

EFFECTS OF AGING ON INHIBITORY LEARNING AND SHORT-TERM MEMORY IN *DROSOPHILA MELANOGASTER*

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ABSTRACT: Two experiments have been performed in young (7 days old), middle-aged (28 days old) and old (49 days old) *Drosophila melanogaster*. In Experiment 1, the inhibitory conditioning of the Proboscis Extension Response (PER) to sucrose was displayed under three Inter-Trial-Interval (ITI) schedules: 1, 2 or 4 minutes. The results did not reveal any age-related impairment of short-term-memory. The PER suppression performance was higher in middle-aged and old flies than in young ones, whatever the ITI. In Experiment 2, the habituation of the PER to sucrose was induced to investigate the hypothesis of an age-related increase of the non associative processes involvement (sensory adaptation, motor fatigue) in the PER suppression. The results showed that once such peripheral effects were removed, suppression performances no longer varied with age.

RESUME: Deux expériences ont été réalisées chez des drosophiles jeunes (7 jours), d'âge moyen (28 jours) et âgées (49 jours). Dans l'expérience 1, l'inhibition conditionnée de la Réponse d'Extrusion du Proboscis (REP) au sucre a été étudiée sous trois conditions expérimentales différant par la durée de l'Intervalle Inter-Essais (ITI): 1, 2 ou 4 minutes. Les résultats ne révèlent pas d'atteinte liée à l'âge de la mémoire à court terme. Les performances de suppression de la REP sont plus élevées chez les mouches d'âge intermédiaire et âgées que chez les jeunes, quel que soit l'ITI. Dans l'expérience 2, l'habituation de la REP au sucre a été étudiée pour tester l'hypothèse d'une augmentation liée à l'âge de l'implication de processus non-associatifs (adaptation sensorielle, fatigue motrice) dans la suppression de la REP. Les résultats montrent que lorsque de tels effets périphériques ne sont pas en jeu, les performances de suppression ne varient plus avec l'âge.

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Over the last few decades, *Drosophila* has been one of the principal invertebrate model in the experimental gerontology area. Although mammals provide biological models closer to human beings, their use encounters some sensitive variables (motivation level, psychomotor abilities) which make the study of age-linked variations of biological or behavioral traits more difficult. The *Drosophila* fruitfly offers several major advantages as an experimental model since the generation time is short, the reproductive capacity is high and its genetic, physiological and biochemical characteristics are well-known. Especially in experimental gerontology research, the main practical benefit of the use of *Drosophila* lies in its short life duration which remains below three months with the standardized rearing-temperature of 25°C.

Indeed, many studies dealing with the age-linked changes at different levels of organization (molecular, cellular, physiological) have already been performed on *Drosophila*. We already know that the biological characters related to fitness (viability, reproductive activity) decline with age (Review in Boulétreau-Merle, 1988), as well as some behavioral capabilities, such as the spontaneous locomotor activity rate or the ability to climb up a vertical surface (Review in Le Bourg et al., 1993). Some studies have also been devoted to the search of possible age-linked changes in learning abilities. A single experimental situation has been used until now, based upon the conditioning suppression of the Proboscis Extension Response (PER) to sucrose. The authors (Brigui et al., 1990; Fresquet and Médioni, 1993) showed that the ability to suppress the PER, and so to reach a learning criterion, decreased after a middle-age (28 days old). Moreover, in a more simple learning situation, such as the habituation of the PER to sucrose, where no associative component is involved, the acquisition is delayed as well after middle age (Fois et al., 1991). Taken together, these results suggest that aging specifically impairs the central inhibitory abilities, making middle-aged and old flies less able to suppress an unconditioned response, whereas associative capacities might be preserved from major aging damages.

The age-linked deterioration of inhibitory capacity has also been reported in rodents, where aged animals have greater difficulty suppressing a response in passive avoidance learning situations (Lamberty and Gower, 1990; Fagioli et al., 1992). Even in humans, the "behavioral rigidity" often associated with aging, consisting in an increasing inability to modify previously learned or habitual behavioral patterns, could be ascribable to a lack of inhibition (Dean and Bartus,

1988).

Therefore, age-related damages in central inhibitory mechanisms seem to be wide-spread across species. Likewise are age-related impairments in Short-Term-Memory (STM) often observed. By using a multi-trial maze learning tasks where the Inter-Trial-Interval (ITI) duration can be lengthened, some studies in rats (Soffié and Giurgea, 1988; Dells et al., 1992) showed that the longer was the ITI, the slower did old animals learn.

