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Centrophobism in *Drosophila melanogaster*

I. Behavioral modification induced by ether

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Summary. The term 'centrophobism' is introduced to describe a newly discovered modification of search behavior in the walking fruitfly, *Drosophila melanogaster:* the avoidance of the center of an arena after diethylether narcosis. Evidence for the effect is obtained by comparison of the tracks of etherized and non-etherized flies under the influence of olfactory attractant around the center of the arena (Fig. 3). The tracks can be distinguished by their mean radial distance from the central district of the arena. 'Centrophobia' denotes the relative difference of the distances of etherized flies and non-etherized controls (Fig. 4).

Etherized flies avoid the center of the arena in spite of the attraction of olfactory, thermal or visual cues. The avoidance is significant even in the absence of conspicuous sensory cues for the discrimination of center and surround. The centrophobia obtained in the arena can be used to estimate the efficacy of attractants in the non-etherized control flies (Figs. 6, 7).

The lowest possible dose of ether sufficient to elicit narcosis is sufficient to induce centrophobia. None of the other prevalent insect anaesthetics, CO_2, N_2 and cold, substitutes ether in the present experiments (Figs. 8, 9).

Centrophobia arises immediately after ether narcosis. Once induced the effect lasts apparently undiminished for the life time of the flies (Fig. 9).

Centrophobia has been found in either sex of the 9 strains tested so far (Fig. 5). Four strains including mutants deficient in wing formation (*vestigial*) or learning (*dunce*) show either temporal decline or partial suppression of centrophobia. The anomalous properties are actually due to enhanced spontaneous centrophobism in the non-etherized control groups of these strains (Fig. 10).

Introduction

Analysis of locomotor behavior has led to the discovery of a striking after effect of ether narcosis in the fruitfly, *Drosophila melanogaster:* the apparently irreversible avoidance of the center of an arena which is described in the present account. The physiological background of the phenomenon of center avoidance is the objective of a second paper (Götz and Biesinger 1985).

Two expressions will be introduced to illustrate this aspect of search behavior in the arena: The general term *'centrophobism'* denotes the manifestation of center avoidance in flies which may, or may not have been under ether narcosis at any time before the trial. The specific term *'centrophobia'* denotes the relative difference between post-narcotic and pre-narcotic centrophobism. This difference will be used to quantify the aftereffect of ether narcosis. The purpose of the two expressions is to describe observations rather than attribute center avoidance to a phobia-like mental state of the flies.

Material and methods

Most of the present experiments relate to a local wild type 'Kapelle' (*WT KAP*) which was collected in 1973. Several other strains of *Drosophila* were used in comparative experiments, notably the standard wild types 'Berlin' (*WT BLN*), 'Canton S' (*WT C-S*) and 'Oregon R' (*WT O-R*). To prevent flight, the wings were clipped, at low temperature (+4 °C), near the anterior cross-vein. Possible side effects of this procedure were avoided by the use of the mutant *vestigial* which lacks functional wings. This mutant was backcrossed nine times to its original background of wild type 'Berlin' (*vg BLN*).

Unless otherwise stated cultures of flies of the same age were divided into an anaesthesia group and a control group. The flies of the anaesthesia group were exposed either to peroxide-free diethyl ether (vapor pressure ≤ 60 kPa) for 20–150 s, or to CO_2, N_2 or cold for somewhat longer. The dosage is

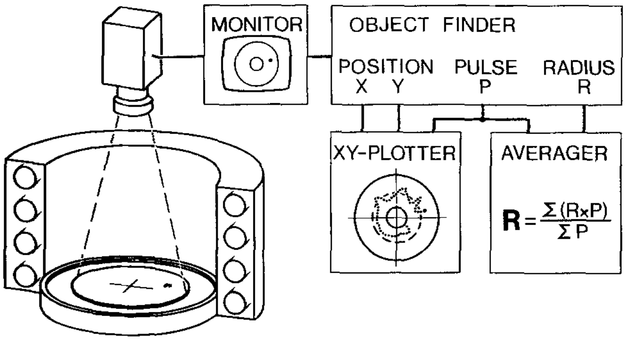

Fig. 1. Video system for the analysis of the tracks of the walking fruitfly, *Drosophila melanogaster*. A 58 cm^2 ring-shaped area of the circular disk in the center of the illuminated arena is imaged onto a scanning device developed in cooperation with M. Herre and H. Wenking. The block diagram illustrates the mode of operation used to obtain both, a copy of the track and the average radius R of its deviation from the center of the disk

