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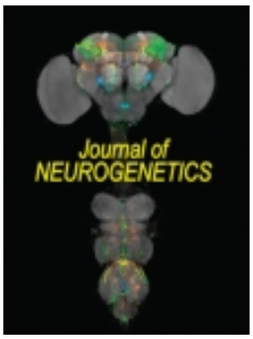

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Short Communication

Stress Resistance Conferred by Neuronal Expression of Dominant-Negative Rac in Adult *Drosophila melanogaster*

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Abstract: How does brain coordinate physiological and behavioral responses to achieve survival in adverse environment is intriguing yet complicated. During studies of the small G protein Rac's role in learning and memory, the authors unexpectedly observed that neuronal expression of dominant-negative Rac in adult *Drosophila* remarkably enhanced the survival of animals in various stress conditions, including oxidation, desiccation, starvation, and heat. The elevated stress resistance was not accompanied by a reduction in female fecundity or a change in whole-body lipid storage. The observation therefore implies the involvement of small G protein Rac in neuronal regulation of global stress responses.

Keywords: *Drosophila*, Rac, stress resistance

INTRODUCTION

Rac is a member of Rho GTPases. This family of small G proteins are best known for their roles in orchestrating actin cytoskeleton rearrangement and have been recognized as master regulators for a wide variety of cellular processes, ranging from phagocytosis of macrophages to morphogenesis of developing neurons (Etienne-Manneville & Hall, 2002). Their physiological functions in the mature nervous system, however, are just starting to be revealed. Taking advantage of the spatiotemporally confined expression system available in *Drosophila* (McGuire et al., 2003), we recently published a paper reporting that early memory forgetting is altered by induced expression of dominant mutants of *Drosophila Rac1* (*Drac1*) (Shuai et al., 2010). During that study, we noticed that neuronal expression of the dominant-negative form could dramatically elevate stress resistance, which is documented in detail below.

MATERIALS AND METHODS

Stress Resistance

All stress assays were performed at 25°C except for the thermal stress test. Flies were segregated by sex at the day before the experiment and were placed in food vials with 20 flies in each.

Paraquat-Induced Oxidative Stress

For oxidative stress test, flies starved for 6 hours were transferred to vials with 2 cm × 3 cm filter paper (Whatman) wetted with 200 μL of 20 mM paraquat (Sigma) in 5% sucrose solution. Percentages of flies that survived at 24 and 48 hours were scored.

Desiccation and Starvation

For desiccation test, flies were placed in empty vials, whereas for starvation test, the vials contained 2 cm × 3 cm filter paper (Whatman) moisturized with 200 μL of distilled water, which was refreshed every day to maintain moisture in the vials. In both tests, dead flies were counted every 6 to 8 hours.

Thermal Stress

Flies were transferred to fresh food vials with 2 cm × 3 cm filter paper (Whatman) and then placed in a 37°C incubator to test the effect of high temperature. Dead flies were counted every 4 to 6 hours.