The purpose of this *Drosophila* study is to find out whether any age-related change may occur in Short-Term-Memory. A decline could be inferred if the acquisition levels reached by old flies decrease as the ITI duration increases, whereas the performance of younger flies remains little or none variable. By using the same inhibitory conditioning as previously done (Brigui et al., 1990; Fresquet and Médioni, 1993), we are able to vary the ITI and so, to compare the performance reached by several age-groups at different ITI schedules. Our learning procedure is an anterograd pavlovian conditioning (Médioni and Vaysse, 1975) based upon the systematic association between the Unconditioned Stimulus (US - Sucrose) and a negative reinforcer (Quinine). The US induces the PER while the quinine acts right after as the punishment of the response releasing. The conditioned inhibition of the PER to sucrose improves over trials as the learning of the association between the US and the negative reinforcer develops.

The previous studies (Brigui et al., 1990; Fresquet and Médioni, 1993) showed that middle-aged (28 days old) and old flies (49 days old) have greater difficulty learning the conditioned inhibition to sucrose, compared to young flies (7 days old). Either the acquisition speed, measured as the number of trials needed to reach an acquisition criterion, or the final acquisition level, measured as the total number of PER suppressions, decreases after middle-age and no longer changes to the age of 49 days old. In the present experiment, the learning of the conditioned inhibition will be tested at various ITI durations and an aging effect on STM may be revealed if the differences in the level of acquisition attained by young (7 ± 2 days old), middle-aged (28 ± 2 days old) and old flies (49 ± 2 days old) increase as the ITI lengthens.

In a second experiment, the non-associative learning of the habituation of the PER to sucrose will be used to assess the possible role of various peripheral mechanisms (sensory adaptation, motor fatigue...) in the PER suppression. Experiment 2 will be designed to determine whether non-associative processes become more important with age.

EXPERIMENT 1 - METHODS

Subjects and apparatus

Adult *Drosophila melanogaster* males of the wild-strain "Meyzieu" (France) were maintained by mass-mating on a standard nutritive medium (agar, sugar, corn-meal and killed yeast) enriched with live yeast. Eggs from parents 4-5 days old, laid for approximately 15 hours, were set in batches of 25 into 80-ml vials supplied with 15 ml of the usual medium and live yeast. After 9-10 days of development, virgin males were transferred after etherization, in batches of 15, to vials containing the S.101 synthetic medium of Pearl et al. (1926) enriched with live yeast and renewed twice a week. Flies were reared in the experimental room under controlled temperature ($25\pm 0.5^{\circ}\text{C}$), relative humidity ($85\pm 5\%$) and photoperiod (L:D = 12:12; 250 lx).

Under such rearing conditions, no significant mortality (lower than 5%) was observed until 30 days old and plotting the longevity curve (see Figure 1) reflects a normal aging pattern in the sample (rectangular shaped curve), excluding accidental or premature deaths. In males, the mean longevity (50% of the sample still alive) is 49.26 ± 1.01 days and the maximum longevity is 73 days ($n = 150$).

In each experiment, three age groups were compared: young (7 ± 2 days old), middle-aged (28 ± 2 days old) and old (49 ± 2 days old). Any variation detected in the characters under study between young and middle-aged flies could be attributed to a true aging effect, since no significant differences in mortality rate were observed between these two ages. By contrast, any change observed between young or middle-aged flies and old ones might be related either to a true aging effect, or to a selective mortality linked to the behavior under study (see Figure 1).

The conditioning device had been described in detail by Médioni and Vaysse (1975). One fly was tested at a time, walking at a fixed point on a dark-shaded revolving drum (4 mm/s) continuously rinsed with distilled water. The black pathway was interrupted at six equidistant points where white rectangular areas of Whatman® filter paper soaked in stimulating solutions were laid down. When the fly walked across the filter-paper, the solutions were detected by its tarsal chemoreceptors, so releasing the PER. The solutions remained out of reach of the proboscis and could not be ingested. The fly being trained was observed through a stereomicroscope ($\times 25$) and any proboscis extension, whether complete or not, was scored as a response.

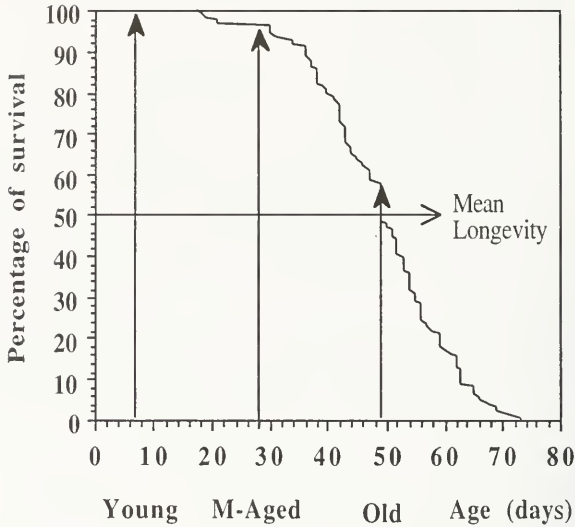


Figure 1. Longevity curve of the males of the wild-strain Meyzieu reared at 25°C. The correspondence between the rate of mortality and the age-group tested is shown.

