Biogerontology **5:** 431–443, 2004.

Research article

Male *Drosophila melanogaster* flies exposed to hypergravity at young age are protected against a non-lethal heat shock at middle age but not against behavioral impairments due to this shock

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Received 10 May 2004; accepted in revised form 6 July 2004

Keywords: aging, *Drosophila melanogaster*, hormesis, Hypergravity, learning, locomotor activity, longevity, phototaxis, stress resistance

Abstract

Previous studies have shown that exposing flies to hypergravity (3 or 5 g) for two weeks at young age slightly increases longevity of male flies and survival time at 37 °C of both sexes, and delays an age-linked behavioral change. The present experiments tested whether hypergravity could also protect flies from a non-lethal 37 °C heat shock applied at young, middle or old age (2, 4 or 6 weeks of age). Various durations of exposure at 37 °C had similar deleterious effects on climbing activity, spontaneous locomotor activity and learning in flies that lived or not in hypergravity at young age. Therefore, hypergravity does not protect the behavior of flies from a deleterious non-lethal heat shock. Hypergravity increased longevity of virgin males and decreased that of mated ones; it also increased longevity of virgins at 25 °C, the usual rearing temperature, but not at 30 °C. Thus, the positive effect of hypergravity on longevity is observed only if flies are not subjected to living conditions decreasing longevity, like mating and high temperature. Finally, 4 weeks-old males that lived in hypergravity at young age lived slightly longer (+15%) after a non-lethal heat shock (60 or 90 min at 37 °C) than flies that always lived at 1 g, but this positive effect of hypergravity was not observed in females or in older males. Therefore, all these results show that hypergravity exposure can help male middle-aged flies recovering from a heat shock, but does not protect them from behavioral impairments linked to this shock: a mild stress occurring at young age can partially protect from a moderate stress at middle age.

Introduction

Exposure to mild stresses, such as hypergravity (HG: gravity levels higher than 1 g, the Earth gravity level) or heat shock, may slightly increase longevity (Le Bourg and Minois 1997; Le Bourg et al. 2001). A mild stress disturbs the homeostasis of the organism but does not kill it. The animal subjected to a mild stress must cope with it through an adaptive response (Minois and Rattan 2003). Contrarily to heat shock, exposure to HG is not encountered by flies living in the wild. However, exposure to HG increases the weight of flies and is thus a means to disturb their homeostasis and probably to increase their metabolic rate (discussion in Le Bourg and Fournier 2004), without increasing the rearing temperature. Besides the positive effects of mild stresses on longevity, a positive effect on aging, as deduced from a delayed behavioral aging, is not always observed. For instance, exposure to HG at young age delays the age-related decline of climbing activity in *Drosophila melanogaster* (Le Bourg and Minois 1999), but a mild heat shock does not (Le Bourg et al. 2001).

Exposure to a mild stress may also increase resistance to a strong stress. For instance, survival time to heat is increased after pre-treatment with heat (e.g. in *C. elegans*: Cypser and Johnson 2002; in *D. melanogaster*: Le Bourg et al. 2001; Hercus et al. 2003) or exposure to HG (e.g. Le Bourg and Minois 1997). However, that does not mean that mild stresses with positive effects on resistance to strong stresses would also have an effect on resistance to moderate stresses, i.e. on non-lethal stresses. Assessing stress resistance only by using lethal stresses might be considered as poorly relevant to the study of aging: in everyday life, people are scarcely subjected to strong stresses, e.g. spending 1 h at −20 °C, and this is even truer for elderly who usually avoid dangerous ways of life. By contrast, people, and particularly elderly, are at risk to be confronted with various moderate stresses, such as a sudden temperature drop during the winter. Elderly have a lower resistance to these stresses than young people, and these stresses are thus of a high value for aging research. Mimicking actual conditions of life of elderly when designing experiments on animals could be tentatively reached by studying how they cope with a non-lethal stress after being subjected to a mild stress. Suppose that, in old flies for instance, a moderate stress kills 10% of animals or impairs behavioral performances. If a mild stress applied before that moderate stress would lower that rate to, say, 1% with also beneficial effects on behavior, it would provide protection against stresses occurring at old age. Such a mild stress could also have positive effects on resistance to strong stresses. Thence, it would be useful to study the effect of exposure to a mild stress not only on resistance to strong stresses, but also to moderate stresses (see for a discussion Le Bourg 2003)

A first part of this article reports effects of exposure to HG at young age on resistance to a non-lethal heat shock (37 °C) occurring either shortly after the end of exposure to HG or two weeks later. Resistance to a non-lethal heat shock was assessed by observing its effect on behavioral traits, namely climbing activity, spontaneous locomotor activity and learned suppression of positive phototaxis. Because one shock duration may affect one behavior but not another one, this duration was selected to impair the behavior under study and varied accordingly. Similarly, some experiments used two durations of exposure to HG, namely one or two weeks. One–week of exposure to HG increases survival time at 37 °C (e.g. Le Bourg and Minois 1997) and a two-week exposure increases both survival time at 37 °C and longevity of males (e.g. Le Bourg et al. 2000).

A second part of this article reports results on longevity after two weeks in HG, using living conditions decreasing longevity. In a first experiment, flies were either virgin or mated. In a second one, flies were transferred to a higher temperature (30 °C) than the usual one (25 °C) after having lived in HG. These two living conditions can be considered as providing a moderate stress because they decrease longevity but do not kill the flies, as it is the case with exposure to 37 °C, for instance. Both experiments tested whether the previously observed positive effect of HG on longevity of males is still observed or whether it is increased or decreased under these conditions of moderate stress. In a last experiment, the effect of a single heat shock (60 or 90 min at 37 °C at 4, 5 or 6 weeks of age) on subsequent longevity was tested. While HG increases survival time to heat shock (Le Bourg and Minois 1997), it is unknown whether it protects from a hard but non-lethal heat shock. Only a very few flies, if any, die during 60 or 90 min heat shocks but preliminary experiments have shown that longevity is reduced after such shocks.

Materials and methods

Flies

This study used wild-type flies of the Meyzieu strain. This strain was caught in the wild in 1976 near the city of Lyon in France and is reared at lab since that time. Flies lived in an incubator (25 ± 0.5 °C; fluorescent lamps on from 08.00 to 20.00 h; ca 200 lux), except for the period in the room containing the centrifuge (see below). Experimental flies were obtained as follows. Eggs laid during a 15 h period by 50 five-day-old pairs of flies were transferred in batches of 25 into 80 ml glass vials. These vials contained the standard agar-sugar-corn meal-killed yeast medium enriched with live yeast. At emergence, virgin flies with a 9 days duration of development were transferred in groups of 15 of the same sex to 20 ml polystyrene vials (Polylabo 5111, France) containing the same medium and a drop of live yeast. Flies were transferred one day later either to 1, 3.02 or 5.02 g (1, 3 and 5 g in the following). Vials were kept vertically within the centrifuge rotating at 102 ± 0.2 rpm. The centrifuge, containing the 3 and 5 g groups, and the 1 g groups were in a room 25 ± 0.5 °C, lit from 08.00 to 20.00 (ca 200 Lux). At one or two weeks of age, depending on the experiment, flies were transferred to the incubator previously described. In all experiments, flies of each vial were transferred without anesthesia to a new one containing fresh medium twice a week.

