Table II. The effect of injection of mitomycin C into 96-h-old wild-type larvae of *Drosophila melanogaster* on the differentiation of the 4 scutellar macrochaetae (n = 43)

Bristle organ	Complete (%)	Socket only (%)	Shaft only (%)	Missing (%)
Left anterior scutellar	2.3	0	65.1	32.6
Right anterior scutellar	0	0	74.4	25.6
Left posterior scutellar	4.7	2.3	62.8	30.2
Right posterior scutellar	7.0	2.3	60.5	30.2
Average	3.5	1.2	65.6	29.7

Table III. Comparison of the frequency of observed and expected bristle organ aberrations in the homozygous mutant *shaven-depilate* of *Drosophila melanogaster* upon injection of mitomycin C into 96-h-old larvae

4 Scutellar bristle organs	Complete (%)	Socket only (%)	Shaft only (%)	Missing (%)
Observed (n = 41)	11.6	56.1	14.0	18.3
Expected	2.0	2.7	37.2	58.1

affected. Other experiments[7] have shown that the pattern of bristle organ modification is the same, whether mitomycin C is injected into wild-type larvae or into larvae of the phenotypically wild-type stock *red*; $sv^{de}/T(3;4)89E$.

To test whether the phenotypic effects of mitomycin C on wild-type and sv^{de} flies are cumulative, we compared the frequency of observed bristle organ modifications in the mutant sv^{de} upon injection of mitomycin C and the expected frequency of bristle organ aberrations. Such an expected frequency was calculated by multiplying the frequencies of the different bristle organ aberrations in non-treated sv^{de} mutants with those of mitomycin C treated wild-type flies. The results are depicted in Table III. It is evident that the expected and the observed frequencies for bristle organ modifications are not in accord (χ^2-Test: $p < 0.001$). On the one hand, too many complete bristle organs and sockets without shafts are formed, on the other hand there are not enough missing bristle organs and bristle shafts without sockets. The same disagreement between expectation and experimental result has also been noted upon injection of nitrogen mustard into wild-type and sv^{de} flies[7].

The data presented indicate that the bristle organ primordia of sv^{de} flies are much more resistant towards the detrimental effect imposed by mitomycin C than in wild-type flies. The reason for this relative resistance against the injuring effect of the drug is not known.

Zusammenfassung. Bei *Drosophila melanogaster* erlaubten genetische Methoden die Konstruktion eines balancierten Stammes, bei dem homozygote «*shaven-depilate*» (sv^{de}) Fliegen bereits im Larvalstadium als solche erkennbar sind. Die Primordien der Borstenorgane der Mutante sv^{de} sind gegenüber einer weiteren Schädigung der Borstendifferenzierung, die durch Injektion von Mitomycin C in verpuppungsreife Larven bewirkt wird, signifikant weniger empfindlich als Borstenprimordien des Wildtyps.

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Blockage of LSD Binding at its High Affinity Site on Synaptosomal Membranes by 1-Methyl-1,2,5,6-Tetrahydropyridine-N,N-Diethyl- carboxamide

SMYTHIES et al.[1] in an attempt to delineate the activity of hallucinogenic agents based on classical structure-activity relationships discovered a most interesting phenomenon related to the compound 1-methyl-1, 2, 5, 6-tetrahydropyridine-N, N-diethylcarboxamide (THPC). The structure of this compound as shown in Figure 1 indicates its relationship to LSD through the D-ring of the LSD molecule.

These investigators had postulated, on the basis of structure activity comparisons, that THPC in sufficiently high concentrations should, in fact, cross the blood brain barrier and act as a competitive antagonist of the hallucinatory activity of LSD. In order to test this hypothesis the modification of rodent behavior produced by LSD was quantified using Bovet-Gatti profiles on a Sidman avoidance schedule[2]. Having established these profiles on rats given LSD the ability of THPC to block this behavior was tested by premedicating the rat with a dose of THPC equivalent to 15 mg/kg of the drug. The drug was given i.p. approximately 45 min before 0.1 mg/kg of LSD was given to the animal. Results of these studies clearly indicated that THPC brought about an almost complete abolition of the behavioral disruption produced by LSD intoxication.

Since we have been working for some time in our laboratory characterizing the properties of the high affinity LSD binding site on synaptosomal membranes from rat brain, we were intrigued by the idea of obtaining molecular (rather than behavioral) evidence for the blockade of LSD binding by THPC.