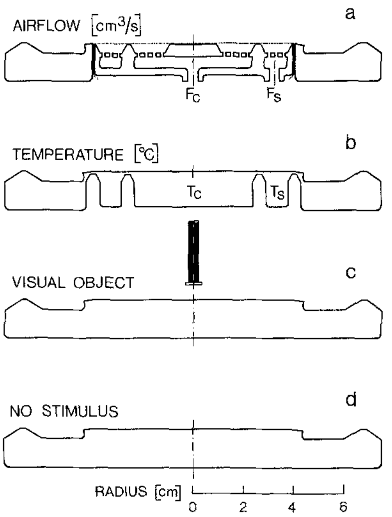

Fig. 2a–d. Cross-sections of four interchangeable disks on which a flightless test fly is kept walking during the arena experiments. The water-filled moat preventing the escape of the fly is shaped to diminish the optic contrast at the horizon. In **a** the disk is covered by muslin mounted as in an embroidery frame. Air containing the odor of a fermenting banana-yeast mixture is released, with a flow rate of 2 cm^3/s, from the central compartment (F_c) or, occasionally, from the surrounding compartment (F_s) of the centerpiece. In **b** center and surround of the metallic disk are held at different temperatures (T_c, T_s). In **c** a rod-shaped black object is suspended above the center which is blanked by the scanning device. A white endpiece conceals the visual object from sight when the fly is below the rod. Finally, in **d** there are no significant sensory cues for the discrimination of center and surround

measured as the *half time of narcosis* $t_{1/2}$. This is the time after which one half of the anaesthetized flies recovered co-ordinated leg movements in the absence of agitation. In some experiments, the total dosage of ether, CO_2, N_2 or cold was increased by repeated treatment and specified by the sum of subsequent half times of narcosis. The different groups of flies are denoted by the time of the following successive events: *hatching* (H), *narcosis* of the anaesthesia group (N), *amputation* of the wings (A), onset of food *deprival* (D) and *trial* (T). The time interval between any two events is derived accordingly: t_{T-H}, for instance, denotes the age of the flies at the time of the trial, t_{A-N} the time elapsed between narcosis and amputation, t_{T-D} the starvation time, and so on. Paired anaesthesia and control groups were equivalent with respect to all of the time intervals which do not relate to narcosis. In general, each fly of these groups was tested only once.

To investigate the influence of previous anaesthesia, single flies were taken alternately from paired anaesthesia and control groups. The fly to be tested was placed in the center of the arena in Fig. 1, and allowed to run freely for 200 s on an elevated circular disk of 4.4 cm radius. This disc is surrounded by a water-filled moat which prevents the fly from escaping. The light diffusing inner wall of the arena and the surrounding four toroid-shaped dc-driven fluorescent lamps are cut away, in the figure, to show the details of the experimental setup. The luminance in the arena was of the order of 400 cd/m^2. The track of the fly was scanned within a 3.4 cm wide annulus of the surface of the disk. The video scanning device consists of a 2/3″ plumbicon camera (Philips LDH 26) in combination with an object finder (Götz 1980; Bülthoff et al. 1982) which was adapted to the particular requirements of the present trials. The object finder determines the XY-coordinates of the position of the fly within the annulus and delivers a sequence of pulses to a point plotter as long as the fly is moving. The plotter records subsequent positions along the track within the annulus as a dotted line. The control mode of the pulse rate determines the information obtained from the counted number of pulses per track. This number corresponds to the time period of locomotor activity if the pulse rate is constant, or to the length of the track if the pulse rate is proportional to the walking speed. The radius of the recorded positions, $(X^2+Y^2)^{1/2}$, was derived electronically. Its average R was used to determine, in relative units, the *mean radial distance* $0 \leq D \leq 1$ of the fly from the inner boundary of the annulus according to $D = (R - R_{min})/(R_{max} - R_{min})$. In this expression, $R_{min} = 1.0$ cm is the inner radius of the annulus whereas $R_{max} = 4.4$ cm is the outer radius of both annulus and disk. The disk could be exchanged to introduce different sensory cues for the discrimination of center and surround. The cross-sections a–d in Fig. 2 show four versions of the arena experiment.

Results

How to demonstrate 'centrophobia'

Figure 3 illustrates the striking effect of ether on the locomotor behavior of *Drosophila* in the presence of banana odor. The track on the left is from a fly which received a comparatively short ether narcosis four days before the trial. Most of the time this fly kept walking near the outer boundary of the disk. Excursions towards the center are comparatively rare events which occur even less frequently in the average track of previously etherized flies. The track on the right is from a non-etherized control fly which confines its exploratory movements to the surface of the inner compartment from which the odor is released. Excursions to the periphery of the disk are absent in the selected

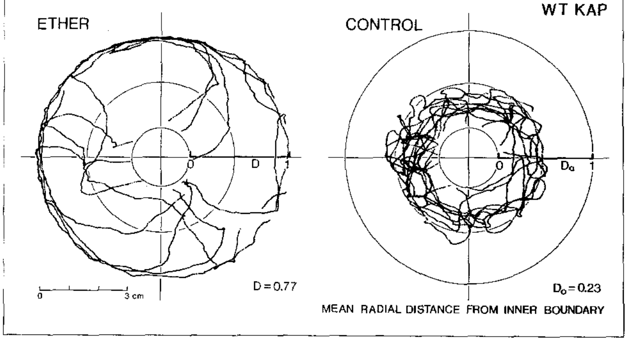

Fig. 3. Two arena experiments in which a fly with shortened wings was allowed to walk for 200 s on the permeable floor of the disk in Fig. 2a. The three circles indicate the surface of the disk and the invisible borders of the central compartment from which banana odor was continuously released. The automatic recording of the tracks was blanked within a central area of 3 cm^2 and at locomotor velocities below 0.1 cm/s, respectively. The flies were equally reared 7 d old female siblings which were treated similarly with respect to food deprival ($t_{T\text{-}D} = 23$ h) and wing amputation ($t_{T\text{-}A} = 5$ h). The track of the control fly on the right shows exploration of the odor source as the prevailing trait of behavior. The different behavior of the ether fly on the left illustrates the centrophobism acquired by a very short undisturbed narcosis ($t_{1/2} = 8$ min) which has been induced several days before the actual test ($t_{T\text{-}N} = 4$ d)

example. However, such excursions do occur in the average track and become increasingly frequent with time.