Procedure

Under weak ether anesthesia, experimental flies were affixed by the notum to the tip of a needle with a droplet of synthetic varnish. The needles were then stored within a closed moistened box (100% Relative Humidity) where flies underwent an hydric diet for an average of forty five hours (see Results Section). This deprivation period was required for the PER to be unconditionally released by sucrose.

Thereafter, the response threshold to sucrose was individually determined with a procedure derived from the psychophysical method of increasing intensity of stimuli. Each fly was stimulated with an ascending scale of sucrose molar solutions (1/512 mol, 1/256 mol, 1/128 mol, 1/64 mol, 1/32 mol, 1/16 mol, 1/8 mol). The range of effective concentrations was first determined roughly by using a two-step increase between two consecutive stimulations. Two trials per concentration were given at a 1-min inter-trial-interval if either no PER or two PER were observed, but three trials if only one response was released. The threshold was considered as crossed when two responses occurred. Then the just lower concentration, which was omitted in the two-step increase, was tested and considered as the threshold value if 2 PER were still observed. The flies which responded to the minimal dilution 1/512 mol were rejected because their threshold remained

unknown ($\leq 1/512$ mol) and their physiological state was usually poor. Otherwise, a sucrose solution eightfold more concentrated than that of the individual threshold was used during the training session. Hence, flies which did not respond to the $1/8$ mol dilution were discarded because the 1 mol value was the highest usable sucrose solution. With such a procedure, using a threshold constant multiple, all the experimental subjects could be trained with a PER releaser of approximately the same effectiveness, if not of equal physical intensity.

Pre-training: One minute after the response threshold determination, each fly was submitted to four pre-training trials (1 min ITI), each including a sucrose stimulation immediately followed (within 2.5 s) by a distilled water neutral stimulation (Médioni et al., 1978). Flies which failed to respond to each pre-training trial, as well as subsequently to the first training-trial, were discarded. This preliminary stage allowed us to reject animals precociously affected by non-associative processes (sensory adaptation, motor fatigue, habituation to sucrose) leading to the disappearance of the PER to sucrose.

Training: One minute after completing the pre-training, the learning session started for 24 acquisition trials arbitrarily defined as 6 consecutive 4-trial blocks. For each trial, the fly was walking at a fixed-point on the humid pathway for approximately 30 seconds as the drum turned and then it was stopped for a rest interval until the next trial. At half-way, the fly crossed a paper soaked in a sucrose solution and right after (within 2.5 seconds) another one soaked in a 10^{-1} mol quinine chlorhydrate solution (Negative Reinforcer).

The conditioning was displayed according to three acquisition schedules differing by the ITI duration: 1, 2 or 4 minutes. Note however that the duration of the rest period between two consecutive trials varied accordingly but not the fixed-point walking time.

Sixteen young (7 ± 2 days old), middle-aged (28 ± 2 days old) and old flies (49 ± 2 days old) were tested in each acquisition schedule (three ITI), giving nine experimental groups.

The individual acquisition performance of the conditioned inhibition was the number of PER suppressions recorded either at the end of the 24 training trials, or for each 4-trial block.

RESULTS

Duration of deprivation and threshold to sucrose

The deprivation times required to meet the threshold criteria varied with age: $F(2, 141) = 15.82, p < 0.0001$. Young flies had to be deprived longer than middle-aged and old ones: 49.69 hr (± 0.45 SE), 41.01 hr (± 1.34 SE) and 42.48 hr (± 1.44 SE) respectively. This result showed that the sensitivity to inanition increased noticeably with age, being most probably linked to the general physiological state.

The response threshold to sucrose also varied with age: $F(2, 141) = 10.12, p < 0.0001$. The mean threshold value was close to 1/32 mol in 7-day-old flies and increased to 1/16 mol in older flies. This age-related decrease in reactivity to sucrose might be due to the loss of some gustative tarsal chemoreceptors, as suggested by Stoffolano (1975) in the blowfly *Phormia regina*. Consequently, the proportion of flies discarded for a threshold to sucrose that was too high ($> 1/8$ mol) increased with age: $\chi^2(2) = 33.80, p < 0.0005$, whereas the number of flies discarded for a threshold that was too low ($\leq 1/512$ mol) decreased with age: $\chi^2(2) = 11.66, p < 0.001$ (see Table 1). Note however that these age-related differences in the reactivity to sucrose were eliminated during the training by stimulating all the flies with a constant multiple of their threshold.