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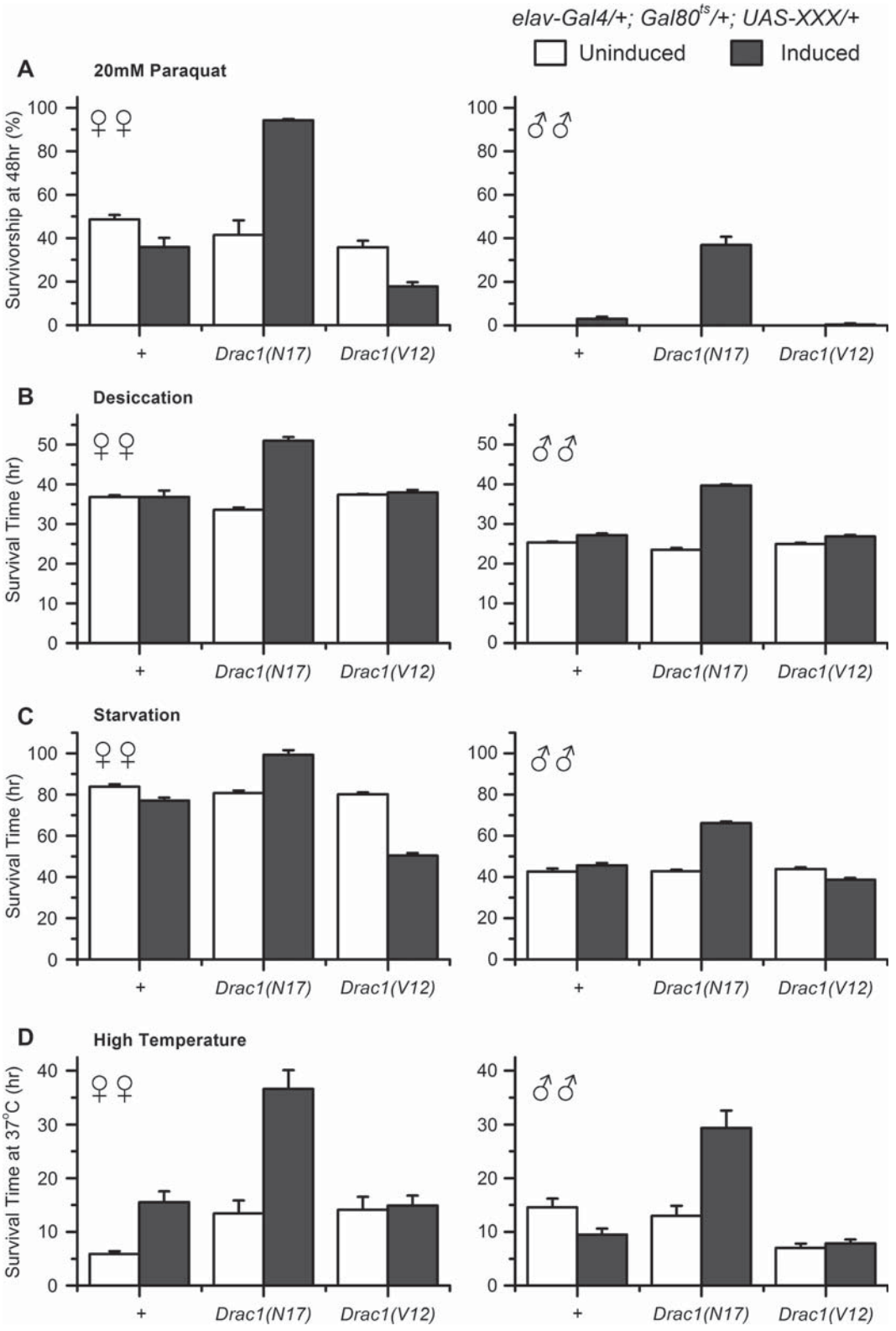

Figure 1. Elevated resistance to various stresses after heat-shock induced expression of Drac1(N17) in adult neurons. Flies were tested for responses to (**A**) oxidative stress induced by 20 mM paraquat ($N = 6$, total of 120 flies); (**B**) desiccation ($N = 6$, total of 120 flies); (**C**) starvation ($N = 8$, total of 160 flies); (**D**) thermal stress in high temperature ($N = 22$, total of 440 flies). Survival time was calculated as average of the group. Data were shown as mean ± SEM. In paraquat test in **A**, none of the male flies in the uninduced group survived to 48 hours.

Fecundity

Five virgin females from each indicated group were crossed with 10 w^{1118} *(isoCJ1)* control males. For 10 days following the heat-shock period, flies were transferred daily. The number of eggs laid in each vial was counted. Fecundity was then calculated as the number of eggs laid per female per day.

Triglyceride Assay

One fly from each indicated group was homogenized in 100 μL of phosphate-buffered saline (PBS) solution. For triglyceride measurement, 10 μL of the resulting homogenate was mixed with 190 μL of Triglyceride Reagent (catalog no. Y015C; Beijing BHKT Clinical Reagent, China) in a microplate. After incubation for 10 minutes at 37°C, OD_{550} was measured in the microplate reader and compared with the standardization curve. Triglyceride levels were shown as μg per fly.