Heat shock

Flies were transferred just before shock from their rearing vials to empty 14.2 ml vials (model 95939, Polylabo, France), the plug containing absorbent cotton wetted with some drops of distilled water. Flies were subjected to heat shock in a water-bath set at 37 °C either for 30, 60, 75, 90 or 120 min, depending on the experiment.

Climbing activity

In the climbing activity test, flies were individually placed in a vertical vial subjected to a mechanical stimulation and the highest height reached during 20 s after cessation of the stimulation was recorded. Climbing scores decrease with age and this test is routinely used in aging research for many years (e.g. Miquel et al. 1972; Feany and Bender 2000). The procedure has been described in detail (Le Bourg and Minois 1999); however, the stimulation intensity used by Le Bourg et al. (2001, rotating speed of the vial: 130 rpm) was used in the present experiment. Flies were kept in HG for one week and tested for their climbing activity, as well as 1 g flies of the same age, after being subjected or not to a heat shock (30 min at 37 °C just after removal from the room of the centrifuge). Climbing activity was not observed two weeks after the end of HG exposure, because HG by itself may increase climbing scores at this age (Le Bourg and Minois 1999). Climbing activity was observed during the afternoon (delay between the end of heat shock and experiment: 150–400 min) and 30 flies with intact legs were used for each combination of sex, heat shock and gravity level ($n = 360$).

Spontaneous locomotor activity

Spontaneous locomotor activity is the occurrence of motions in quiet flies. It has been shown repeatedly, using the procedure described below, that spontaneous locomotor activity scores decrease with age (e.g. Le Bourg and Minois 1999). The procedure used here is similar to that of this previous article. Flies were kept or not in HG for one or two weeks and were subjected or not to a heat shock (30, 60 or 90 min at 37 °C). The heat shock occurred just after removal from the room containing the centrifuge if flies were observed at young age (1 or 2 weeks), or two weeks later if observed at middle age (4 weeks).

After the heat shock, heat-shocked and control flies were individually transferred without anesthesia to 70 ml Pyrex vials (length: 20 cm, diameter: 2.4 cm, Bibby Sterilin 612.26, England) containing the S-101 medium (Pearl et al. 1926) and a drop of live yeast. The vials were put in one stand made of five vertical rows, each one containing up to 20 horizontally set vials. An empty vial separated males from females. The intensity of light, measured near the vials, was about 450 Lux. Flies were observed during 10 rounds of observations starting at every 11 min interval from 10.15 h for the 30 min heat shock condition, and from 13.30 h for the 60 and 90 min ones. Each fly was observed in turn for a maximum of 6 s and recorded as active or inactive using a computer. Activity (quoted 1) is defined as any motion (walking, attempt to fly), preening being however considered as inactivity (quoted 0). The individual score may thus vary between 0 (no observed activity) and 10 (fly active at each observation).

The number of observed flies was up to 16 in each group of gravity, sex and heat shock condition (heat-shocked or not heat-shocked) the first day of observation. Flies were again observed the next day using the same procedure, but the number of observed flies could decrease because some flies stuck to the food or died before the second experiment: these few flies were discarded from the results. These few flies cannot be used in the statistical analysis because one of the factor of the analysis was the day of observation, i.e. the day of shock and the day after.

Learning

A previous article has described a learning task (Le Bourg and Buecher 2002). The task consists in training individual flies into a T-maze to suppress their natural positive phototactic tendency. Flies have to choose between a lighted arm, leading to a lighted vial containing a filter paper wetted with an aversive quinine solution, and a darkened arm leading to a darkened vial with no aversive stimulus. Most of young flies of both sexes have an increased tendency during a 16-trials training session to choose the darkened vial when the lighted vial is associated with quinine, while most of flies tested with a dry lighted vial repeatedly choose this vial. No effect of age has been observed on this learning task (Le Bourg 2004). Material and methods have been described in detail in Le Bourg and Buecher (2002). In all experiments reported below the lighted vial contained a paper wetted with a quinine solution, and the darkened vial was dry and contained no paper.

The present experiments tested whether heat shock could modify learning scores and if exposure to HG could protect from this effect. A preliminary experiment was done to determine the length of heat shock to be used. Two or four week-old flies were subjected to a heat shock (30, 45, 60, 75, 90 or 120 min) in the morning and to learning in the afternoon. Five flies were used for each age, sex and length of shock group.

Once a length of heat shock was shown to have some effect on learning score, the learning of flies that have lived or not in HG for two weeks was observed at 2 or 4 weeks of age after they were subjected to heat shock. Five flies were used for each age, sex and length of shock group.

Longevity of mated and virgin flies

At emergence, virgin flies were transferred in groups of 16 flies of the same sex or in groups of 8 males and 8 females to the 20 ml vials previously described. Flies were transferred one day later either to 1, 3 or 5 g and, at two weeks of age, they were transferred at 1 g into an incubator (25 ± 0.5 °C; fluorescent lamps on from 08.00 to 20.00 h; ca 200 lux). Dead flies were recorded daily up to the death of the last fly. The number of flies was about 120 for each sex, gravity and mating condition.

Longevity at 25 or 30 °C

At emergence, virgin flies were transferred in groups of 15 flies of the same sex to the 20 ml vials previously described and, one day later, to 1, 3 or 5 g. At two weeks of age, they were transferred at 1 g, either into an incubator set at 25 °C or into another one set at 30 °C (fluorescent lamps on from 08.00 to 20.00 h; ca 200 lux). Dead flies were recorded daily up to the death of the last fly. The number of flies was about 75 for each sex, gravity and temperature condition.

Longevity after a non-lethal heat shock

At emergence, virgin flies were transferred in groups of 15 flies of the same sex to the 20 ml vials previously described and, one day later, to 1, 3 or 5 g. At two weeks of age, they were transferred at 1 g into an incubator set at 25 °C (fluorescent lamps on from 08.00 to 20.00 h; ca 200 lux). At 4, 5 or 6 weeks of age, flies were heat-shocked at 37 °C for 60 or 90 min and thereafter transferred to their vials. Dead flies were recorded daily from the day following heat shock up to the death of the last fly. For each combination of sex, age, gravity and length of heat shock, 3 vials of about 15 flies were used and the experiment was replicated. The number of flies was thus about 90 for each sex, age, gravity and length of heat shock condition. However, this number was lower at 6 weeks of age, due to the natural mortality occurring before that age.