The threshold values (mmol) and the fasting durations were not correlated at any age: young group, $r(48) = -0.14, ns$; middle-aged group, $r(48) = +0.13, ns$; old group, $r(48) = +0.05, ns$.

The number of flies discarded for failing to respond at each pre-training trial was very low whatever the age (see Table 1).

Table 1. Number of discarded flies before the training in Experiment 1.

Criterion of elimination	Age (in days)		
	7	28	49
Threshold $> 1/8$ mol	38	68	167
Threshold $\leq 1/512$ mol	23	8	5
Pre-training selection	2	1	1

Suppression performance

We have verified first at the individual level that the conditioning

scores were neither correlated with the fasting durations nor with the threshold values. A three-way analysis of variance with two main factors (Age and ITI) and one repeated factor (Trials) showed that both Age and Trials had significant effects on the number of PER suppressions: for the Age effect, $F(2, 135) = 10.69$, $p < 0.0001$, and for the Trials effect, $F(5, 675) = 27.04$, $p < 0.0001$. The interactions between Age and Trials on the one hand, ITI and Trials on the other hand, also had significant effects on the number of suppressions: for the Age \times Trials interaction, $F(10, 675) = 6.31$, $p < 0.0001$, and for the Trials \times ITI interaction, $F(10, 675) = 1.99$, $p < 0.04$.

This analysis revealed that the suppression performance increased with age, whatever the ITI duration. Even though a decrease might be noticed in the final acquisition level reached by 49 days old flies under the 4 minutes ITI schedule, such difference was not statistically significant (see Figure 2).

On the other hand, the suppression performance improved across trials in the three age-groups while oldest flies remained able to display the highest number of suppressions throughout the training (see Figure 3).

Finally, a deleterious effect of the lengthening of the ITI might be established by analysing the learning curves. Indeed, the acquisition was delayed, in the three age-groups, when the ITI reached 4 minutes (see Figure 4).

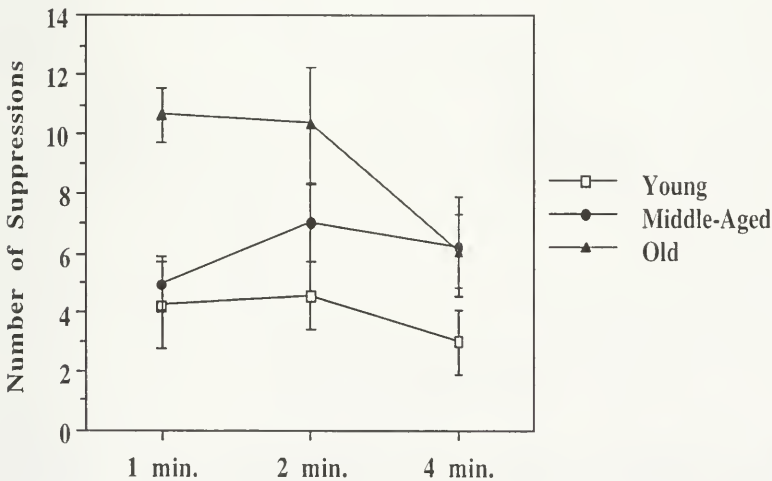


Figure 2. Experiment 1: Mean (and SE) acquisition performance as a function of age and Inter-Trial-Interval (abscissae), at the end of the training.

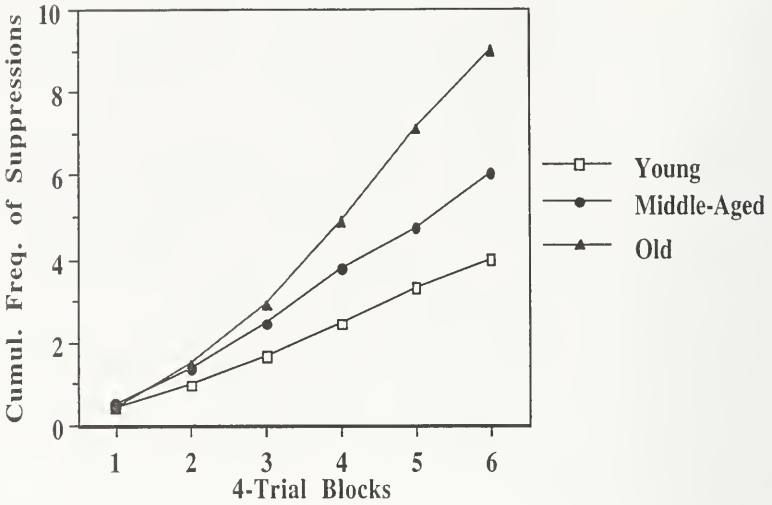


Figure 3. Experiment 1: Cumulative frequency of suppressions all along the 24 training trials, as a function of age.