The most remarkable fact about the ether effect is its persistence over at least four days which may have prevented its detection in laboratories where ether is routinely used to collect flies. The present example shows that the olfactory response of *Drosophila* in a classical arena experiment (Otto 1951) can be improved considerably by avoiding ether narcosis at any age.

How to quantify 'centrophobia'

Ether-induced centrophobia can be described by the relative difference in the mean radial distances of the tracks of a test group of etherized flies (D) and a control group of non-etherized flies (D_0) according to $C = (D - D_0)/(D + D_0)$ where $-1 \leqq C \leqq 1$. A typical experiment requires a number of flies which were reared and maintained under similar conditions, and divided into a test group and a control group each comprising an average of 5 flies of the same sex. Figure 4 shows the histograms of the weighted averages of D, D_0 and C, respectively, as derived from the tracks of 240 *WT KAP* flies. The results illustrate the persistence and the reliability of the centrophobia in tests made about two weeks after temporary ether narcosis.

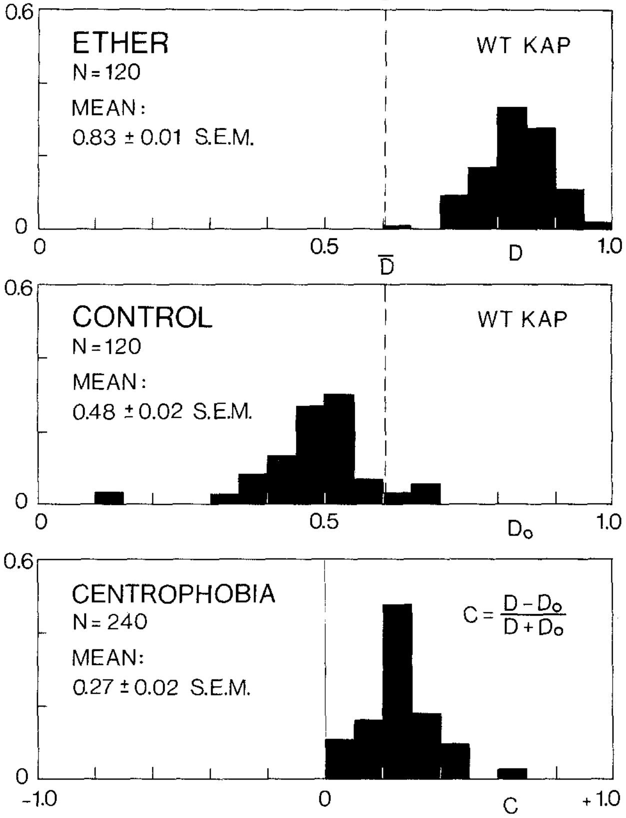

Fig. 4. Distribution of the mean radial distances of the tracks of identically treated groups of etherized (D) and non-etherized (D_0) siblings of wild type 'Kapelle' under attraction of banana odor in the center of the arena. Experiments as in Fig. 3. Average time between trial and narcosis $t_{T\text{-}N} = 13$ d. The increase of D beyond the mean distance in case of equidistributed probability of position in the arena ($\bar{D}$) excludes irreversible olfactory desensitization as a possible explanation of ether-induced centrophobism. The term 'centrophobia' (C) has been introduced to specify the relative difference between the centrophobism of etherized and non-etherized flies. The distribution of C in the lowermost diagram was obtained by evaluation of corresponding mean radial distances in the two upper diagrams

Comparison of the mean radial distances in Fig. 4 indicates another important property of the long lasting ether effect: The broken vertical line in the upper two diagrams denotes the calculated mean radial distance $\bar{D}$ of a walking fly as expected on the assumption of equidistributed probability of position within the annular test field in the arena. Olfactory desensitization of the etherized flies would explain a transition from $D_0 = 0.48$ to the calculated mean distance $\bar{D} = 0.60$ rather than to the actual mean distance $D = 0.83$. The investigated groups of etherized flies avoid the center of the arena unambiguously ($P < 10^{-8}$), and with remarkably low scatter of independent experimental results. The term 'centrophobia' has been chosen accordingly.

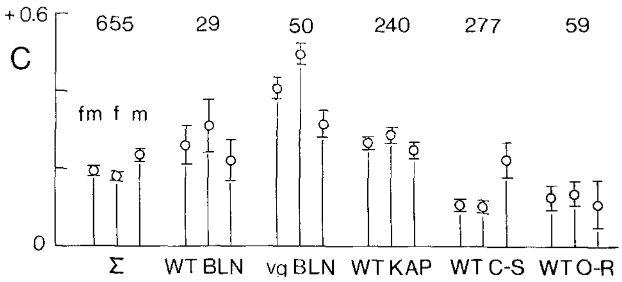

Fig. 5. Ether-induced centrophobia in different strains of *Drosophila*. Means, and standard errors of the means, were derived from the tracks of females (*f*), males (*m*) and both (*fm*), respectively. Technical details as in Figs. 3 and 4. From left to right: Weighted averages from 5 strains, wild type 'Berlin', wing-deficient mutant *vestigial* (background 'Berlin'), wild type 'Kapelle' (data from Fig. 4), wild type 'Canton S' (2 sub-strains, see Fig. 10), and wild type 'Oregon R'. Number of experiments per strain is given on top of the bars