Statistical analysis

Data were analyzed with ANOVAs. A square-root transformation was applied to climbing scores before to be analyzed with a 3-way factorial ANOVA testing for the effect of gravity, sex, heat shock and all interactions. Square-roots of spontaneous locomotor activity scores were analyzed with 4-way repeated measures ANOVAs testing for the effect of gravity, sex, heat shock and day of observation (repeated factor), and all interactions. Learning scores were analyzed with 4-way repeated measures ANOVAs testing for the effect of gravity, sex, heat shock and blocks of trials (repeated factor). Since each ANOVA showed that the scores increased with the order of blocks of trials, the results of this factor are not reported below. Longevity data elevated to power two were analyzed in each sex with 2-way factorial ANOVAs testing for the effect of gravity, living condition (mated or virgin status in one experiment and temperature in another one), and their interaction. However, a square-root transformation was applied to the longevity data of flies subjected to a heat shock and these data were analyzed in each age group (heat shock at 4, 5 or 6 weeks of age) with 3-way factorial ANOVAs testing for the effect of gravity, sex and duration of heat shock (60 vs. 90 min). For the sake of clarity, figures and means report not transformed data.

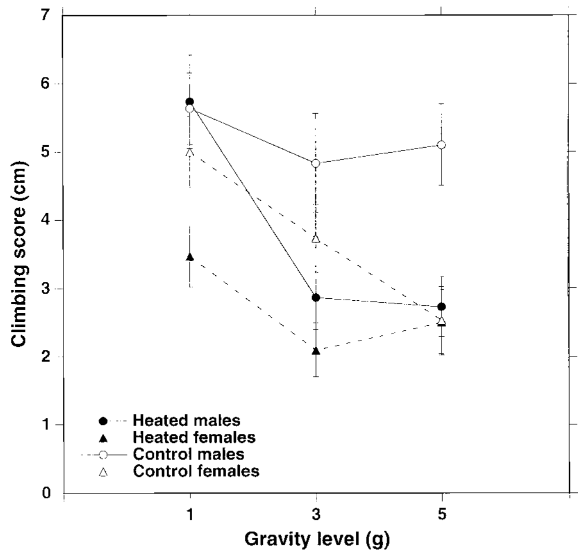

Figure 1. Effect of a one-week exposure to HG on mean climbing scores ± SEM of 8 days-old flies subjected or not to a 30 min heat shock at 37 °C before experiment. The climbing score is the maximal height reached by a fly 20 s after the end of a mechanical stimulation applied to the vial containing the fly. This score may vary between 0 and 16 cm, the height of the vial. Each point is the mean of 30 flies.

Results

Climbing activity

Females had lower climbing scores than males (Figure 1, $F(1, 348) = 14.95$, $P < 0.0001$) and climbing scores were lower in flies that have been subjected to HG ($F(2, 348) = 13.96$, $P < 0.0001$). Heat shock decreased climbing scores ($F(1, 348) = 16.65$, $P < 0.0001$). All interactions were non-significant, even if the second-order interaction ($F(2, 348) = 2.31$, $P = 0.1009$) seems to indicate that heat-shocked males have lower climbing scores if they have lived in HG, while heat shock has no effect in females if they have lived at 5 g. To sum up, the whole picture shows that heat shock decreases climbing scores roughly to the same extent in flies that have lived or not in HG prior to heat shock. In other words, HG does not protect flies from heat shock, as far as climbing activity is concerned, but, rather, seems to impair scores of males. Since the short heat shock used here (30 min at 37 °C) is sufficient to impair climbing scores of flies that have been kept or not in HG for one week, there is no reason to test longer durations of heat shock or exposure to HG.

Spontaneous locomotor activity

Thirty minutes heat shock

In a first experiment, a one-week exposure to HG was used and activity was observed at 8 days of age, just after removal from centrifuge, and the day after. Males had lower activity scores than females (Figure 2a, $F(1, 178) = 32.40$, $P < 0.0001$) and the scores were slightly higher if flies have lived at 1 g ($F(2, 178) = 3.87$, $P = 0.0227$). Heat-shocked flies had lower scores than control ones ($F(1, 178) = 6.84$, $P = 0.0097$). This effect was due to females (sex by shock interaction: $F(1, 178) = 43.39$, $P < 0.0001$) and heat shock slightly increased the scores of males. The scores were higher the second day of observation ($F(1, 178) = 37.72$, $P < 0.0001$) and this effect was due to females (sex by day interaction: $F(1, 178) = 28.21$, $P < 0.0001$). Not unexpectedly, the shock by day interaction ($F(1, 178) = 18.13$, $P < 0.0001$) and the sex by shock by day second-order interaction ($F(1, 178) = 29.45$, $P < 0.0001$) were also significant. All other interactions were not significant. To sum up, heat shock transiently decreased the activity scores of females, and slightly increased those of males. Females that