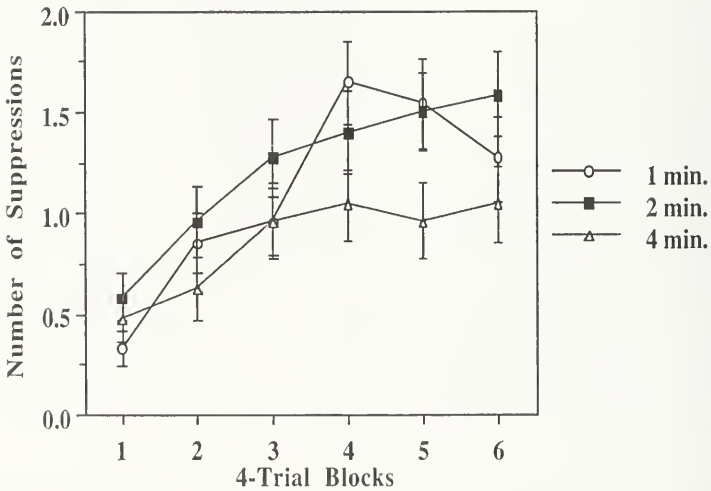


Figure 4. Experiment 1: Mean (and SE) acquisition performance all along the 24 training trials as a function of Inter-Trial-Interval, the three age-groups being pooled.

DISCUSSION

The results of this experiment are quite surprising, mainly due to the age-linked increase in the acquisition performance. Such an age-related variation has not before been reported, as for example in Brigui et al. (1990) who showed in middle-aged and old flies a greater difficulty in acquiring the inhibitory conditioning. Also, an age-related decrease in the ability to suppress the PER to sucrose was stated either in a visual discrimination inhibitory conditioning (Fresquet and Médioni, 1993) or in habituation learning (Fois et al., 1991). These previous experiments were all performed thru the same experimental procedure, including first a determination of the threshold to sucrose then a pre-training, prior to the learning session. Therefore, in all these studies a certain pourcentage of the initial samples had to be discarded on the basis either of the threshold value or of the pre-training score, as we did in this first experiment. Indeed, from the beginning of Experiment 1, the proportions of flies that were kept until the end of the learning session amounted respectively in the young, middle-aged and old group to: 43.24%, 38.40% and 21.72%. These proportions are very close to the ones reported in Fresquet and Médioni (1993) where the acquisition scores were computed on 47.06% of young flies, 26.66% of middle-aged flies and 23.88% of old flies. Such data were not published in the two other studies (Brigui et al., 1990; Fois et al., 1991) but we may assume that the outcomes are quite the same since the experimental design was not much different. Consequently, the prerequisite selection could not account for the difference between Experiment 1 and the other studies of reference in the variation observed with age.

From the previous experiments dealing with inhibitory conditioning, authors concluded that the central inhibition mechanisms were the target of aging damages, excluding any possible impairment of the associative processes. Indeed, the evaluation of the efficiency of associative and inhibitory processes could be dissociated in the discrimination procedure (Fresquet and Médioni, 1993) and only the latter were impaired with age. Moreover, in the habituation task (Fois et al., 1991) where no associative component was involved, the age-related decline in the suppression ability was observed again from a middle age.

The purpose of Experiment 1 was as well to investigate a possible age-related change in short-term memory. One might indeed expect, if any impairment in short-term memory occurs with age, that the age-

related differences in acquisition will increase with the lengthening of the ITI. No such result was obtained since neither the duration of the ITI itself, nor the ITI by Age interaction, had a significant effect on the acquisition levels. Even though the performance level reached by the oldest group decreased noticeably with the 4-min ITI, it remained higher, at least, than the mean performance level of youngest animals.

However, a damaging effect of the lengthening of the ITI was revealed by analysing the course of acquisition, which was delayed under the 4-min ITI. Since the speed of acquisition was decreased to the same extent in the three age-groups under this time-condition, no age-related differences in the efficiency of memory could be put forward. Otherwise, it is worth noting that the acquisition curve is the most regular and reaches its highest value with the 2-min ITI whatever the age. These observations may remind the studies made by Jost (1897) and Piéron (1909) on habituation in invertebrates, showing that optimum intervals allow more rapid acquisition and better retention, whereas shorter or longer ITI yield a decrease in these performances. We thus would assume that the 2-min ITI is more convenient for acquiring such inhibitory conditioning when compared to a shorter (1-min) or a longer (4-min) interval. Indeed, Médioni (1986) had previously reported higher suppression performances in young (7 days old) *Drosophila* tested with a 2-min ITI compared to 1 minute.