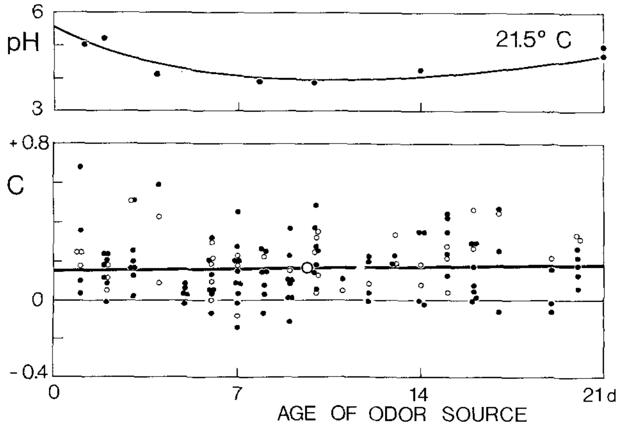

Fig. 6. Use of centrophobia as an assay of olfactory attraction in *Drosophila*. Experiments as in Fig. 3. The example shows the effect of gradual decomposition of a fermenting banana-yeast mixture both on the pH of the odor source and on the attraction of the odor for all of the groups of non-etherized females (filled circles) or males (open circles) from the strains in Figs. 5 and 10. Attraction for either sex seems to be statistically independent of the state of the odor source at room temperature

Disposition to 'centrophobia' in different strains

Figure 5 shows the means of centrophobia C derived from the 655 tracks of 471 females and 184 males of five different strains. The results suggest the following:

1. Centrophobia can be induced in all strains tested so far. The quantitative differences between the ether effects of the strains in Fig. 5 are within the limits of interstrain variation observed in other behavioral paradigms.

2. Quantitatively different effects have been found also in closely related sub-strains of the wild type Canton-S (*WT C-S* in Fig. 5). The mean response $C=0.273\pm0.026$ SEM obtained with 72 females and 16 males of a sub-strain held in Tübingen ($WT\ CS_T$) is about six times larger than the mean response 0.046 ± 0.014 SEM obtained with 189 females of a sub-strain from Freiburg ($WT\ CS_F$). The different responses of the sub-strains are discussed in the last two sections of the results.

3. The ratio C_f/C_m of centrophobia of females and males seems to be greater than 1 in all of the strains tested so far. Ratio, and corresponding standard error, of the ether effect in different sexes are, respectively, 1.40 ± 0.45 in the wild type Berlin (*WT BLN*), 1.57 ± 0.19 in the wing deficient mutant *vestigial* (*vg BLN*), 1.16 ± 0.14 in the wild type Kapelle (*WT KAP*), 1.28 ± 0.29 in the wild type sub-strain Canton-S Tübingen ($WT\ CS_T$), and 1.24 ± 0.75 in the wild type Oregon-R (*WT O-R*). The preponderance of female centrophobia is obscured, in Fig. 5, by the data from the wild type sub-strain Canton-S Freiburg ($WT\ CS_F$). Males of this sub-strain have not been tested, so far. However, the weakly reacting females contribute 72% of the female tracks in the combined *WT C-S* group, or 40% of the female tracks accumulated in the summary Σ on the left of the figure.

4. The flight deprived wild type Berlin (*WT BLN*) and the flightless mutant *vestigial* on the same genetic background (*vg BLN*) are qualitatively equivalent in their response to ether. Accordingly, neither flight deprivation by amputation of the normally developed wing tips of the wild strains nor the low temperature anaesthesia applied for these purposes can be considered as prerequisites for centrophobia. The ether-induced behavior is essentially invariant to the state of the wings and their sense organs. This is reminiscent of male courtship behavior which is similarly resistant to wing amputation (Cook 1973).

Olfactory attraction in non-etherized flies

The properties of the ether effect in *Drosophila* suggest the use of centrophobia C as a measure of the olfactory responses of non-etherized flies with reference to the responses of etherized flies. This interpretation of centrophobia is converse to the previous definition: The flies in the 'control group' assume the status of test flies while the flies of the 'ether group' have to provide the reference data. In Fig. 6 we used the centrophobia of *Drosophila* to specify the attraction of the odor of 0–3 weeks old banana-yeast samples during the course of fermentation and decomposition. Banana odor is known as an efficient wide-band attrac-