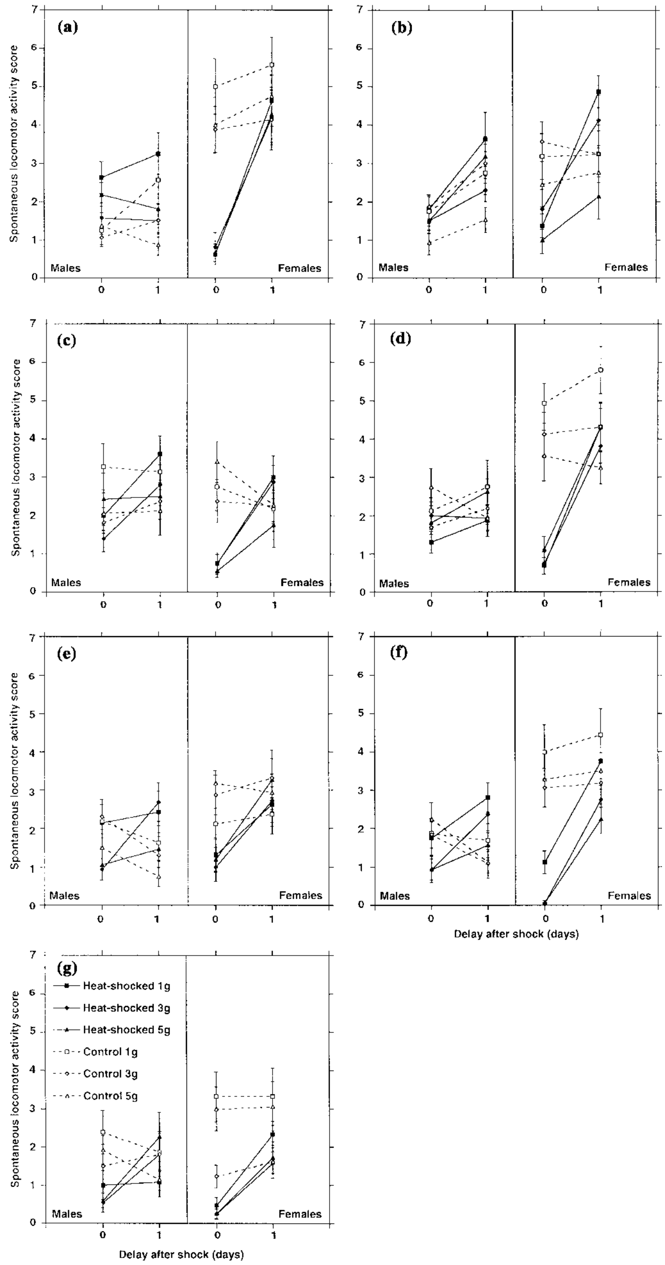

Figure 2. Effect of a one-week or a two-weeks exposure to HG on mean spontaneous locomotor activity scores ± SEM in flies subjected or not to a 37 °C heat shock. The activity score is the number of observations when the fly was active and may vary between 0 and 10, the number of observations. The same flies were observed the day of heat shock (0 on abscissa) and the day after (1 on abscissa). Each point is the mean of ca 16 flies. (a) One-week exposure to HG. Flies lived or not in HG and were subjected or not to heat for 30 min at 8 days of age, just after removal from the centrifuge. (b) Two-week exposure to HG. Flies lived or not in HG and were subjected or not to heat for 30 min at 15 days of age, just after removal from the centrifuge. (c) Two-week exposure to HG. Flies lived or not in HG and were subjected or not to heat for 30 min at 29 days of age, two weeks after removal from the centrifuge. (d) Two-week exposure to HG, 60 min heat shock at 15 days of age. (e) Two-week exposure to HG, 60 min heat shock at 29 days of age. (f) Two-week exposure to HG, 90 min heat shock at 15 days of age. (g) Two-week exposure to HG, 90 min heat shock at 29 days of age.

lived in HG for one-week displayed a similar activity decrease due to heat shock as 1 g ones, which shows that HG did not protect them from heat. It could be that a longer stay in HG is necessary to observe protecting effects of HG.

In a second experiment, a two-week exposure to HG was thus used and activity was observed at 15 days of age, just after removal from centrifuge, and the day after. The results (statistical analysis not shown, but see Figure 2b) were roughly similar to the previous ones but not shocked females had lower scores than at one-week of age. This effect of age explains why the effect of heat shock was not significant in the present experiment. However, heat shock affected SLA scores of females as in the previous experiment.

Protecting effects of HG are not observed at two weeks of age in females that have spent two weeks in HG and males are not affected by heat shock. The experiment was thus reiterated at an older age, because old flies are more affected by heat than young ones (Le Bourg et al. 2001). A two-week exposure to HG was used and activity was observed at 29 and 30 days of age, two weeks after removal from centrifuge. Similar results to previous ones were observed (Figure 2c, statistical analysis not shown): females, which were affected by heat shock, were not protected from it if they had lived in HG.

Therefore, a 30 min heat shock transiently decreased the activity scores of two- and four-week old females and those that lived for two weeks in HG were not protected from heat. A similar conclusion was reached when flies were exposed to HG for only one-week. Heat shock had only slight effects in males. Using a longer heat shock is thus of interest to possibly observe deleterious effects of heat and protective effects of HG.

Sixty minutes heat shock

A two-week exposure to HG was used and activity was observed at 15 days of age, just after removal from centrifuge and the day after. In a second experiment, a two-week exposure to HG was used and activity was observed at 29 and 30 days of age, two weeks after removal from centrifuge. Females that lived in HG were not protected from deleterious effects of heat shock, as previously shown, and the effect of heat shock on the SLA score of males was weak (Figure 2d and e, statistical analyses not shown). Flies were thus subjected to a 90 min heat shock to possibly observe decreased SLA scores in males.

Ninety minutes heat shock

A two-week exposure to HG was used and activity was observed at 15 days of age, just after removal from centrifuge, and the day after. In a second experiment, a two-week exposure to HG was used and activity was observed at 29 and 30 days of age, two weeks after removal from centrifuge. These two experiments showed that, here again, flies that have lived in HG were not protected from heat shock (Figure 2f and g, statistical analyses not shown). This was observed in females, as in previous experiments, but also in 4 week-old males which were also affected by heat shock. The whole series of experiments measuring SLA thus shows that HG never protected flies from the effect of heat shock on SLA. However, the effects of heat shocks, when observed, were transient.

Learning

A preliminary experiment was carried out to determine what duration of heat shock modifies learning scores. Two week-old flies were subjected to 0, 30, 45 or 60 min at 37 °C in a first experiment and to 0, 75, 90 or 120 min at 37 °C in a second experiment. The same procedure was used for 4 week-old flies.

No effect of heat shock was observed in the first experiment using a 0–60 min shock range (sex, age, and shock factors not significant, statistical analysis not shown but see Figure 3a and b), in accordance with a study of conditioned courtship using a 30 min heat shock at 37 °C (Nikitina et al., 2003). In the second experiment, many 4 week-old flies died during the 120 min heat shock. This duration was thus not used with these flies and the results of 2 and 4 week-old flies were analyzed separately. Concerning younger flies, the shock had no effect on learning scores, but the sex by shock interaction ($F(3, 32) = 4.28$, $P = 0.012$) showed that no effect was observed in females and that the learning scores of heat-shocked males decreased with longer shocks (Figure 3a). Concerning older flies, the shock had no significant effect on learning scores. However, as for young flies, it seems that no effect was observed in females and that the learning scores of heat-shocked males tended to decrease with longer shocks, but the sex by shock interaction was not significant (Figure 3b). This preliminary experiment has thus shown that long heat shocks can decrease learning scores, i.e. flies have more difficulties to learn avoiding a lighted vial associated with an aversive stimulus when they are heat-shocked for a long time. A control experiment showed that heat-shocked (75, 90 or 120 min at 37 °C) and not shocked young flies have the same low avoidance of the lighted vial when it is dry (mean: 1.55 avoidance during 16 trials, 5 flies for each sex and length of shock group), i.e. when the vial contains no aversive stimulus (data not shown). In other words, the decreased learning score after a long heat shock is not due to a decreased positive phototaxis.