EXPERIMENT 2

Experiment 2 was undertaken to test whether peripheral, uncontrolled processes may have influenced the age-linked increase in the number of suppressions previously reported in Experiment 1. For that purpose, the three age-groups were tested in the habituation of the PER to sucrose as following a procedure which included single sucrose stimulations (not associated with a negative reinforcer).

Habituation may be defined as the waning of the PER to the monotonous repetition of the sucrose stimulation. However, once the PER is no longer released after a variable amount of training, one still needed to establish the nature of the mechanisms underlying the disappearance of the response. Indeed, we made the difference between "true habituation" which referred exclusively to a centrally controlled stoppage of the PER and "pseudo-habituation" which involved peripheric mechanisms such as sensory adaptation to sucrose or motor fatigue of the proboscidian effectors. Previous studies in *Drosophila*

have indeed demonstrated the central origin of habituation process. Duerr and Quinn (1982) induced habituation by applying sucrose stimulations to the right prothoracic tarsus and showed that responsiveness was depressed to subsequent stimulations to the left prothoracic tarsus for at least 10 minutes. These authors affirmed that the interaction between stimuli to contralateral legs took place centrally. Thereafter, Bouhouche et al. (1993) used an habituation procedure based on ipsi- and contralateral sucrose stimulations to compare wild-type and mutants *Drosophila*, these latter being characterized by a disorganized protocerebral bridge and a partial loss of the fibers connecting the central complex with the central brain. After having checked that motor activity and sucrose reactivity were normal in mutants, authors reported abnormally low habituation scores. These results were interpreted through a defect in processing the information between the two brain hemispheres and such a reduced inter-hemispheric communication impaired the ability to inhibit the PER when stimulations were unilaterally applied.

No such habituation procedure involving unilateral stimulations was used in Experiment 2 but instead, a dishabituation test was performed at the end of the training. The presentation of a dishabituating stimulus (yeast smell) simultaneously with the sucrose stimulation disclosed the origin of the disappearance of the PER during training: its re-appearance in the dishabituation test revealed the involvement of central processes, mediating a "true habituation" learning; if the PER was not restored during the dishabituation test, then we could reliably infer that peripheral mechanisms were mainly involved in the PER disappearance and that animals were just no longer able to display a proboscis extension ("pseudo-habituation"). Such peripheral mechanisms could be sensory adaptation to sugar and/or a muscular fatigue of the proboscidian effectors.

The comparison, across the three age-groups, of the respective proportions of truly habituated vs. pseudo-habituated flies has permitted ascertaining whether the role of peripheral mechanisms in the PER suppressions became more important with age. If such a positive relation could be established, then the higher numbers of suppressions reported with age in Experiment 1 might not reveal a higher efficiency of the inhibitory abilities.

METHOD

Subjects and apparatus

The experimental subjects came from the same wild-strain (Meyzieu) and were reared in the same conditions as in Experiment 1. Also, same apparatus was used.

Procedure

Threshold determination: After the deprivation period was completed, the individual threshold to sucrose was measured by using a simpler procedure derived from that of Experiment 1. It was still based upon the psychophysical method of increasing intensity of stimuli except each sucrose concentration was tested only once in order to minimize the number of pre-exposures to this stimulus. Subsequently, the sucrose concentration used during training was only twofold higher than the individual threshold, in agreement with one of the parametric characteristics of habituation learning defined by Thompson and Spencer (1966): "the weaker the stimulus, the more rapid and/or more pronounced is habituation. Strong stimuli may yield no significant habituation".

Therefore, the animals were discarded before training either if they responded to the minimal concentration tested (1/512 mol) because their physiological state was usually poor and their threshold remained unknown, or if they did not respond to the highest concentration (1/2 mol).