Life Span Assay

Crosses were reared at 18°C. Three-day-old progeny were segregated by sex under light anesthesia, and housed in a 30°C incubator at a density of around 40 flies per food vial. Flies were transferred to fresh food vial every 2 to 3 days and the number of dead flies was recorded.

RESULTS AND DISCUSSION

Adult-onset neuronal expression of dominant-negative Drac1(N17) (Luo et al., 1994) was as described previously (Shuai et al., 2010). Male flies bearing *UAS-Drac1(N17)* were crossed with females bearing pan-neuronal *elav-Gal4* (Lin & Goodman, 1994)) and *tubulin-Gal80*^{ts} (*Gal80*^{ts}) (McGuire et al., 2003). The crosses were raised at 18°C. Three-day-old progeny were collected and equally divided into two groups, one as the induced group, which was subjected to heat shock at 30°C for 3 days to induce the transgene expression, the other as the uninduced group, which was kept continuously at 18°C and served as control. After the heat-shock period, both groups were allowed to recover at 25°C for 3 hours and then were subjected to stress tests along with the similarly treated parental control *elav-Gal4/+; Gal80*^{ts}*/+*.

As shown in Figure 1, the induced group of *elav-Gal4/+; Gal80*^{ts}*/+; UAS-Drac1(N17)/+* flies had a remarkable tolerance to all stresses tested, including paraquat-induced oxidative stress, desiccation, starvation and high temperature. These flies with Drac1(N17) expressed in the adult neurons usually survived 50% to 100% longer than controls in these adverse conditions. This generalized stress resistance was attributed to Drac1(N17) expression, since similarly induced expression of constitutively active Drac1(V12) (Luo et al., 1994) did not enhance stress resistance, but rather diminished survival in some stress conditions (Figure 1A–D).

