



Fig. 3. The free glutamate/total free amino acid concentration ratio is shown on the Y-axis. Each tissue is shown at 2, 4, 10 and 25-day periods following crushing (between DR1 and DR2 as in Figure 1) on the X-axis. The arrow points to the control value glutamate/free amino acid concentration ratio obtained in each particular time period. The bar for each tissue on the X-axis represents the glutamate/free amino acid concentration ratio, and the line above this is the standard deviation. The significance of the difference between control and crush tissue was tested by the Student's *t*-test. The number of X's at the base of each bar represents the level of significance of this difference; X = 5%, XX = 2.5% and XXX = 0.5% level of significance.

crushing, not on absolute grounds (per g tissue), but on a relative basis (expressed as the ratio of free glutamate/total free amino acid concentrations). Perhaps, however, a significant free glutamate concentration decrease could be detected at times earlier than 25 days if a very large sample size was used with data expressed per g tissue only. Although this may suggest that there is a decrease in free glutamate in the dorsal sensory neuron following injury, some additional difficulties must be considered before this conclusion is reliable. Distal to the injury there is complete degeneration of the separated axons<sup>17</sup> with phagocytic satellite cells breaking down axonal debris<sup>18</sup>. The proteolytic activity of the satellite cells may cause free glutamate increases which cancel any decreased axonal glutamate when measured on a tissue basis. Proximal to the injury, the intact axons regrow in an attempt to reinnervate the end organ<sup>17</sup>. It is not yet known whether the total free amino acid increase observed proximally here is neuronal or extraneuronal in nature, although free glutamate is decreasing relative to total free amino acids at a great distance proximal to the crush. Therefore, the results of this study<sup>1</sup>, and the fact that glutamate is such an active substance of widespread importance<sup>6</sup>, indicate that one must ask the following three questions concerning such data. 1. How important are such observations in showing whether or not glutamate is a synaptic transmitter? 2. How specific are such crush effects on free glutamate levels where one is reasonably sure it is a synaptic transmitter as opposed to where it is not a transmitter? 3. What amino acid changes following axonal crush or section are associated with the axons and which are associated with satellite cells?

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<sup>18</sup> M. SINGER and M. C. STEINBERG, *Am. J. Anat.* 133, 51 (1972).

## Long-Chain (Z)-9-Alkenes are 'Psychedelics' to Houseflies with Regard to Visually Stimulated Sex Attraction and Aggregation

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**Summary.** Three different tests on houseflies (*Musca domestica* L.) revealed that both pheromone-free pseudoflies and male partner flies exhibit in the presence of mixtures of long-chain (Z)-9-alkenes or pure (Z)-9-tricosene enhanced releasing effects for two optical cues, which stimulate male houseflies to mating strikes and houseflies of both sexes to aggregation.

Behavioral responses of long-chain (Z)-9-alkenes in the male housefly, *Musca domestica* L. are believed to be sex attraction<sup>1-3</sup>, sex stimulation<sup>3</sup>, excitement, mating and orientation<sup>4</sup>. Furthermore, aggregation of both sexes of houseflies is attributed to (Z)-9-tricosene<sup>5,6</sup>. Several homologous compounds show pheromone activity<sup>3,4</sup>, especially a 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene<sup>4</sup>. Although it is well known that visual stimuli elicit strong sex attraction<sup>7</sup> and aggregation<sup>8</sup>, the mutual interaction between visual and olfactory stimuli in the housefly has not been established<sup>9</sup>.

In the present study, combinations of several (Z)-9-alkenes were tested to elucidate their effect on the behavior of houseflies in conjunction with visual stimuli. 3 types of tests were carried out, each in the absence of pheromones ('standards') and in the presence of pheromones: 1. The well established pseudofly test in Petri

dishes<sup>2,3,10</sup>, in which one pseudofly is exposed to 2 male houseflies; 2. a modified pseudofly test in Petri dishes, in which two pseudoflies are exposed to 1 male housefly; and 3. a large-scale test using pseudofly-flypaper strips. In 2. as well as in 3. pseudoflies containing no pheromones were employed as 'controls' in the presence of pseudoflies impregnated with pheromones.