Training: One minute after the threshold measurement was completed, the habituation training began for, at most, 32 training trials (i.e. eight four-trial blocks) separated by a 1 minute intertrial-interval. Each trial included a sucrose stimulation followed, within 2.5 seconds, by a distilled water stimulation. The training was stopped as soon as a criterion of acquisition defined as 3 PER-suppressions within a 4-trial block was reached. Flies which failed to reach the acquisition criterion within 32 training trials were considered as non-learners. Otherwise, the dishabituation test was performed one minute after the end of the training. The first dishabituation trial included the sucrose stimulation and a simultaneous diverting event (yeast smell). If the PER was then restored, the fly was considered as "truly habituated" since we might reliably infer a central leading to the suppression of the response. If no PER was observed, a second trial was presented one minute later,

including the single sucrose stimulation, and the fly was considered as truly habituated if the PER was then elicited. If no PER had been released, then the peripheral mechanisms (sensory adaptation, motor fatigue) were believed to have caused the response disappearance during training and no habituation learning was assumed. These flies were designated as "Pseudo-habituated".

The experiment went on until 16 truly habituated young (7 ± 2 days old), middle-aged (28 ± 2 days old) and old flies (49 ± 2 days old) were collected. The experimenter was blind to the age of the fly during experiment.

RESULTS

Duration of deprivation and threshold to sucrose

Twenty-nine to thirty hours of deprivation were needed on the average, in each age-group, to meet the threshold criteria: $F(2, 107) = 2.22$, ns. The durations of deprivation recorded here were shorter than those in Experiment 1 (above forty hours). This difference might be explained by the higher threshold limit accepted in Experiment 2 (until $1/2$ mol against $1/8$ mol in Experiment 1).

The response threshold to sucrose did not vary with age: $F(2, 107) = 1.35$, ns, the mean threshold value being close to $1/8$ mol in the three age-groups. We noticed however a higher proportion of young and old flies discarded for a too high reactivity threshold ($> 1/2$ mol), compared to middle-aged ones: $\chi^2(2) = 11.12$, $p < 0.0001$ (see Table 2).

Table 2. Number of discarded flies before the training in Experiment 2.

Criterion of elimination	Age (in days)		
	7	28	49
Threshold $> 1/2$ mol	33	6	30
Threshold $\leq 1/512$ mol	4	2	5

The absence of age-linked variation in the durations of deprivation required or in the thresholds to sucrose might be related to the use of the simpler threshold measurement (a single trial per concentration) since the more extensive procedures previously used, in Experiment 1 as well as in Brigui et al. (1990), allowed such variation to be reported.

The threshold values (mmol) and the durations of deprivation were

not correlated when the data of the three age-groups were pooled: $r(144) = +0.15$, ns.

Distribution of experimental subjects

Of the 110 flies which completed the experiment, 24 (21.82%) failed to reach the acquisition criterion (see Table 3). The proportions of these non-learners did not differ in age: $\chi^2 = 2.44$, ns. These flies, most probably, were slower to learn the habituation but might not have been affected by peripheral mechanisms since they continued to respond frequently during training.

On the other hand, 38 flies (34.54%) fell into the "pseudo-habituated" category and their proportions in each age-group were not significantly different: $\chi^2 = 3.73$, ns (see Table 3). Even if a weak increase might be noticed in the proportion of oldest pseudo-habituated flies, our result did not allow us to conclude that the involvement of peripheral mechanisms in the PER suppression increased with age.

Table 3. Distribution of the experimental subjects at the end of the training in Experiment 2.

Categories	Age (in days)		
	7	28	49
Habituation	16	16	16
Pseudo-Habituation	9	10	19
Criterion not reached	11	6	7

Habituation performance

A one-way analysis of variance performed on the three truly habituated samples did not reveal an age-linked variation in the number of trials needed to reach the acquisition criterion: $F(2, 45) = 0.46$, ns (see Figure 5).

The habituation performance (number of trials to criterion) was not correlated with the durations of deprivation nor with the threshold values (the data of the three age-groups being pooled).

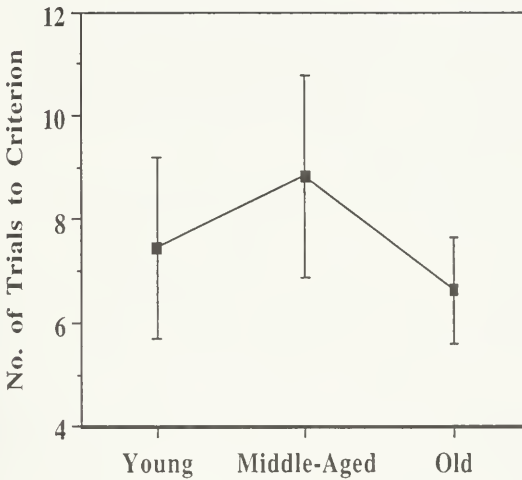


Figure 5. Experiment 2: Mean (and SE) number of trials needed to reach the acquisition criterion in the truly habituated groups, as a function of age.