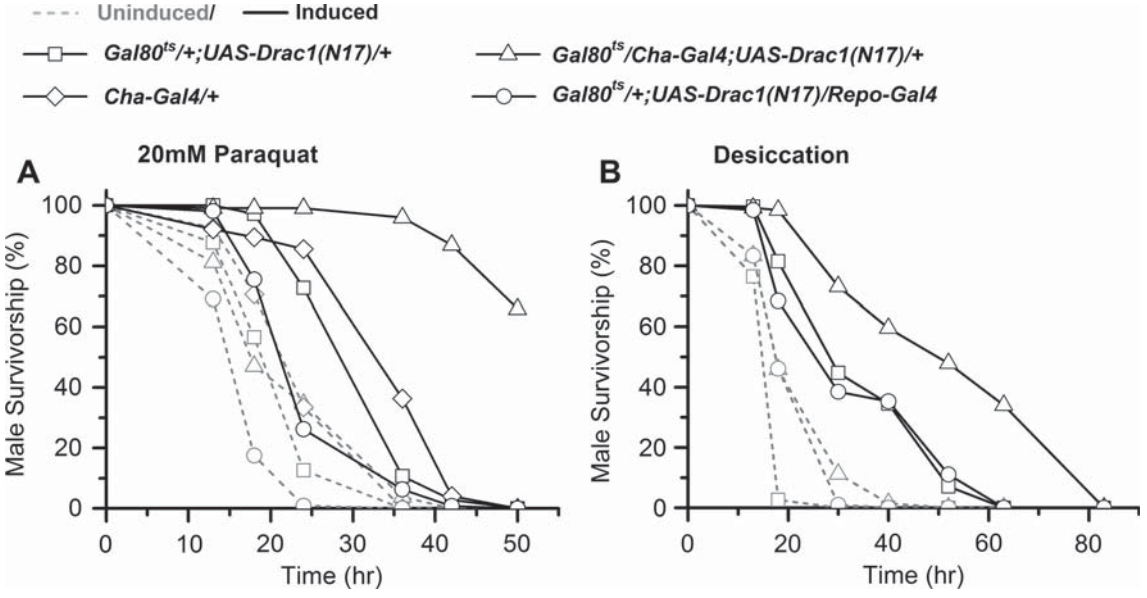

Figure 2. Stress resistance conferred by neuronal but not glial expression of Drac1(N17). Male survival curves in 20 mM paraquat (**A**) and desiccation (**B**) were depicted for the uninduced (gray) and induced (dark) groups of each genotype. Total number of flies for paraquat and desiccation test: *Gal80*^{ts}*/+; UAS-Drac1(N17)/+* (uninduced, 129 and 144; induced, 140 and 248), *Gal80*^{ts}*/Cha-Gal4; UAS-Drac1(N17)/+* (uninduced, 69 and 265; induced, 101 and 244), *Cha-Gal4/+* (uninduced, 108 and --; induced, 77 and --), *Gal80*^{ts}*/+; UAS-Drac1(N17)/Repo-Gal4* (uninduced, 145 and 267; induced, 147 and 189). *Cha-Gal4/+* was not tested in desiccation because insufficient progeny were collected during the experiment.

