—
0	20	16.0	—	—
50	20	15.5	0	0
500	20	146.7	150	7.5
500	25	173.2	175	7.0

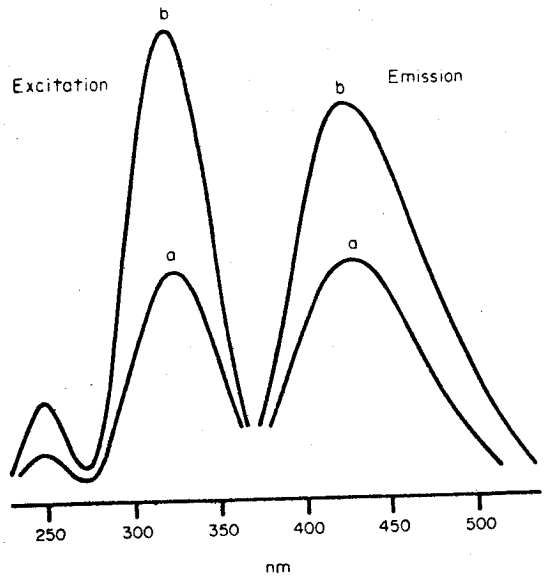

Fig. 1. Fluorescence spectra of LSD in 0.004 N HCl. Emission-spectra are scanned at fixed excitation (330 nm), excitation-spectra at fixed emission (430 nm). a: extract of 1 ml solution containing 100 ng LSD; b: brain extract of 25 ants fed for 2 days with sugar-water containing 500 μg/ml LSD.

mum average values of about 150 pg per brain from 12–24 hr after drug uptake. After 42 hr however, the drug content decreased to 1/3 (about 50 pg per brain) of this maximum value.

In a different series of experiments, [^{3}H]THC was given orally to ants (in sugar-water containing 10 vol% Tween-80). Despite the emulgator, the solubility of THC was bad. Therefore the drug content in each stock solution was determined by counting its radioactivity. Accordingly, the feeding solutions contained 570, 590, 660 and 750 μg/ml [^{3}H]THC respectively. To obtain comparable results, all values were normalized to a theoretical drug concentration in the food of 100 μg/ml.

The radioactivities were determined at 9 different time intervals (from 1 hr to 10 days) after drug intake. In experiments continuing more than 3 days after drug application, the ants were fed with sugar-water on days 3, 5, and 7. At each time the radioactivity of 6 or 8 samples containing the homogenate of 5 brains were measured and averaged. For background determination, we used samples containing the brain homogenate of two control-fed ants.

The radioactivity of samples with brains of [^{3}H]-THC-fed ants amounted to peak values of more than 500 cpm net from 24–48 hr after drug intake, whereas the lowest average (70 cpm net) was found 1–2 hr after drug intake. After 10 days, we still were able to observe a radioactivity of 90 cpm net, which clearly exceeded background values (20–25 gross cpm). The drug contents of the brains, normalized to a THC-concentration of 100 μg/ml food are drawn in Fig. 3.

The drug content in ant brains increased steeply during 24 hr from drug intake. The maxima of 750–800 pg THC per brain were reached after 24–48 hr. Subsequently the drug content decreased to about 1/3 (220 pg per brain) after 6 days.

DISCUSSION

In these investigations, the uptake of hallucinogenic drugs into the brain (head ganglia) of ants was examined quantitatively for the first time.

Fluorescence spectrophotometric measurements showed, that LSD, which was available to the ants during a period of 2 days in a concentration of 500 μg/ml in sugar-water, concentrated in the brain to an extent of about 7 ng. However, the sensitivity of this method proved to be not sufficient to ascertain the time course of drug uptake and elimination. Therefore we subsequently used tritiated drugs, which were detectable in the nervous system even after a single application of a non-toxic dose. The amount of radioactivity found in the brain homogenates does not distinguish between unchanged and metabolized

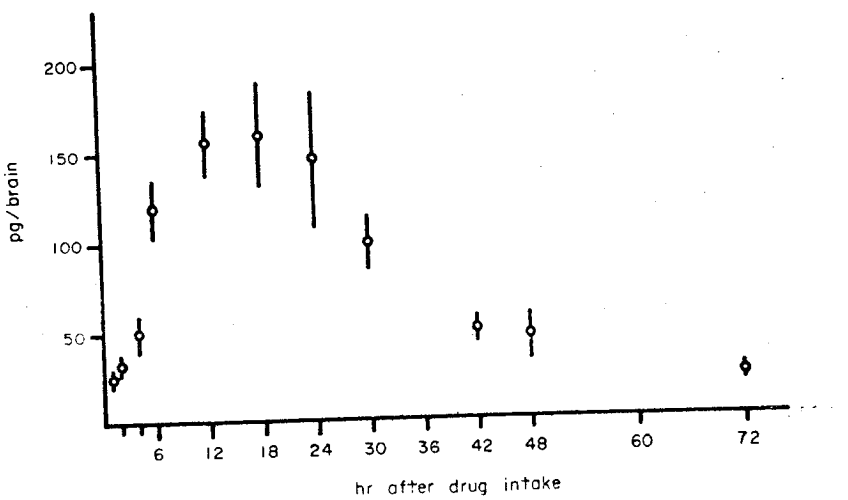

Fig. 2. Drug contents in ant brains at different times after a single feeding with sugar-water containing 100 μg/ml [^{3}H]LSD. (◊ mean ± S.E., n = 10).