DISCUSSION

Experiment 2 was undertaken to study the role of peripheral mechanisms (sensory adaptation, motor fatigue) in PER suppression. In consideration of the results of Experiment 1 revealing an age-related increase in the number of PER suppressions, we needed to determine the proportion of suppressions ascribable to central processes vs. that to peripheral ones in order to learn whether these latter might induce an

increasing proportion of suppressions as flies age. The results of Experiment 2 revealed that the number of pseudo-habituated flies did not vary significantly with age, leading us to conclude that peripheral mechanisms are not of greater importance in aged flies. A slight increase however was noticeable in the proportion of oldest pseudo-habituated flies but it does not induce a significant variation on the basis of the samples collected.

Up to now, the only studies dealing with that question in a similar learning situation are from Médioni and Vaysse (1975) and Vaysse and Médioni (1976). These authors established in young flies (7 days old) a ratio of approximately 1 suppression out of 5 ascribable to a sensory adaptation to sucrose, as well as 20% derived from pseudo-conditioning

effects, i.e. from the single repetition of the negative reinforcer unless it was associated with the sucrose stimulation. Ultimately, 40% of suppressions may be related to peripheral factors in young flies. Such a proportion is much too large in itself and would badly affect the results if it were to increase with age.

Finally, Experiment 2 also allowed us to compare the habituation performances across the three age-groups. Even if more simple than the inhibitory conditioning since no associative component is involved, habituation refers to central abilities as cleared from the effects of peripheral mechanisms. No age-linked variation could be stated in the ability to reach the acquisition criterion. This result might be put in relation with an hypothesis of a rising influence of peripheral mechanisms in aged flies because their suppression performances are no longer higher than those of younger flies as soon as the incidence of any peripheral factor is removed. On the other hand, this result is not in accordance with a previous one (Fois et al., 1991) showing a decrease in the habituation speed from a middle age.

GENERAL DISCUSSION

Two experiments have been performed in young, middle-aged and old *Drosophila melanogaster*. Experiment 1 aimed at investigating possible aging effects on short-term-memory abilities required in the inhibitory conditioning acquisition. No significant decrease in performance was produced in aged flies by lengthening the ITI, preventing us from claiming any impairment of short-term-memory. Only a slight decrease was noticeable in the oldest group when the ITI reached 4 minutes, which might let us expect that the use of longer intervals might reveal some memory deficiencies. Damaging effects of aging on memory processes have been reported in mammals either in maze learning (Soffié and Giurgea, 1988; Dellu et al., 1992; Lindner et al., 1992) or in discrimination learning (Goodrick, 1968; Bartus et al., 1978; Cavoy and Delacour, 1993). In these studies, the differences across age-groups in acquisition levels increased as the ITI was lengthened. In invertebrates, a study performed in the nematode *C. elegans* (Beck and Rankin, 1993), based upon a mechanosensory reflex habituation learning, led to opposite results since old animals displayed the higher acquisition rates with the longest ITI.

On the other hand, Experiment 1 revealed an age-linked increase in the number of PER suppressions, whatever the ITI duration. Such a

result was not consistent with the ones of previous studies in *Drosophila* dealing with comparable conditioning procedures (Brigui et al., 1990; Fresquet and Médioni, 1993) where higher suppression performances were reported in young flies. Literature in rodents as well gives evidence of an age-linked impairment of central inhibitory abilities. Indeed, some studies in mice or rats have shown the increased difficulties of old animals to learn a passive avoidance task (Lamberty and Gower, 1990; Fagioli et al., 1992; Mondadori et al., 1992). To try to understand our present results, we then hypothesized of a greater susceptibility in old flies to peripheral mechanisms (sensory adaptation to sucrose, motor fatigue) which might have increased their suppression performances. Thus we designed Experiment 2 to test this hypothesis. The results however were not so easy to interpret since they showed on the one hand that the proportion of pseudo-habituated flies did not significantly increase with age and on the other hand that once the peripheral effects were removed, the suppression performance no longer differed with age. Such a result in the habituation learning is consistent with the one reported in a previous study (Le Bourg, 1983) where young (7-8 days old) and old (41-42 days old) *Drosophila* were compared in a somewhat different procedure. They are however different from those of a more recent study (Fois et al., 1991) showing that flies were slower to habituate from a middle age.

Finally, as a first attempt in *Drosophila* we did not reveal aging effects in short-term-memory. Future experiments may plan still to use longer ITI to try again to assess the existence of such aging effects. At the same time, the possible influence of various peripheral effects on learning will have to be taken into account. Henceforward, the design of future experiments should include a comparison between experimental and their yoked control groups in order to be aware of the involvement of non associative mechanisms in any conditioning situation.

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