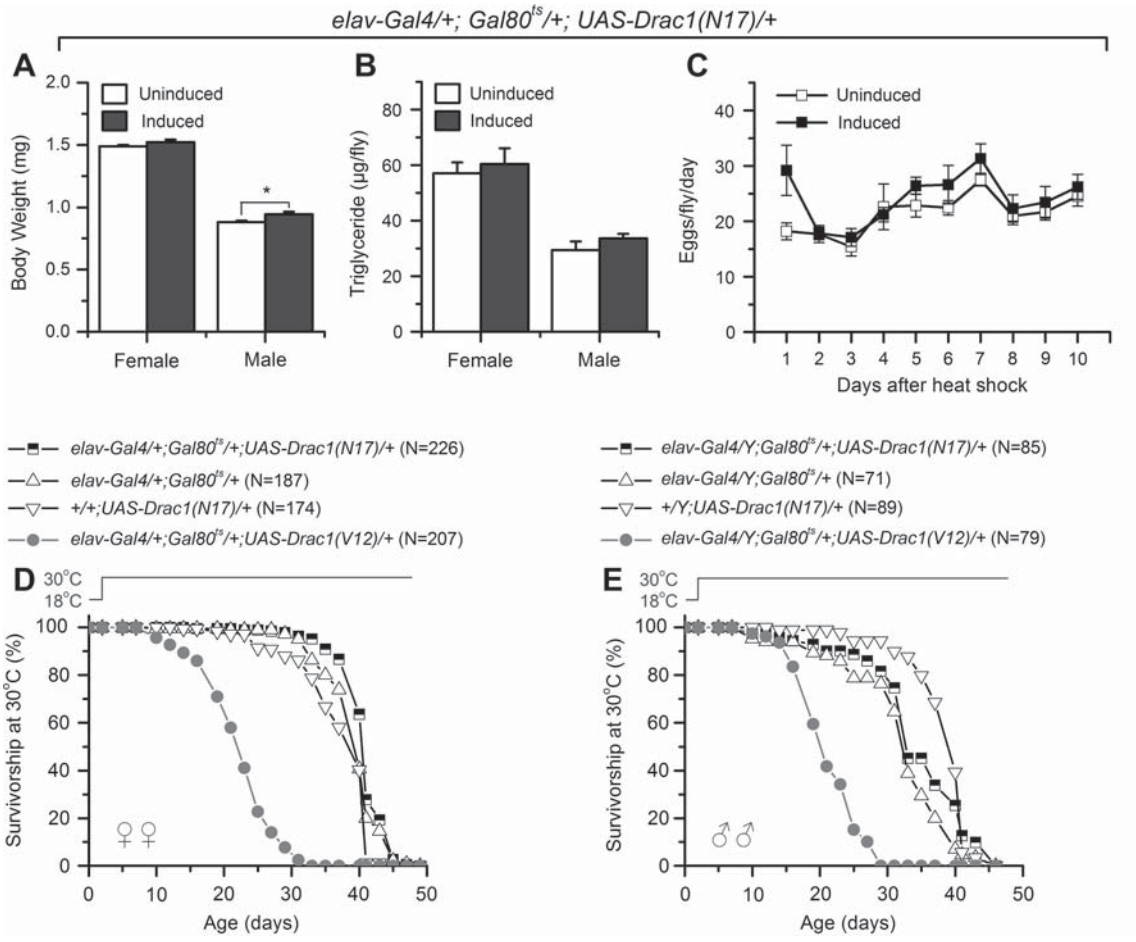

Figure 3. Measurements of energy storage, reproduction, and life span. (**A**) Wet weight. A marginal but statistically significant increase was found for the induced male flies (ANOVA, $p = .02$). $N = 8$, total of 80 flies. (**B**) Whole-body triglyceride levels. $N = 8$, total of 8 flies. (**C**) Female fecundity for the induced group was not compromised in 10 days following the heat-shock period. Fecundity at the first day was even higher than the uninduced control. However, that difference could also be attributed to nonspecific effect of the heat-shock treatment. $N = 8$, total of 40 flies. Data were shown as mean ± SEM. (**D**) Female and (**E**) male life spans at 30°C. The number of flies in each group is indicated in the figure.

We further confirmed the phenotype by using another widely expressed neuronal Gal4 specific for cholinergic neurons, *Cha-Gal4* (Salvaterra & Kitamoto, 2001). Male flies were tested for responses to 20 mM paraquat (Figure 2A) and desiccation (Figure 2B). It is noteworthy to mention that in this set of experiments the induced groups have better survivorship regardless of the genotypes. Presumably, the mild stress during the 3-day treatment at 30°C triggers certain protective mechanisms to acclimatize flies to stress survival (Tatar et al., 1997). Nonetheless, effects of Drac1(N17) expression are still clearly discernible on top of the nonspecific consequence from induction treatment itself. The induced group of *Gal80^{ts}/Cha-Gal4; UAS-Drac1(N17)/+* flies survived significantly longer than controls undergoing the same heat-shock treatment, whereas the uninduced group had a survival curve comparable to controls (Figure 2A, B). The glia-specific *Repo-Gal4* (Sepp et al., 2001) was included as an additional control, which did not show similar heat-shock dependent enhancement of stress resistance (Figure 2A, B).

The mechanism of the elevated stress resistance is yet to be known. The body weight of Drac1(N17)-expressing flies was largely comparable to their uninduced controls, although a marginal but statistically significant increase was found in males (Figure 3A). The level of triglyceride, the primary storage form of lipid, was not altered either (Figure 3B). The fecundity of female flies as determined by the number of eggs laid per fly per day was not compromised compared to that of control (Figure 3C). Thus, the obtained results do not appear to support explanations such as increase in energy storage or tradeoff in reproduction.

Generalized stress resistance is intimately connected with postponed aging and has been observed in numerous long-living *Drosophila* mutants (Lin et al., 1998; Wang

et al., 2004). We therefore performed preliminary life span measurements. To allow for continuous transgene expression through the lifetime, the measurements were performed at 30°C. Female and male data were shown in Figure 3D and E. A shortened life span was observed after expression of constitutively active Drac1(V12), but no obvious beneficial or detrimental effects were detected for dominant-negative Drac1(N17). The persistent nondiscriminable inhibition of Rac activity could possibly compromise some other neuronal function and eventually cancel the beneficial effects from stress resistance, which might explain the negative results on Drac1(N17). It would be of necessity in the future study to identify the specific signaling cascade mediating Drac1(N17)'s effect on stress responses and further test its relationship to aging.

In summary, we report the observation that induced expression of Drac1(N17) specifically in adult neurons confers fruit flies generalized stress resistance. The enhanced stress survival could arise from protection of neurons from damage during stress insult, which is supported by a recent mammalian study showing that depletion of Rac1 activity protects retinal rod cells from photooxidative stress (Haruta et al., 2009). Alternatively, accumulating evidence now emphasizes that the brain can sense and evaluate the environmental status, and then bring about adaptive change in physiology of the whole organism through the neuroendocrine/hormonal system (Libert & Pletcher, 2007). The normal triglyceride level and female fecundity do not favor a global change in energy storage and reproduction; however, it still awaits to be fully determined whether Rac signaling could possibly interact with humoral control in a more perplexing way and affect stress responses through systematic mechanisms.

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