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SHORT COMMUNICATION

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Hormetic protection of *Drosophila melanogaster* middle-aged male flies from heat stress by mildly stressing them at young age

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Abstract Previous studies have shown that exposing flies to hypergravity (3*g* or 5*g*) for the first 2 weeks of adult life slightly increases longevity of male flies and survival time at 37°C for both sexes, and delays an age-linked behavioral change. The present experiment tested whether the hypergravity could also protect flies from four successive deleterious non-lethal heat shocks at 4 and 5 weeks of age. Males that lived in hypergravity for the first 2 weeks of adult life lived slightly longer (ca. +15% or 1.2 day) after heat shocks (30 min or 45 min at 37°C) than flies that always lived at 1*g*, but this positive effect of hypergravity was not observed in females. Therefore, hypergravity exposure at young age can help the male flies recovering from a heat shock at older ages.

Introduction

Exposure to mild stresses, such as hypergravity (HG: gravity levels higher than 1 g, the Earth's gravity level) or heat shock, may slightly increase longevity in *Drosophila melanogaster* flies (Le Bourg and Minois 1997; Le Bourg et al. 2001). A mild stress disturbs the homeostasis of the organism but does not kill it, forcing the animal to implement an adaptive response. Thus, a mild stress can improve the functional ability of the organism and even increase longevity: this phenomenon is called hormesis (Minois and Rattan 2003). 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 hypergravity at young age delays the age-related decline of the climbing activity in *D. 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 lethal stress. For instance, survival time to heat is increased after pre-treatment with heat (e.g, in *Caenorhabditis 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). It is of interest to know whether a mild stress could also protect against deleterious but non-lethal stresses because animals are often subjected to non-lethal stresses, such as a sudden temperature rise. A previous article (Le Bourg et al. 2004) has shown that 4-week-old male flies living in HG at young age lived slightly longer (+15%) after a single deleterious non-lethal heat shock (60 min 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.

The aim of the present article was to investigate whether flies living in HG at young age could be protected against successive heat shocks shorter (30 min or 45 min at 37°C) than those used in our previous study and at an older age (more than 4 weeks).

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 in the lab since that time. Flies lived in an incubator (25±0.5°C; fluorescent lamps on from 0800 to 2000 hours; ca 200 lux), except for the period spent in the room containing the centrifuge (see below).

Experimental flies were obtained as follows. Eggs laid during a 15-h period by 50 5-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-day duration of development were transferred in groups of 15 of the same sex to 20-ml polystyrene

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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 at 25±0.5°C, lit from 0800 to 2000 hours (ca. 200 lux).

At 2 weeks of age, flies were transferred to the incubator previously described. Flies of each vial were transferred without anaesthesia to a new one containing fresh medium twice a week.

Longevity after a non-lethal heat shock

Flies were subjected to four heat shocks from 4 weeks of age (30 min or 45 min in a water-bath set at 37°C, twice a week for 2 weeks; five replicates for each length of heat shock). For each replicate, all flies were born the very same day. The first replicate was done during the first half of April 2004, the second one in the second half of the same month, the third one in September 2004, the fourth and fifth ones in November 2004.

Flies were transferred just before the heat 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. After each shock, flies were transferred back to their vials. Dead flies were recorded daily from the day following the first heat shock up to the death of the last fly. For each combination of gravity level, sex, and length of heat shock, three vials of about 15 flies were used. However, due to a technical failure, only two vials of 1*g* males heat-shocked for 30 min were used for the first replicate.

Results

Flies living in HG for 2 weeks at young age were subjected to four heat shocks from 4 weeks of age. These shocks were non-lethal but deleterious, because they decreased the longevity (mean longevity ± S.E.M. after the first shock: 30-min groups pooled: 10.65±0.12 days, 45-min groups: 8.95±0.10 days, not heat-shocked flies living for about 20 days). For each sex, the longevity after the first shock was analyzed with a factorial ANOVA (gravity, length of heat shock and replicate factors). Figure 1 reports the longevity curves after the first heat shock.