**Material and methods.** The test materials (I-VI), as shown in Table I, contained various levels of biologically active hydrocarbons, such as (Z)-9-heneicosene<sup>3,4</sup>, (Z)-9-docosene<sup>3,4</sup>, (Z)-9-tricosene<sup>1-6</sup>, (Z)-9-tetracosene<sup>3,4</sup>, and (Z)-9-pentacosene<sup>4</sup>.

1. The short range action of long-chain (Z)-9-alkenes on the sex attraction of male houseflies was studied using the pseudofly test in Petri dishes<sup>2,3,10</sup>. Covered Petri dishes, 9 cm in diameter, each with 2 male houseflies, 5 to 10 d old, from laboratory culture and 1 'pseudo-

fly' (knot made from black shoelace), placed in the center, were arranged on a table in 3 groups. The tests were carried out at 25°C between 14.00 and 16.00 h under bright daylight and each fly was used for a single test only. A group of 3 observers counted, each at 6 dishes in any single 30 min session, the number of mating strikes from one fly to the accompanying fly as well as to the pseudofly, which was pretreated with the test material.

2. The extent of short range orientation of male houseflies to olfactory stimuli by long-chain (Z)-9-alkenes was assayed in a modified pseudofly test in Petri dishes. Male houseflies were tested individually in Petri dishes, 9 cm in diameter, containing 2 knots made from black shoelace, which were separated by 5 cm from each other. In half of the dishes, both pseudoflies were treated with 10 µl of hexane and the solvent was allowed to evaporate. In each of the remaining dishes, one knot was treated with 10 µl pure hexane and the other with 100 µg of (Z)-9-tricosene<sup>12</sup> dissolved in 10 µl hexane. After evaporation of the solvent, the tests were carried out at 28°C under the same conditions as above. The number of mating strikes to 'left' and 'right' pseudoflies was counted separately by a group of 4 observers, each at 6 dishes in any single 30 min session.

3. The influence of long-chain (Z)-9-alkenes on the aggregation behaviour of male and female (1:1) house-

flies was investigated using 'pseudofly-flypaper' strips as traps. In this test system the pseudoflies were printed on Aeroxon® flypaper strips (4 × 80 cm) in the form of 300 black spots of 5 mm diameter, per strip. For assays, batches of 5,000 to 12,500 houseflies from laboratory cultures were kept on a sugar plus water diet in a 142 m³ test room with 4 windows. When the flies were 5 to 7 days old, 10 pseudofly-flypaper strips were hung from the ceiling (3 m high), separated by 1 m from each other. In each test series, 10 traps were exposed once to the flies between 10.00 and 16.00 h at about 23°C. Each group of flies was taken for 2 consecutive tests only. In the absence of (Z)-9-alkenes, 10 standard series were run in order to determine the basic average percentages of captures at each of the ten trap positions. The sequence of pseudofly-flypaper strips, impregnated with the different mixtures of (Z)-9-alkenes and of control strips investigated simultaneously, was changed in all four test series.

*Results.* 1. The data obtained in the tests with pseudofly-Petri dishes, as given in Table II, show that in standards the average number of homosexual mating strikes was 2 times higher than copulative jumps on the pseudofly. In the presence of long-chain (Z)-9-alkenes, however, the number of mating strikes onto male flies was increased 2 to 3.6 times and onto pseudoflies 4.4 to 6.6 times. Thus, the total number of mating strikes between fly-fly and fly-pseudofly reached the same absolute level.

2. In the modified version of the tests in pseudofly-Petri dishes, 192 males were assayed individually for standards, both pseudoflies in each dish being free of (Z)-9-alkenes. The total number of mating strikes to 'left' and 'right' pseudoflies was 600 and 535, respectively. The double dummy tests on 192 male flies, each in Petri dishes containing one pseudofly, impregnated with 100 µg of (Z)-9-tricosene<sup>12</sup>, and the second pseudofly for control, were run simultaneously. The total number of copulative strikes was 2,043 on knots treated with (Z)-9-tricosene, and 1,236 on knots for control. Thus, the number of mating strikes compared to standards, run in the absence of (Z)-9-tricosene, was increased 3.6 and 2.2 times, respectively. In one fourth of all tests (48 males) carried out with (Z)-9-tricosene, the number of copulative strikes on the pseudofly impregnated with (Z)-9-tricosene (606 jumps) was almost equal to that on the control dummy (575 jumps).