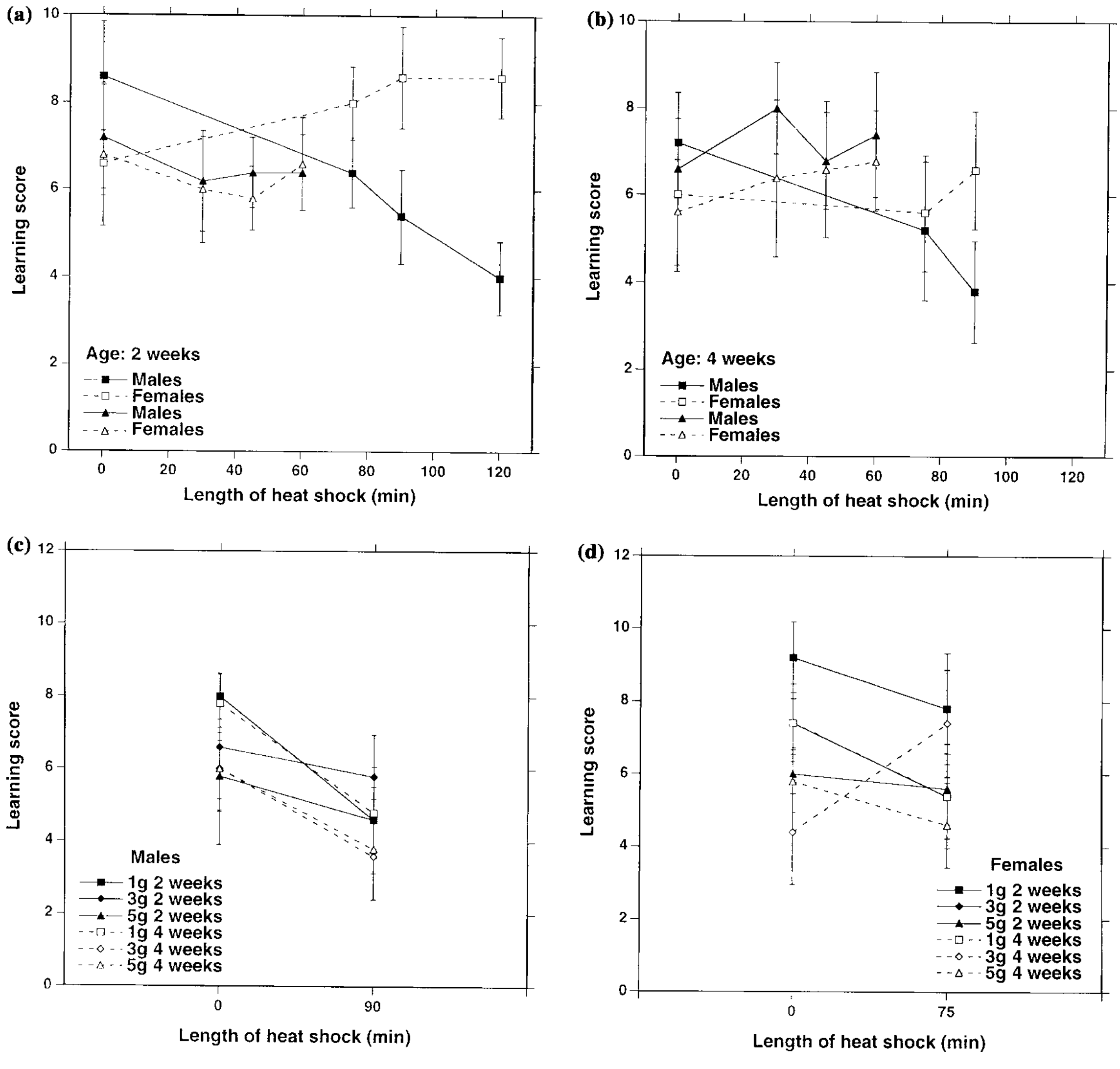

Figure 3. Effect of heat shock on learning score (mean number of photonegative choices) ± SEM. The learning score may vary between 0 and 16. Flies were trained with a lighted vial containing a paper wetted with a quinine solution as an aversive stimulus on each of 16 trials. Each point is the mean of five flies. (a) Two-week-old flies were subjected to a 0,30, 45 or 60 min heat shock (triangles) or to a 0, 75, 90 or 120 min one (squares). (b) Four-week-old flies were subjected to a 0, 30, 45 or 60 min heat shock (triangles) or to a 0, 75 or 90 min one (squares). (c) Two-week exposure to HG. Male flies lived or not in HG and were subjected or not to heat for 90 min at 15 or 29 days of age (respectively, 2 and 4 weeks on the figure). (d) Two-week exposure to, HG. Female flies lived or not in HG and were subjected or not to heat for 75 min at 15 or 29 days of age.

We thus decided to use a 90 min heat shock but it rapidly appeared that too many heat-shocked females were unable to walk into the maze. In such conditions, we decided to use a 75 min heat shock in females and a 90 min one in males, because this last shock had some effect on learning scores in males (Figure 3a and b) and we analyzed separately the results of males and females.

In males, the ANOVA showed that the learning score decreased in heat-shocked flies (Figure 3c, $F(1, 48) = 9.21$, $P = 0.0039$). The gravity and age effects and all interactions were not significant. In other words, heat shock decreased learning score but flies that had lived in HG were not protected against this effect at any age.

In females, the gravity effect was marginally significant (Figure 3d, $F(2, 48) = 2.98$, $P = 0.0605$). The shock and age factors were not significant as well as all interactions. Therefore, these results confirm that a 75 min heat shock has no negative effect on learning score of females: in such conditions, it is obviously impossible to bring to the fore any positive effect of HG.

This experiment thus shows that at 2 and 4 weeks of age, flies that have lived in HG at young age are not protected against the negative effect of heat shock on learning score when this negative effect exists.

Longevity of mated and virgin flies

Previous experiments have repeatedly shown that HG has no positive effect on longevity in females, by contrast to what occurs in males (e.g. Le Bourg et al. 2000), and we thus analyze below the results of each sex separately.

Females kept at 5 g for the first two weeks of imaginal life lived shorter than flies kept at 1 or 3 g (Figure 4a, $F(2, 714) = 4.40$, $P = 0.0126$) and, as expected, mating decreased longevity of females ($F(1, 714) = 47.92$, $P < 0.0001$). The non-significant gravity by mating condition interaction ($F(2, 714) = 2.77$, $P = 0.063$) indicated that the deleterious gravity effect was mainly observed in mated females.

No gravity effect was observed in males (Figure 4b, F close to 1) and, as expected, virgin males lived longer than mated ones ($F(1, 678) = 252.85$, $P < 0.0001$). The gravity by mating condition interaction ($F(2, 678) = 8.66$, $P = 0.0002$) indicated that longevity increased with the gravity level in virgin males and decreased in mated ones.

Thus, the effect of HG on longevity is positive in virgin males, mainly at 5 g, and negative in mated ones, mainly at 5 g. In females, HG has no effect in virgins and a negative one in mated flies, mainly at 5 g. It may be concluded that, in each sex, the deleterious effect of mating on longevity is observed at each gravity level. In other words, HG exposure at young age does not protect from the deleterious effects of mating on longevity. Moreover, this deleterious effect appears to be reinforced if flies lived at 5 g. It seems therefore that this HG level, which increases longevity in virgin males (Figure 4b), is deleterious if flies are subjected to a living condition decreasing longevity.

Longevity at 25 or 30 °C

As for the previous experiment, the results of each sex were analyzed separately.