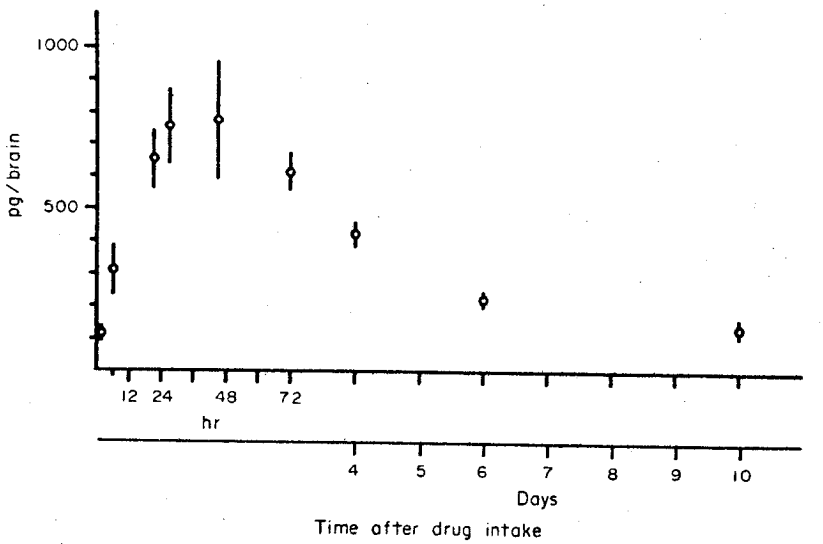

Fig. 3. Drug contents in ant brains at different times after a single feeding with sugar-water containing 100 μg/ml [^{3}H]delta-9-tetrahydrocannabinol and 10 vol.% Tween-80 (♀ mean ± S.E. n = 6 or 8).

drugs. Therefore the term "drug content" used in this context is not quite correct.

Liquid scintillation studies of brain homogenates from ants fed with sugar-water containing 100 μg/ml [^{3}H]LSD showed peak values from 12 to 24 hr after drug application which corresponded to LSD contents of about 150 pg per brain. This is distinctly below expectations based on the preceding fluorimetric results. Two reasons might be responsible for this discrepancy: (1) The drug was offered only once, unlike in the first series of experiments with free access for 2 days. (2) The concentration of 100 μg/ml LSD caused no toxic effects. Therefore we can suppose that the mechanisms of resorption from intestine to hemolymph and into the brain and of elimination from the nervous system are intact. In contrast, ants fed on sugar-water containing 500 μg/ml LSD during 2 days, showed severe poisoning. However, it might be possible that in this case LSD accumulates in the brain to a larger extent because of damage to their blood-brain barrier.

If we take into consideration, that hungry ants eat about 3.6 μl of sugar-water (estimated by weight gain), the highest LSD contents of 150 pg per brain corresponded to 0.04% of the ingested drug amount. The brain weight (0.3 mg) is about 2% of the whole body weight (15 mg). In conclusion we detected only low portions of the ingested [^{3}H]LSD in the brain, and the maximum wasn't reached before 12–24 hr after drug intake. Within the next 6 hr (30 hr after food intake) the drug content diminished by 1/3 and decreased in the next 12 hr (42 hr after drug intake) down to 30% of the peak values.

After feeding with [^{3}H]THC the highest degrees of radioactivity in the brain were reached between 24–48 hr. The peak values corresponded to drug contents of about 800 pg per brain (related to drug concentrations of 100 μg/ml THC in the food). This amounts to about 0.22% of the ingested drug dosage. From this maximum the values were reduced within the next 24 hr (72 hr after food intake) by only 20% and 3 days later (6 days after food intake) we were still able to detect 30% of the peak content in the brain.

THC is taken up by the nervous system of ants to a larger extent than LSD and remains there for a longer time. This finding corresponds with results in mammals after intravenous and intraperitoneal application of LSD and THC (Diab *et al.* 1971; Gill & Jones, 1972; Ho *et al.*, 1972; Layman & Milton, 1971; Rosencrans *et al.*, 1967). The size, charge, and the polarity of a molecule are responsible for its ability to penetrate through membranes and into the brain. The THC molecule is lipophilic and crosses the blood-brain barrier much more easily than LSD molecules with their polar carboxy- and amino-groups. Insects, despite their open hemolymph circulatory system possess some kind of a "blood-brain barrier" (Pichon, 1974). It is composed of the glia cells and the neural laminae of the nervous tissue. Thus the higher drug concentration attained by THC compared to LSD in head ganglia of ants may be mainly attributed to differences in physico-chemical properties of the two substances.

Finally, it should be noted that a strikingly long time is required for the peak levels of drug concentration in the ant's brain to be reached. This might be connected to the fact that the drugs were administered orally. In ants, the ingested food first passes through the oesophagus into the crop. This is some kind of "social stomach", from where the food may be regurgitated and then fed to 8–10 hungry nestmates. Alternatively, the food passes to the ant's midgut to be absorbed there. This process is very slow. Gösswald (1959) fed [^{32}P]sodiumphosphate mixed with honey-water to worker ants. He found only 1% of the total radioactive food consumed after 55 min, and only 20% after 4½ hr, in the midgut. After 1 day, the radioactivity was distributed throughout the whole body and then completely disappeared within 4 days after intake.

This parallel between delayed forwarding of the food and delayed drug uptake into the brain suggests that the resorption of LSD and THC does not take

place in the crop, but at a later stage in the intestine. This may be an explanation for the delayed accumulation of the drugs in the brain of ants.

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