3. All pseudofly-flypaper strips aggregated both sexes of houseflies, males predominantly at the upper part and females predominantly at the lower part. Table III shows that in each case the number of males trapped was 2.7

Table I. Composition of mixtures of the (Z)-9-alkenes tested

Test material <sup>a</sup>	Percentages of (Z)-9-alkenes									
	C <sub>16</sub>	C <sub>17</sub>	C <sub>18</sub>	C <sub>19</sub>	C <sub>20</sub>	C <sub>21</sub>	C <sub>22</sub>	C <sub>23</sub>	C <sub>24</sub>	C <sub>25</sub>
I		2		1.5		2.5		88		6
II		0.5		0.5		54		38		7
III						1.5		97		1.5
IV	4		22		11		59		4	
V	0.5		1		60		33		5.5	
VI	Trace				3		96		1	

<sup>a</sup>Prepared from the seed oils of *Brassica napus* (I and IV), *Simmondsia chinensis* (II and V) and *Tropaeolum majus* (III and VI)<sup>11</sup>.

Table II. Effect of long-chain (Z)-9-alkenes on male houseflies in tests in pseudofly-Petri dishes<sup>a</sup>

Test material <sup>b</sup>	Number of mating strikes <sup>c</sup> on	
	Male fly	Pseudofly
Standard	268	107
I	542 (2.0)	526 (5.0)
IV	657 (2.5)	701 (6.6)
Standard	363	149
II	816 (2.2)	808 (5.4)
V	774 (2.1)	652 (4.4)
Standard	189	130
III	684 (3.6)	693 (5.3)
VI	597 (3.1)	501 (3.9)

<sup>a</sup>Tests according to 2<sup>3</sup>, 3<sup>10</sup>. <sup>b</sup>10 µl hexane (for standards) or 100 µg (Z)-9-alkenes dissolved in 10 µl hexane were applied to each pseudofly and the solvent was allowed to evaporate. <sup>c</sup>Counted for 30 min using 168 male flies; in parentheses, times increased compared to standards tested simultaneously.

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<sup>2</sup> W. M. ROGOFF, G. H. GRETZ, M. JACOBSON and M. BEROZA, *Ann. entom. Soc. Am.* 66, 739 (1973).  
<sup>3</sup> D. A. CARLSON, R. E. DOOLITTLE, M. BEROZA, W. M. ROGOFF and G. H. GRETZ, *J. agric. Food Chem.* 22, 194 (1974).  
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<sup>5</sup> D. A. CARLSON and M. BEROZA, *Envir. Entom.* 2, 555 (1973).  
<sup>6</sup> P. B. MORGAN, I. H. GILBERT and R. L. FYE, *Fla. Entom.* 57, 136 (1974).  
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<sup>8</sup> R. WIESMANN, *Mitt. Schweiz. entom. Ges.* 33, 121 (1960).  
<sup>9</sup> I. RICHTER, *Naturwissenschaften* 61, 365 (1974).  
<sup>10</sup> W. M. ROGOFF, A. D. BELTZ, J. O. JOHNSON and F. W. PLAPP, *J. Insect. Physiol.* 10, 239 (1964).  
<sup>11</sup> H. K. MANGOLD, H. BECKER and I. RICHTER, in preparation.  
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Table III. Effect of long-chain (Z)-9-alkenes on both sexes (1:1) of houseflies in tests with pseudofly-flypaper strips\*

Test material <sup>b</sup>	No. of traps	Total number of flies captured	Ratio (male/female)	Times increase of captures (%)
Standards	100	4248	2.7	
Controls	16	1312	3.0	1.8
I	4	467	5.7	2.4
II	4	679	3.3	2.5
III	4	442	2.7	2.4
IV	4	398	3.5	2.5
V	4	462	3.4	1.7
VI	4	657	3.9	2.7

\*Tests on 55000 houseflies (total) at 22 to 24°C in 14 six-hour test series using 10 flypaper strips, each, with 300 black spots (5 mm Ø).