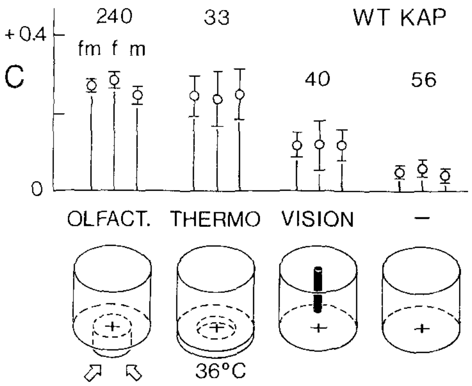

Fig. 7. Ether-induced centrophobia under the influence of different cues for the discrimination of center and surround in the arena. Notation and results of the olfactory tests as in Fig. 5. The options shown in Fig. 2a–d have been used to present the following cues on the circular disk of the arena. From left to right: Olfactory attractant at the center, thermal repellent at the surround, visual attractant at the center, and none of these cues. Centrophobia in *Drosophila* is obviously not olfaction-specific. The etherized flies avoid the center of the arena even in absence of conspicuous sensory support

tant (Otto 1951) comprising at least 183 identified and 167 unidentified volatile substances (Tressl et al. 1969). However, as this odor defies standardization there is a risk of obtaining unreproducible olfactory responses. The regression line of the 139 weighted results in the lower diagram of Fig. 6 coincides with the horizontal direction $C = \text{const}$. As an attractant to *Drosophila*, the banana-yeast odor is obviously invariant to ageing. This statement holds for females as well as males, and has been confirmed also for single strains. The results obtained, so far, seem to justify the use of non-standardized odor in the present experiments.

Is 'centrophobia' olfaction-specific?

If ether-induced centrophobia were sufficiently explained by irreversible interactions with chemosensory centers, it would probably be best to try to elucidate this effect with appropriate electrophysiological methods (Boeckh 1962; Kaissling 1971, 1977; Venard and Pichon 1981, 1984). However, the action of ether in *Drosophila* is not restricted to the olfactory system. This is shown by modifying the experimental design according to Fig. 2a–d. The results in Fig. 7 were obtained with *WT KAP* flies of either sex. The data on olfaction correspond to Fig. 5. The effect of the *attractant* in the central compartment of the disk (Fig. 2a) can be produced also by a temperature gradient (Fig. 2b) between center (27 °C) and surround (36 °C) which is *repellent* to the flies at the periphery of the disk. A dark object suspended in the middle of a brightly illuminated arena (Fig. 2c) prompts the flies to commute, more frequently, between center and surround. The alternating attraction and repulsion exerted by the visual object reflects the fixation-antifixation dichotomy in *Drosophila* (Götz 1980; Bülthoff et al. 1982). Centrophobia is still distinctly present under these conditions. Surprisingly, a small but significant centrophobia ($C = 0.054 \pm 0.015$ SEM, $P < 4 \times 10^{-4}$) remains even in the absence of conspicuous sensory cues for the discrimination of center and surround (Fig. 2d). To remove possible scent marks the disk was made of glass that could be thoroughly rinsed between subsequent tests. As this procedure did not noticeably diminish the effect, the remaining centrophobia cannot be attributed to residual chemosensory cues. The result of this experiment defies simple explanations.

Could it be that ether treatment of the test flies has induced the attraction to specific signals from the periphery of the disk? To answer this question, the water filled moat of the experiment of Fig. 2d was replaced by an invisible annular heat barrier of about 57 °C. This increases the fluctuation of the mean radial distances. However, the centrophobia $C = 0.033 \pm 0.018$ SEM, $P < 0.04$, derived from the tracks of 50 flies of the strain *WT KAP* seems to conform with the results obtained in the presence of rim-specific signals. In a converse experiment, the disk of Fig. 2d was covered by rough-textured plain wall paper. On its way across this substrate the test fly encounters, randomly on either side, numerous projections of surface. Such encounters raise the level of optomechanical stimulation. Concurrently, the visual and tactile discontinuities of the rim should become less conspicuous, and less attractive, to the fly. The centrophobia $C = 0.081 \pm 0.016$ SEM, $P < 2 \times 10^{-4}$, derived from the tracks of 12 females of the strain *vg BLN* is not reduced under these conditions. Accordingly, centrophobia does not seem to result from ether-induced attraction to either the humidity of the moat or the visual and tactile discontinuities of its rim.

Is 'centrophobia' ether specific?

To answer this question, 65 flies of the flightless mutant *vg BLN* were divided into four different anaesthesia groups and one control group. The anaesthetics were applied at the age of about 2.7 d. The application was repeated up to three times in order to obtain similar cumulative half times of narcosis $t_{1/2}$ for the ether group (25 min), the CO_2-group (28 min), the N_2-group (27 min) and

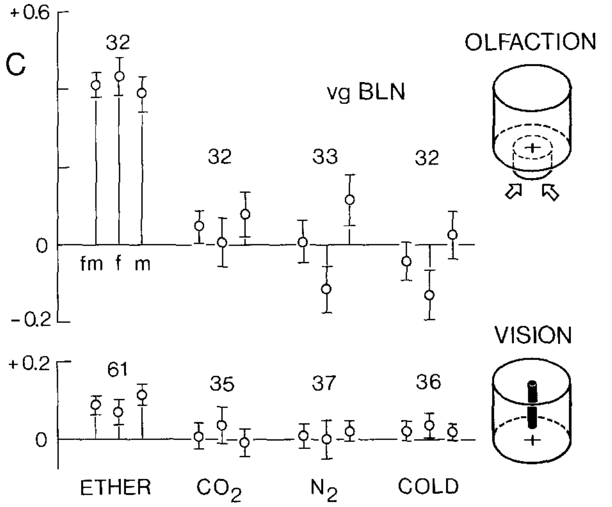

Fig. 8. Substitution of ether by the anaesthetics CO_2, N_2 or cold in centrophobia experiments with the flightless mutant *vestigial*. This mutant was chosen to avoid cooling required for flight-preventing wing operation in normal flies. The cumulative half time of undisturbed narcosis of the anaesthesia groups in each of the four experiments was about 26 min. Attractant at the center of the arena was either an olfactory cue (upper diagram) or a visual object (lower diagram). Notation and results of the upper left experiments as in Fig. 5. Particular properties of ether narcosis are required to elicit centrophobia in *Drosophila*

the 'cold'-group (25 min). Each of the flies was tested under olfactory stimulation (Fig. 2a), and under visual stimulation (Fig. 2c).