In males, longevity was lower with the 45-min heat shocks ($F(1, 1060) = 48.94$, $p<0.0001$; means ± S.E.M. of, respectively, 30 and 45 min groups: 10.63±0.19 days and 9.15±0.15 days). Males living at 5*g* at young age lived longer than those who have lived at 3*g* or 1*g* ($F(2, 1060) = 13.40$, $p<0.0001$; means ± S.E.M. of, respectively, 1, 3 and 5*g* groups: 9.48±0.23, 9.40±0.20, 10.71±0.21 days). The replicate effect was significant, as well as the interaction between the replicate and gravity factors (statistical analysis not shown, but see Fig. 2), showing

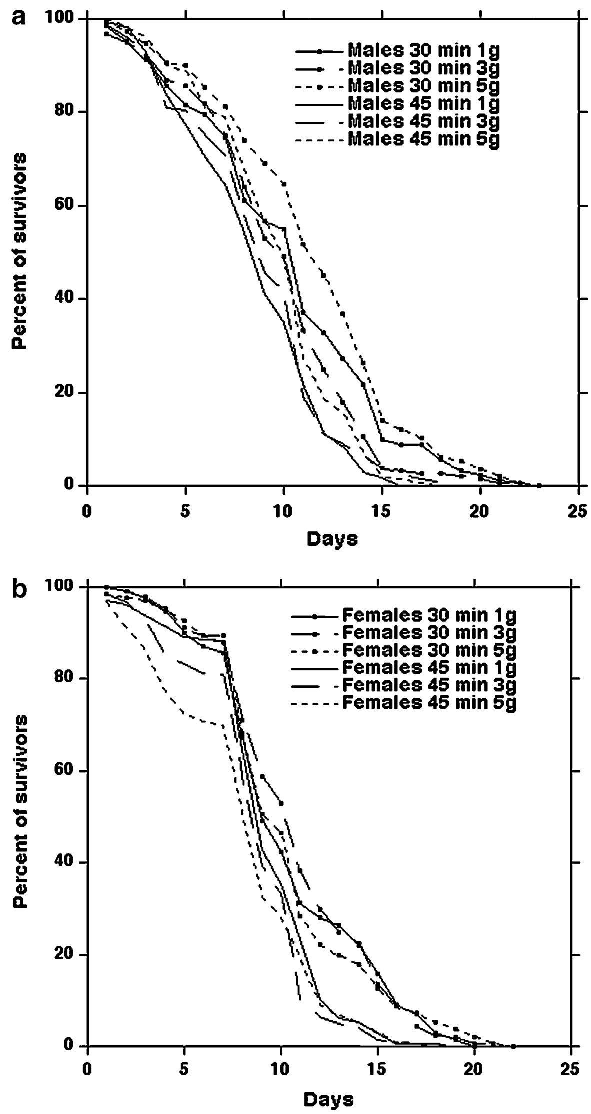

Fig. 1 Survival curves after 30 min or 45 min heat shocks of male (**a**) and female (**b**) flies which lived in HG at young age or always lived at 1*g*. Day 0 is the day of the first heat shock

that HG had no positive effect if heat had moderate effects on longevity; the other interactions were not significant.

In females, longevity was also lower with the 45-min heat shocks ($F(1, 1232) = 122.17$, $p<0.0001$; means ± S.E.M. of, respectively, 30 and 45 min groups: 10.67±0.16 days and 8.79±0.13 days) and HG females lived slightly shorter than 1*g* ones ($F(2, 1232) = 5.52$, $p=0.0041$; respectively for 1, 3 and 5*g* females: 9.96±0.17, 9.84±0.18, 9.36±0.20 days), the gravity effect being stronger with the long heat shock (gravity by shock interaction: $F(2, 1232) = 4.07$, $p=0.0172$). It has been shown that positive effects of mild stress can be not observed in females (Le Bourg and Minois 1997; Vaiserman et al. 2003). The replicate effect was significant, as well as all interactions involving this factor (statistical analysis not shown, but see Fig. 2).

Correlation coefficients between mean longevity of 1*g* flies and deviations from it of 3*g* or 5*g* flies were computed