No effect of gravity was observed in females (Figure 5a, F close to 1). Longevity was higher at 25 °C than at 30 °C, as expected ($F(1, 438) = 588.75$, $P < 0.0001$). The temperature by gravity interaction ($F(2, 438) = 5.62$, $P = 0.0039$) showed that longevity was higher in HG than at 1 g if females lived at 25 °C while no gravity effect was observed in 30 °C females. It is the first time that a positive effect or HG on the longevity of females is observed, and this effect is confirmed if the ANOVA is restricted to females that always lived at 25 °C (compare with Figure 4, Le Bourg and Minois 1997, and Le Bourg et al. 2000). However, the main result is that the opposite trend is observed if females lived at 30 °C.

In males, longevity increased with the gravity level (Figure 5b, $F(2, 432) = 5.25$, $P = 0.0056$) and was higher at 25 °C than at 30 °C, as expected ($F(1, 432) = 595.42$, $P < 0.0001$). The temperature by gravity interaction ($F(2, 432) = 4.36$, $P = 0.0133$) showed that longevity more increased with the gravity level at 25 °C than at 30 °C. In other words, the positive effect of HG was more observed at 25 °C than at 30 °C.

Therefore, in both sexes, a positive effect of HG on longevity was observed if flies lived continuously at 25 °C, while no such effect was observed if they were transferred at 30 °C after the age of

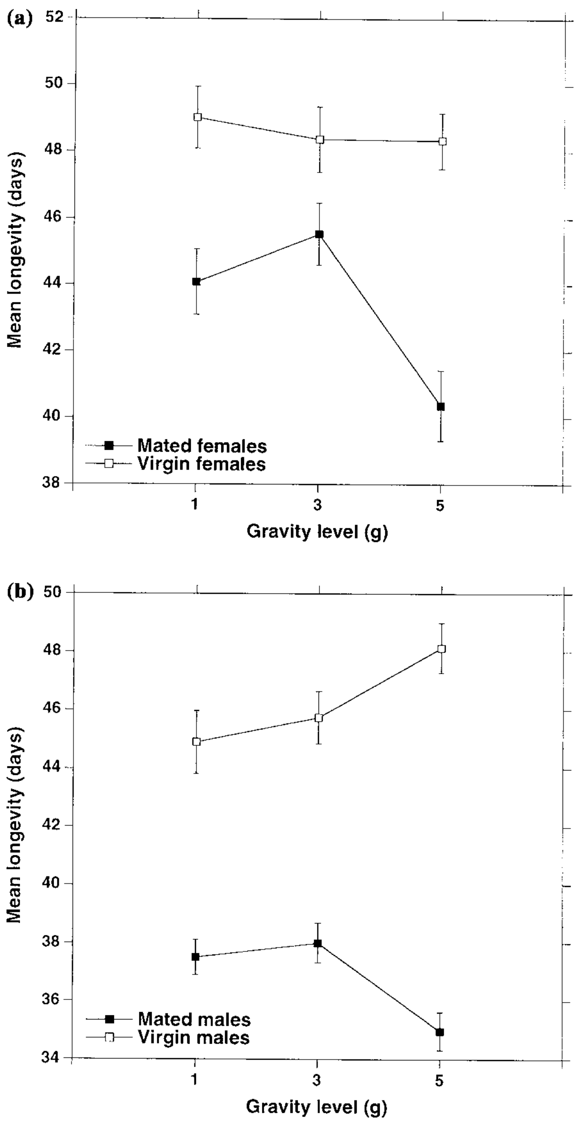

Figure 4. Effect of a two-week exposure to HG on mean longevity ± SEM of mated or virgin females (a) and males (b). Each point is the mean of ca 120 flies.

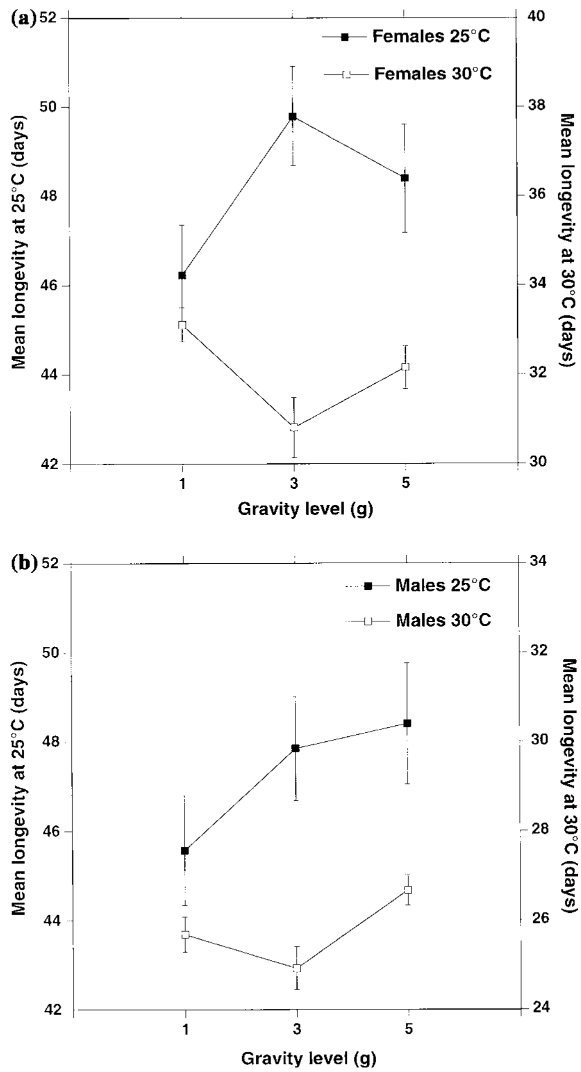

Figure 5. Effect of living at 25 or 30 °C after a two-week exposure to HG at 25 °C on mean longevity ± SEM of females (a) and males (b). For the sake of clarity, a double-Y plot is used. Each point is the mean of ca 75 flies.

removal from HG. Another experiment, using a transfer at 28 °C after a two-week exposure to HG at young age, provided similar results: no positive effect of HG on longevity was observed in both sexes (data not shown). No positive effect of HG on longevity of males is thus observed if flies live in a rather stressful condition, such as a high temperature. A similar conclusion was reached when flies were subjected to mating, another living condition decreasing longevity.

Longevity after a non-lethal heat shock

This experiment tested whether middle-aged and old flies subjected to a non-lethal heat shock better resist to that shock if they have lived in HG at young age, and thus live longer after that shock. Even if 60 or 90 min at 37 °C kill only a few flies, preliminary experiments showed that longevity was reduced after such shocks (see also Le Bourg et al. 2001). As it is expected that the age at heat shock will have a strong effect on resistance to heat

(Le Bourg et al. 2001) and thus on subsequent longevity, the results were analyzed separately for each age of shock.