The results in Fig. 8 were derived from two complete sets of trials made with different flies and on different post-narcotic days. The corresponding average time interval between trial and narcosis was $t_{T-N} = 2.3$ d and 8.4 d, respectively. No significant differences were found between the results of the two sets of trials. Of the anaesthetics tested in these experiments ether is unique in its ability to induce centrophobia in *Drosophila*. There is no hint of similar effects in the groups of flies treated with CO_2, N_2 and cold.

Dosage effect of ether

The dosage of ether is characterized by the half time of narcosis $t_{1/2}$. This is the time after which one half of the flies of an ether group have recovered, spontaneously, from anaesthesia. Under normal conditions, undisturbed narcosis lasts for about 20–30 min, or up to twice the time required under continuous agitation. A time controlled vapor dispenser allowed us to expand the range of possible half times to 5 min $\leq t_{1/2} \leq$ 58 min. All of the flies of the different ether groups recovered within a time interval between 0.8 $t_{1/2}$ and 1.2 $t_{1/2}$.

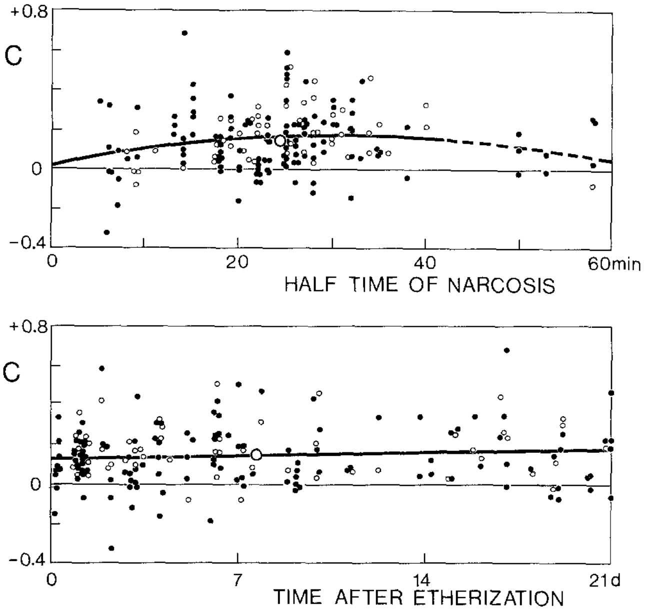

Fig. 9. Induction and retention of centrophobia in *Drosophila*. Each of the diagrams comprises the available results from 1661 flies of different origin. Data represent 178 means of *C*, derived from identically treated groups of etherized and non-etherized females (filled circles) or males (open circles). Regression curves and large circles indicate, respectively, the trend of *C* and the weighted average of the means. Average standard error of the means is ±0.06 units of *C*. To show the dose effect of ether, the results are plotted against the half time of undisturbed narcosis $t_{1/2}$ (upper diagram). To show time course of retention, the same results are given as a function of the time interval between trial and narcosis $t_{T\text{-}N}$ (lower diagram). The lowest narcotic dose of ether can be sufficient to induce sizeable centrophobia in either sex. Once induced, the effect lasts apparently undiminished for the life time of the flies

The upper diagram in Fig. 9 summarizes the effect of dosage on the centrophobia of *Drosophila* as obtained from the experiments in Figs. 5, 7, 8 and 10 including 2 results obtained with an acetylcholinesterase deficient mutant $Ace^{lm\,38}/TM6$ (Greenspan et al. 1980; Hall et al. 1982; Götz and Biesinger 1985). Evaluation of the data leads to the following conclusions:

1. Even the shortest possible narcosis is sufficient to produce, in the experimental paradigm of Fig. 3, significant centrophobia in the order of $C = 0.338 \pm 0.022$ SEM, ($P < 10^{-6}$). Repeated application of a sub-narcotic dosage may amount to ether uptake beyond the threshold of centrophobia. However, the absence of narcosis seems to prevent the induction of the ether effect. This is demonstrated by the abnormally low centrophobia $C = -0.107 \pm 0.056$ SEM in a test group of 17 *WT KAP* females which received, four times in 15 min, the maximum sub-narcotic dosage of ether.

2. Linear regression of the weighted means of

the centrophobia of females as well as males does not show significant linear trends of C in the given range of narcosis. However, the ether effect is not necessarily invariant to dosage. Nonlinear regression seems to indicate an optimum of dosage which corresponds to undisturbed narcosis of about 30 min in females as well as males.

3. The highest doses of ether used in the present experiments were still far below the lethal quantity: without exception, the flies survived this treatment and recovered to seemingly normal locomotion.