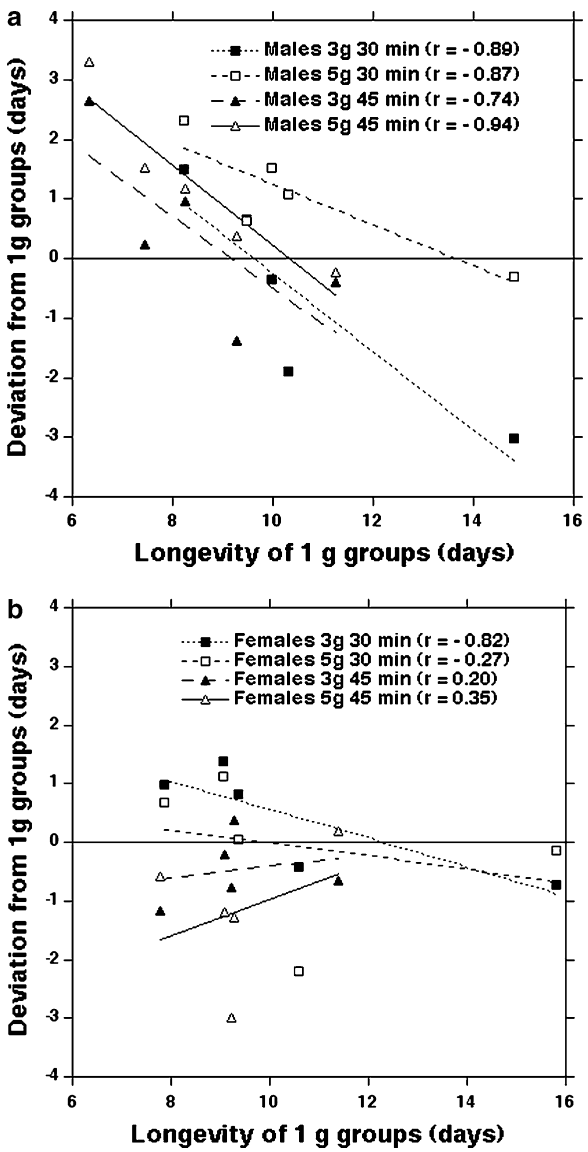

Fig. 2 Difference between the mean longevity after heat shocks of male (**a**) and female (**b**) flies which lived in HG at young age and that of flies always living at 1*g*. For each length of heat shock, five replicates were done (total n=2352). Each point stands for the mean of a replicate

in each group of sex, gravity and length of heat shock. As each fit used the means of five groups, i.e. three d.f., correlation coefficients lower than r=0.87 were not significant. The linear fits show that, in males, those who lived in HG at young age were protected against heat shocks when the deleterious effect of heat in 1*g* flies was important, i.e., when longevity was low in 1*g* flies (Fig. 2), the effect being significant (r=−0.89 or −0.94) or not (r=−0.87 or −0.74). In other words, living in HG at young age had nearly no positive effect if the deleterious effect of heat was moderate (and could even have some negative effect: see the 1*g* replicate living for ca. 15 days), but males took advantage of a stay in HG if the negative effect of heat was important.

By contrast, in females, living in HG at young age did not protect them against heat shocks at older ages (all correlation coefficients were not significant, the highest one being: r=0.82; see Fig. 2 for more details).

Discussion

The goal of this experiment was to know whether mild stresses applied at young age protected from more severe non-lethal stresses at old age. Studying non-lethal stresses is of interest because the 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.

Male flies successively heat-shocked for 30 min or 45 min from the age of 4 weeks lived longer if they had lived in HG for 2 weeks at young age, such an effect being not observed in females. Thus, HG exposure at young age can partially protect older male flies from successive deleterious non-lethal heat shocks. However, even if flies living in HG at young age lived longer than those always living at 1*g*, their lifespan was shorter than that of non-heat-shocked flies.

Some authors have hypothesized that mild stresses could have hormetic effects and help preventing some effects of aging (see, e.g., Human and Experimental Toxicology 20(6), 2001). 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 of hearts of 10-month-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.

Our previous paper (Le Bourg et al. 2004) reported that HG could partially protect flies from a single 60 min or 90 min heat shock occurring at 4 weeks of age but not at an older age. Thus, it was concluded that a mild stress can help flies to cope with heat stress, provided they are not too old, and that the physiological state of old flies probably did not allow them to take advantage from exposure to HG. The present results show that male flies are protected from milder but successive shocks occurring at the ages of 4 and 5 weeks. It thus seems that protection is enhanced against heat shocks for flies even if they are older than 4 weeks of age.

These results show that a mild stress applied at young age can protect older males from deleterious non-lethal heat shocks, but not the females. To the best of our knowledge, this is the first example of a treatment at young age protecting at older ages against a deleterious non-lethal stress. This encouraging result could stimulate the search for more efficient stresses that could be used not only in flies but also in mammals. It seems clear that HG could not be used in mammals because they are less tolerant to HG than flies (review in Le Bourg 1999), but one could use other mild stresses at young age only, for instance, hyperthermia or caloric restriction.

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