Flies heat-shocked at 4 weeks of age

Males lived longer than females, but this tendency was marginally significant (Figure 6a, $F(1, 935) = 3.81$, $P = 0.0512$). Longevity was lower when flies were subjected to a 90 min shock rather than to a 60 min one ($F(1, 935) = 51.67$, $P < 0.0001$, mean ± SEM of 60 min groups: 9.63 ± 0.19 days; mean ± SEM of 90 min groups: 7.70 ± 0.17 days, see Figure 6b for the results of males). The gravity effect was not significant but the sex by gravity interaction showed that the longevity slightly increased with the gravity level in males, while it had no effect in females ($F(2, 935) = 4.50$, $P = 0.0113$). All other interactions were not significant: in other words, the longevity of males increased with the gravity level with both 60 and 90 min heat shocks (Figure 6b). The mean longevity of males after the heat shock was, respectively 8.34, 9.02 and 9.70 days at 1, 3 and 5 g: the longevity was thus 16% higher for the 5 g males when compared to the 1 g ones.

Flies heat-shocked at 5 or 6 weeks of age

The results were rather similar to what was observed when flies were heat-shocked at 4 weeks of age, if we except that no positive effect of HG was observed in males or in females (statistical analysis not shown, but see Figure 6a). Therefore, hypergravity had no positive effect on longevity if 5 or 6 week-old flies were heat-shocked. These results also show that, as expected (e.g. Le Bourg and Minois 1997; Minois and Le Bourg 1999; Semenchenko et al. 2004), stress resistance decreases with age because longevity decreases when the age at heat shock increases (Figure 6a).

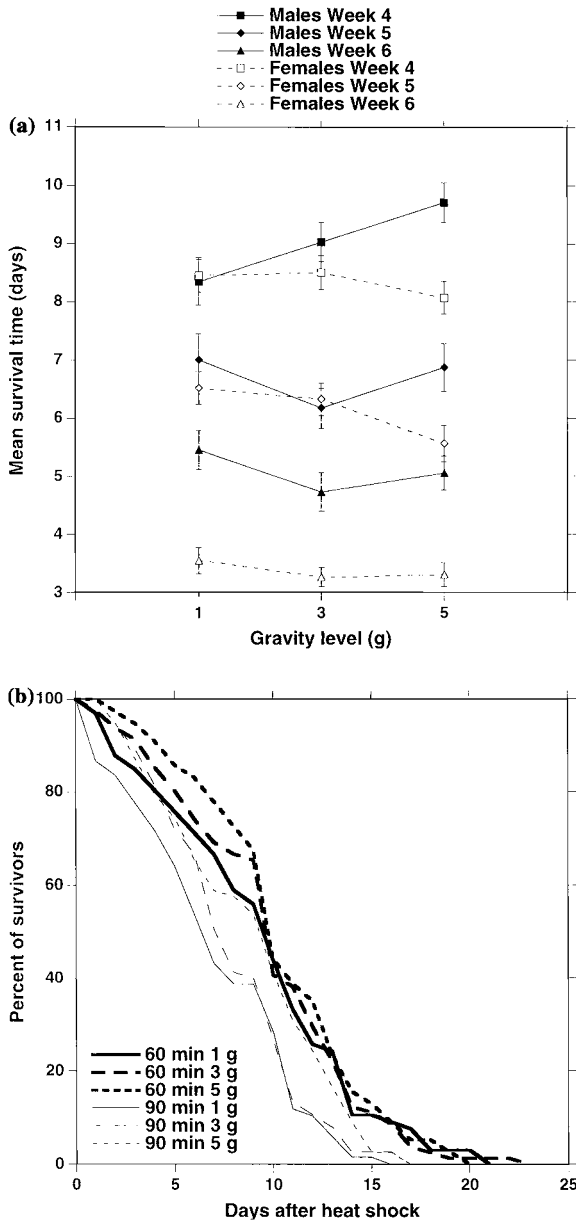

Figure 6. Effect of a two-week exposure to HG on survival time ± SEM after a 60 or 90 min heat shock at 37 °C. (a) Mean survival time at 4, 5 or 6 weeks of age. Each point is the mean of 133–170 flies at 4 weeks of age, 107–165 flies at 5 weeks of age, and 94–147 flies at 6 weeks of age. The 60 and 90 min groups are pooled on the figure. (b) Survival curves of males heat-shocked at 4 weeks of age.

Discussion

The main goal of this project was to know whether a mild stress at young age could protect from a more severe non-lethal stress at old age. Studying non-lethal stresses is of interest because elderly are often confronted with such non-lethal stresses in everyday life, like for instance abrupt temperature falls in winter, while lethal stresses are obviously encountered only once.

Studying the effect of non-lethal stresses on behavior is of concern because measuring longevity only does not give any information about the physiological state of organisms (see for an example of contrasting effects on behavior and longevity Cook-Wiens and Grotewiel 2002). Three behavioral variables have been measured in this study. Climbing activity shows if flies are able to respond to a mechanical stimulus by climbing on the side of a vial. Spontaneous locomotor activity measures the ability of flies to move when no mechanical stimulus is applied. Learning to avoid a lighted area associated with an aversive stimulus is linked to cognitive ability rather than to basic behaviors, like climbing and spontaneous locomotor activities are. Heat shocks (between 30 and 90 min at 37 °C) had negative effects on each of the studied behaviors (Figure 1–3) and living in HG at young age did not protect from a heat shock occurring at young or middle age. In other words, a mild stress known to have positive effects on longevity (in males) and on survival time to heat (in both sexes) does not protect from deleterious consequences of heat shock on behavior at young or middle age.

Concerning longevity, the deleterious effect of mating on longevity was reinforced if flies had lived at 5 g (Figure 4) and living at a higher temperature than the usual one made the positive effect of HG on longevity to vanish (Figure 5). Finally, male flies that were heat-shocked at 4 weeks of age lived longer after this shock if they had lived in HG for two weeks at young age, such an effect being not observed if flies were shocked at 5 or 6 weeks of age (Figure 6). Thus. HG exposure at young age has positive effects on longevity of males if flies have a very peaceful life after having lived in HG (i.e. they are kept virgin or at 25 °C). If flies that have lived in HG are subjected to moderate stress not killing them, such as mating or living at 30 °C, the positive effect of HG can even become a negative one. At variance with this conclusion, living in HG at young age protects from a non-lethal heat shock at middle age but not at old age.

To conclude, HG exposure can help flies recovering from a heat shock, but does not protect them from behavioral impairments linked to this shock; however, HG can impair longevity if flies have not a very peaceful life (no mating, no life at 30 °C).

Some authors have hypothesized that mild stresses could help preventing some effects of aging (see, e.g. the issue 6, volume 20, of Human and Experimental Toxicology, 2001). After some results showing that mild stresses increase resistance to lethal stresses and longevity (see the Introduction, Minois 2000; Minois and Rattan 2003), this hypothesis was the next logical step but the present results only partially confirm it.