<sup>b</sup>2.5 ml hexane, for standards (10 test series) or 125 mg (Z)-9-alkenes dissolved in 2.5 ml hexane, for tests (4 test series) and 2.5 ml hexane, for control strips run simultaneously, were applied to each trap and the solvent was allowed to evaporate.

to 5.7 times higher than that of females, though in the test room the starting ratios of the sexes was 1:1. In the presence of long-chain (Z)-9-alkenes, both traps impregnated with these compounds and those not impregnated caught more flies compared to standards run in the absence of the unsaturated hydrocarbons. The efficiency of the pseudofly-flypaper strips loaded with test material V, which contained the smallest proportion of biologically active (Z)-9-alkenes<sup>3,4</sup> was increased by a factor of 1.7, whereas that of the controls, run simultaneously, was increased by a factor of 1.8. The percentages of captures of all other traps impregnated with (Z)-9-alkenes were increased 2.4 to 2.7 times.

**Discussion.** The results obtained with houseflies show that a wide spectrum of long-chain (Z)-9-alkenes, in varying combinations, exhibit, both qualitatively and quantitatively, the same behavioral responses, depending upon the different visual signals in the tests using pseudofly-Petri dishes and pseudofly-flypaper strips.

Recently, (Z)-9-tricosene has been characterized as a short range sex pheromone<sup>13</sup>. In the present study (Z)-9-tricosene did not give clear evidence for short range

orientation of male houseflies to the source of odor in the modified pseudofly tests using Petri dishes, although it is well known that houseflies pay attention to nearly any odor at a short distance<sup>8</sup>. However, in the presence of long-chain (Z)-9-alkenes the optical stimuli of sex attraction by untreated pseudoflies (controls) exerted an enhanced releasing effect, compared to those in standard tests run in the absence of pheromones.

The conventional tests with pseudofly-Petri dishes also showed unequivocally, that in the presence of long-chain (Z)-9-alkenes the optical stimuli of sex attraction by male partner flies were enhanced, compared to those in the standard tests which were run in the absence of pheromones. Similar observations have been made using benzene extracts of female houseflies<sup>10</sup>.

Similarly, in the tests using pseudofly-flypaper strips, the visual stimuli for aggregation provided by the untreated strips (controls) were potentiated in the presence of high dosages of long-chain (Z)-9-alkenes compared to standards run in the absence of pheromones.

On the basis of all these findings, it is concluded that the threshold for optical cues stimulating houseflies to sex attraction or aggregation is decreased in the presence of long-chain (Z)-9-alkenes in the test systems. Thus, long-chain (Z)-9-alkenes can be considered 'psychedelics' to houseflies with regard to visually stimulated sex attraction and aggregation. The state of expanded sensoric susceptibility is a characteristic effect of psychedelic drugs.

Sex attraction of male houseflies by moving pseudoflies is not changed in the presence of long-chain (Z)-9-alkenes<sup>9</sup>, because the releasing effect of such fast moving dummies is greatly increased compared to static female fly models<sup>7</sup>. In tests using plexiglas olfactometers<sup>1,3,4</sup> or other types of traps<sup>5,6</sup> for the assay of pheromones on houseflies, visual stimuli may have been involved to varying extents.

The results obtained in tests using pseudofly-flypapers show poor captures compared to the total number of flies present, although both olfactory and visual stimuli were provided. Therefore, the use of long-chain (Z)-9-alkenes or other olfactory stimuli<sup>8</sup> seems to be of minor value for control of the housefly.

<sup>13</sup> P. A. LANGLEY, R. W. PIMLEY and D. A. CARLSON, *Nature*, Lond. 254, 51 (1975).

## Sodium Pumps and Galactose Transfer in the Short-Circuited Small Intestine

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**Summary.** In a short-circuited preparation of rat jejunum, there are two sodium pumps, one electrogenic and the other neutral. When energy sources are limited, the total sodium transfer is limited. In the presence of a non-metabolized actively transferred hexose, the electrogenic pump is preferentially used. The neutral sodium pump is only able to function when additional energy is available.

The absorption of Na and certain hexoses by the small intestine are closely linked<sup>1-8</sup>. The situation in the rat jejunum in vitro is complicated by there being two distinct Na pumps, one which is electrogenic and the other which is non-electrogenic<sup>7,9</sup>. Na transferred by the electrogenic mechanism gives rise to a transmural potential difference and is linked in part to active hexose transfer, while that transferred by the non-electrogenic mechanism

is linked to hexose metabolism and causes an increase in fluid absorption. The capacity of the jejunum to transfer Na and the relationship between these two types of Na pump has been investigated using a short-circuiting technique.

**Materials and methods.** White male rats (220-250 g) were anaesthetised with i.p. pentobarbitone sodium (Nembutal). The small intestine was removed and