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Methods. In order to quantify the binding of LSD to its high affinity neuronal membrane binding site the technique of equilibrium dialysis was utilized. The dialysis cells were of an all Teflon design and the system consisted of a complete unit obtained from the Kontron Corp. of Zurich, Switzerland. The halfcell volume was 1 ml and cells were separated by specially washed regenerated cellulose membranes. Various concentrations of tritiated LSD (17.1 Ci/mM) were dialyzed against synaptic membrane preparations[3] in a dialysis medium consisting of 0.005 M *Tris*-HCl, pH 7.4, 0.6% NaCl, and 10 μM $CaCl_2$. Radioactivity measurements of the equilibrated fractions were determined in a toluene-Triton X-100, PPO, dimethyl POPOP cocktail[4] using a Packard Tricarb liquid scintillation spectrometer. In all of our studies lysed rather than intact synaptosomes have been used to preclude the possibility of confusing uptake with binding[5]. Protein concentration was determined by the method of LOWRY[6]. All experiments were performed in the presence of 5×10^{-4} M pargyline, an irreversible monoamine oxidase inhibitor[7] in order to block any drug-enzyme rather than drug-membrane binding.

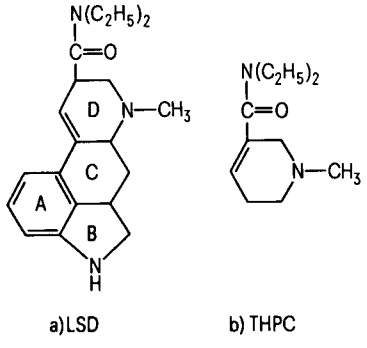

Fig. 1. Structural relationship between a) D-lysergic acid diethylamide and (LSD) b) 1-methyl-1,2,5,6-tetrahydropyridine-N,N-diethylcarboxamide (THPC).

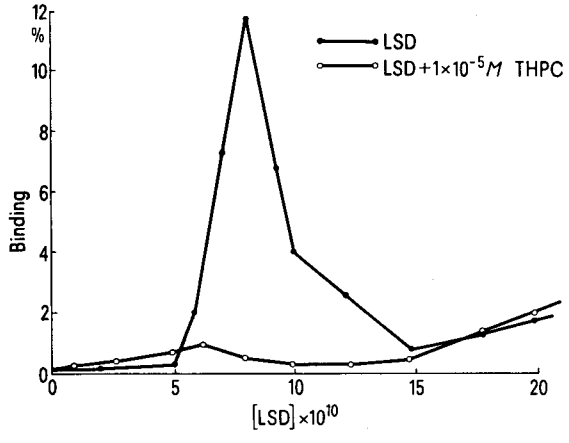

Fig. 2. The effect of 1×10^{-5} M THPC on the binding of LSD to its high affinity synaptosomal receptor. The closed triangles represent the binding of LSD alone at the concentrations noted on the X-axis to isolated synaptosomal membranes while the open circles represent the complete blockade of LSD by 1×10^{-5} M THPC at its high affinity 'receptor' site.

Results. Using these techniques a K_d (dissociation constant) of 2.9×10^{-9} M was obtained for the high affinity LSD-synaptosomal membrane binding site[8]. The concentration (or number) of binding sites for LSD on the synaptosome based on the amount of tissue was calculated from these data and gave a value of 0.058 picomoles/mg of protein. In order to demonstrate the effect of THPC on this high affinity binding the compound was added to the dialysis medium such that its final concentration was 1×10^{-5} M. Dialysis runs were repeated several times with ^{3}H-labeled LSD in both the presence and absence of THPC. The results of a typical experiment are shown in Figure 2. From these data one can clearly see that the binding of LSD to its high affinity site on the synaptosomal membrane is completely blocked by the THPC added to the dialysis medium.

Discussion. The results of this study are most interesting from the standpoint of both behavioral and molecular pharmacology. In fact, these data appear to lend some definition on a molecular basis to the antagonism of the behavioral toxicity of LSD as manifested by the bar pressing activity carried out by rodents. Although our results could not (or should not) be interpreted as indicating the mechanism of LSD toxicity it does bring the investigator closer to an understanding of the real consequences of agonist-antagonist relationships. There are two considerations which, we believe, are important consequences of this study. Firstly, as far as we know, THPC is only one of a very few compounds that have been documented as blockers of the behavioral effects of LSD. Data presented here confirm this blockade at the receptor level. Secondly, the design of THPC as a blocking agent was conceived at the theoretical level and now has indeed been shown to modify the activity of LSD both at the whole animal level and at the neuronal membrane level.

Résumé. Par dialyse d'équilibre on a montré que le 1-methyl-1, 2, 5, 6-tetrahydropyridine-N, N-diethylcarboxamide (THPC) empêche le LSD de se lier aux membranes des synaptosomes. Ce résultat correspond à un effet du THPC qui est de réduire la nocivité de LSD.

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