A similar hypothesis has been made in cardiology. Ischemic preconditioning, i.e. brief periods of ischemia, protects the myocardium from more severe periods of ischemia, but this protection is diminished in hearts from elderly people (Bartling et al. 2003) or middle-aged rats (Honma et al. 2003). Honma et al. (2003) successfully used heat shock as a mild stress in addition to ischemic preconditioning to improve post-ischemic function in hearts from young rats, but this treatment was inefficient in old rats. By contrast, subjecting rats to 6 months of caloric restriction restored the cardioprotective effect of ischemic preconditioning in hearts from 10 months-old rats (Long et al. 2002). It may thus be concluded that the positive effect of ischemic preconditioning can be restored at middle age by some treatments only.

Regarding flies, HG partially protects from a moderate heat shock occurring at middle age but not at older ages. Thus, a mild stress can help flies to cope with heat stress, provided they are not too old. The physiological state of old flies probably does not allow them to take advantage from exposure to HG, as it is observed with ischemic preconditioning (e.g. Bartling et al. 2003), but the Long et al.'s results (2002) bear the hope to overcome this problem in flies too. The observed effect at middle age (Figure 6) is not paramount and one cannot definitively conclude that a mild stress occurring at young age should always protect from moderate stresses occurring at an older age. Moreover, the results allow concluding that, even if HG helps middle-aged males surviving after a heat shock, flies are not protected against sequels of this heat shock in everyday life, as it can be assessed from the observation of their behavior soon after the heat shock (Figure 1–3). However, flies that lived in HG could be protected against behavioral impairments due to the heat shock, contrarily to 1 g flies, provided there is a sufficient delay between the heat shock and the observation of behavior. This experiment remains to be done.

Our results show that there is at least one example of a mild stress that, when applied at young age, protects flies against a stronger shock at an older age. This encouraging result could stimulate the search for more efficient stresses. Such studies could help finding new ways to protect elderly people against threatening stresses at old age like summer heat wave. In France, about 15,000 elderly people died during the few days of the summer heat wave in 2003 (Belmin 2003). The number of deaths in France in 2003 is similar to that observed nearly 20 years before and, in women, the expectation of life at birth stopped increasing (Desesquelles and Richet-Mastain 2004). Preventing deaths caused by heat wave is of a high concern because Earth temperature increases and more heat wave episodes are expected in the future due to global climatic change. One can hope that studies like the present one will give ideas to colleagues working on human aging to prevent the deleterious consequences of stress at old age.

References

Bartling B, Friedrich I, Silber RE and Simm A (2003) Ischemic preconditioning is not cardioprotective in senescent human myocardium. Ann Thorac Surg 76: 105–111

Belmin J (2003) Les conséquences de la vague de chaleur d'août 2003 sur la mortalité des personnes âgées. Un premier bilan *(The consequences of the heat wave in August 2003 on the mortality of the elderly in France. The first overview)*. Presse Med 32: 1591–1594

Cook-Wiens E and Grotewiel MS (2002) Dissociation between functional senescence and oxidative stress resistance in *Drosophila*. Exp Geront 37: 1345–1355

Cypser JR and Johnson TE (2002) Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. J Gerontol Biol Sci 57A: B109–B114

Desesquelles A and Richet-Mastain L (2004) Bilan démographique 2003: stabilité des naissances, augmentation des décès *(Demographic assessment of 2003 in France: the number of births is stable but that of deaths increases)*. INSEE Première, issue 948 (downloadable at www.insee.fr)

Feany MB and Bender WW (2000) A *Drosophila* model of Parkinson's disease. Nature 404: 394–398

Hercus MJ, Loeschcke V and Rattan SIS (2003) Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. Biogerontology 4: 149–156

Honma Y, Tani M, Yamamura K, Takayama M and Hasegawa H (2003) Preconditioning with heat shock further improved functional recovery in young adult but not in middle-aged rat hearts. Exp Geront 38: 299–306

Le Bourg E (2003) Delaying aging: could the study of hormesis be more helpful than that of the genetic pathway used to survive starvation? Biogerontology 4: 319–324

Le Bourg E (2004) Effects of aging on learned suppression of photopositive tendencies in *Drosophila melanogaster*. Neurobiol Aging 25: 1241–1252

Le Bourg E and Buechel C (2002) Learned suppression of photopositive tendencies in *Drosophila melanogaster*. Anim Learn Behav 30: 330–341

Le Bourg E and Fournier D (2004) Is lifespan extension accompanied by improved antioxidant defences? A study of superoxide dismutase and catalase in *Drosophila melanogaster* flies that lived in hypergravity at young age. Biogerontology 5: 261–266

Le Bourg E and Minois N (1997) Increased longevity and resistance to heat shock in *Drosophila melanogaster* flies exposed to hypergravity. CR Acad Sci Paris 320: 215–221

Le Bourg E and Minois N (1999) A mild stress, hypergravity exposure, postpones behavioral aging in *Drosophila melanogaster*. Exp Geront 34: 157–172

Le Bourg E, Minois N, Bullens P and Baret P (2000) A mild stress due to hypergravity exposure at young age increases longevity in *Drosophila melanogaster* males. Biogerontology 1: 145–155

Le Bourg E, Valenti P, Lucchetta P and Payre F (2001) Effects of mild heat shocks at young age on aging and longevity in *Drosophila melanogaster*. Biogerontology 2: 155–164

Long P, Nguyen Q, Thurow C and Broderick TL (2002) Calorie restriction restores the cardioprotective effect of preconditioning in the rat heart. Mech Ageing Dev 123: 1411–1413

Minois N (2000) Longevity and aging: beneficial effects of exposure to mild stress. Biogerontology 1: 15–29

Minois N and Le Bourg E (1999) Resistance to stress as a function of age in *Drosophila melanogaster* living in hypergravity. Mech Ageing Dev 109: 53–64

Minois N and Rattan SIS (2003) Hormesis in aging and longevity. In: Rattan SIS (ed) Modulating Aging and Longevity, pp. 127–137. Kluwer Academic Publishers, Dordrecht

Miquel J, Lundgren PR and Binnard R (1972) Negative geotaxis and mating behavior in control and gamma-irradiated *Drosophila*. Droso Infor Serv 60: 48

Nikitina EA, Tokmatcheva EV and Savateeva-Popova EV (2003) Heat shock during the development of central structures of the *Drosophila* brain: memory formation in the *l(1)ts403* mutant of *Drosophila melanogaster*. Rus J Genet 39: 25–31

Pearl R, Allen L and Penniman WBD (1926) Culture media for *Drosophila*. II. A new synthetic medium and its influence on fertility at different densities of populations. Am Nat 60: 357–366

Semenchenko GV, Khazaeli AA, Curtsinger JW and Yashin AI (2004) Stress resistance declines with age: analysis of data from a survival experiment with *Drosophila melanogaster*.Biogerontology 5: 17–30