4. The centrophobia of the 'average fly' is essentially independent of age at the time of etherization (t_{N-H}). The impressively increased ether resistance during early adulthood (Ogaki et al. 1967; Deery and Parsons 1972) does not seem to interfere with the induction of the ether effect at this age.

Retention of 'centrophobia'

A remarkable property of centrophobia is its persistence in time. The lower diagram in Fig. 9 summarizes the results of the retention tests. The slightly positive time course of the regression line shows consolidation rather than relaxation of centrophobia in a fly representing the average behavior of the different strains. Extension of the statistical analysis to the responses D, D_O, and C of the 'average fly' of either sex has not revealed any significant trend with respect to age ($t_{T\text{-}H}$), time after etherization ($t_{T\text{-}N}$), time after wing amputation ($t_{T\text{-}A}$), starvation-time ($t_{T\text{-}D}$), age at the time of etherization ($t_{N\text{-}H}$) and age at the time of amputation ($t_{A\text{-}H}$), respectively. The centrophobia of the 'average fly' is essentially invariant to the different parameters of time. Evaluation of the present results suggests the following:

1. The longevity of centrophobia and the failure to extinguish this effect with shocks such as cooling, wing amputation and food deprivation point to irreversible effects of ether which cannot be remedied by the fly. Once induced the effect lasts undiminished for at least three weeks, and therefore most probably for the life time of the fly.

2. Centrophobia in *Drosophila* seems to be completely developed in less than 8 h after etherization. The temporal limit for the development of the ether effect has been further reduced to less than 30 min on account of the sizeable centrophobia $C = 0.387 \pm 0.051$ SEM observed immediately after narcosis in a test group of 6 *WT KAP* females. Transition to centrophobia is apparently too fast to be explained by an ordinary process of irreversible cell degeneration.

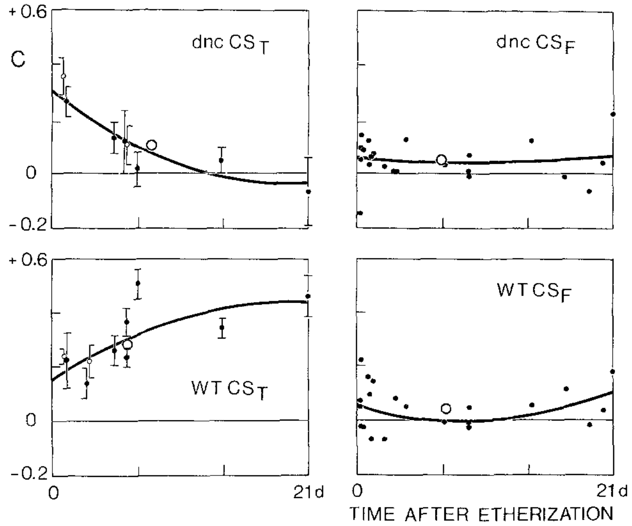

Fig. 10. Retention of centrophobia in sub-strains of the learning mutant *dunce* (upper diagrams) and of the corresponding wild type 'Canton S' (lower diagrams). Each of the diagrams represents a fraction of data shown in the lower Fig. 9. Notation, time scale, regression curves and averages of the means as in this figure. Average standard error of the means drawn without error bars is ±0.05 units of C. Females (filled circles) were used in 96% of the trials. Diagrams on the left show the trend of C in the sub-strains held in Tübingen: Relaxation of centrophobia in the mutant *dnc* CS$_T$ contrasts markedly with consolidation in the corresponding wild type *WT CS$_T$*. The diagrams on the right refer to the sub-strains from Freiburg: The data show partial suppression of centrophobia both in the mutant *dnc CS$_F$* and in the wild type *WT CS$_F$*. The anomalies of C can be explained by the abnormally enhanced spontaneous centrophobism of the control flies. The induced centrophobism of the ether flies is essentially normal in all of the strains tested so far

Anomalous retention of 'centrophobia' in the mutant dunce

The usual association of 'retention' with properties of learning and memory is certainly not justified in the case of induced centrophobia. However, physiological conditions which contribute to the 'leakyness' of memory might as well contribute to the decay of an acquired state of centrophobia. To investigate this conjecture we used the cAMP-phosphodiesterase II-deficient mutant *dunce E* from S. Benzer's laboratory which has been selected because of its low performance in olfactory and chemosensory learning (Quinn et al. 1974; Dudai et al. 1976; Dudai 1979; Byers 1980; Byers et al. 1981; Kauvar 1982). The mutation is held on its original genetic background Canton-S in a number of local sub-strains. The present experiments refer to the tracks of 68 females and 4 males of sub-strain 'Tübingen' (*dnc CS$_T$*) as well as 216 females of sub-strain 'Freiburg' (*dnc CS$_F$*). Figure 10 shows the results obtained with groups of identically treated flies from the sub-strains of *dunce* and the corresponding wild type. As before, the groups are used only once in a test, and up to three weeks after etherization. Comparison of the upper diagrams shows a significant quantitative difference between the means of the centrophobia *C* of *dnc* CS_T (0.105 ± 0.028 SEM) and *dnc* CS_F (0.059 ± 0.015 SEM). A difference in at least the same order of magnitude results from the corresponding data of the wild type sub-strains in the lower diagrams which have been mentioned earlier in this paper.

The most conspicuous result of Fig. 10 is the comparatively fast relaxation of the centrophobia of *dnc* CS_T with a half time of less than 5 days. The decline is highly significant ($P < 10^{-8}$), and is qualitatively different from the consolidation of centrophobia in the corresponding wild type *WT* CS_T as well as in all but one of the strains tested so far. The relaxation found in this particular strain, *vestigial* (*vg BLN*), is less significant ($P < 10^{-3}$) and the half time of centrophobia of either sex is still about 3 times longer than in *dnc* CS_T.

In view of the inhomogeneous results obtained from closely related sub-strains it appears premature to link the decline of centrophobia to the specific deficiencies of retention in the memory of the mutant *dunce*.

Centrophobism without ether

The mean radial distance D_O of the non-etherized flies of a centrophobia experiment is comparatively small if these flies react to attractants in the center of the arena. Most of the reactions to banana odor or tolerable floor temperature or a visual target last for the time period of our standard test of 200 s duration. However, occasional excursions towards the periphery of the arena become increasingly frequent if the test is continued: Accommodation to the conditions in the arena seems to convert the locomotor behavior of non-etherized flies into the behavior of etherized flies. The corresponding transition $D_O \rightarrow D$ of the mean radial distance diminishes the effect of ether-induced centrophobia $C = (D - D_O)/(D + D_O)$. Estimates of the average time constant of this process in 9 different strains are in the order of 500 s. The exceptionally low time constants of < 200 s found in non-etherized flies of the mutant *dnc* CS_F ($P < 0.004$) and the corresponding wild type *WT* CS_F ($P < 0.001$) may explain the absence of sizeable ether effects in the experiments on the right of Fig. 10.

Is the accommodation of non-etherized flies to the conditions in the arena due to the use of scent marks? Scrutiny of the records of numerous tracks suggest the use of scent marks in *Drosophila*. However, removal of the traces at the end of the test did not seem to reset the time course of accommodation.

Does adaptation to the attractants in the center of the arena explain the accommodation effect? Evidence against this conjecture comes from the responses of stationarily reacting flies in experiments on osmotropotaxis (Borst and Heisenberg 1982), heat avoidance (Götz, unpublished) and fixation of a visual target (Götz 1983), respectively. The responses of these flies persist for hours. During a test interval of 200 s significant adaptation is likely to occur neither at the level of signal reception nor at the level of signal perception.

A spontaneous increase of the mean radial distance of non-etherized flies has to be considered also in connection with the anomalous retention of centrophobia in the mutant *dnc* CS_T and *vg BLN*. The decrement of *C* with time after narcosis in the upper left diagram of Fig. 10 could be due to either anomalous relaxation of the ether effect ($D \rightarrow D_O$), or spontaneous centrophobia ($D_O \rightarrow D$), or both simultaneously. Partition of the decrement of *C* into its constituents shows that the etherized mutants do not actually regain their previous behavior. About 60% of the decrement in the two mutants result from a gradual increase of distance D_O in the non-etherized flies. Only about 40% of the decrement are probably due to a gradual decrease of distance *D* in the etherized flies. This decrease is significant only in the mutant *vg BLN* ($P < 0.004$). The retention of the ether effect is not significantly disabled by the hereditary defects of the mutant *dnc* CS_T.

Discussion

Judging from the ubiquity of diethylether as the preferred anaesthetic in *Drosophila* research it is not easy to understand why ether-induced centrophobism apparently escaped earlier attention. Two possible reasons deserve to be mentioned:

1. The phenomenological similarity between ether-induced and spontaneous centrophobism, both noticeable by an increase of the corresponding mean radial distances (D, D_O) beyond the expected distance $\bar{D}$ in the case of equidistributed probability of position on a circular disk. In a very long test of more than 200 s duration the ether effect may be masked by the effect of spontaneous locomotor accommodation to the arena. A com-

mon physiological basis of these effects is likely to exist, but remains to be found.

2. The irreversibility of the aftereffect of ether narcosis in all of the strains which have been tested so far. Much if not all of the induced centrophobism is retained, for the rest of the life, even in strains where decline (*dnc* CS_T, *vg BLN*) or low level (*dnc* CS_F, *WT* CS_F) of centrophobia *C* suggest the opposite. (The anomalous properties of *C* in the strains of Fig. 10 are actually due to increased spontaneous centrophobism in the non-etherized controls.) Irreversibility of the change in behavior may have prevented the detection of induced centrophobism in etherized flies. The spontaneous centrophobism in non-etherized flies does not seem to be irreversible: The centrophobism acquired during gradual accommodation to the arena is not retained after prolonged interruption of the experiment.

So far, centrophobism has been described as a new trait of search on the platform of the arena. However, the underlying change in the behavior of *Drosophila* is likely to appear in other experimental situations as well. The recommendation of ether treatment in one such test (Ringo 1971) deserves caution and should not be generalized: A few seconds of exposure to ether vapor during the past may reliably suppress, in the freely walking fly, the responses to olfactory, thermal or visual attractants or repellents. Ether uptake weeks before the experiment, and far below a hazardous dosage, sufficiently explains the striking extinction of response in a classical olfaction test (Otto 1951).

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