Chronic stress elevates telomerase activity in rats

Annaliese K. Beery[1,*], **Jue Lin**[2], **Joshua S. Biddle**[3], **Darlene D. Francis**[3], **Elizabeth H. Blackburn**[2] and **Elissa S. Epel**[4]

[1]*Robert Wood Johnson Health & Society Scholar at University of California at San Francisco, San Francisco, CA 04143 and University of California at Berkeley, CA 94720, USA*
[2]*Departments of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, CA 94158-2517, USA*
[3]*Departments of Public Health and Helen Wills Neuroscience Institute, University of California at Berkeley, Berkeley, CA 94720-1650, USA*
[4]*Department of Psychiatry, University of California at San Francisco, San Francisco, CA 94143, USA*
Author for correspondence (abeery@smith.edu).

The enzyme telomerase lengthens telomeres— protective structures containing repetitive DNA sequences at chromosome ends. Telomere shortening is associated with diseases of ageing in mammals. Chronic stress has been related to shorter immune-cell telomeres, but telomerase activity under stress may be low, permitting telomere loss, or high, partially attenuating it. We developed an experimental model to examine the impacts of extended unpredictable stress on telomerase activity in male rats. Telomerase activity was 54 per cent higher in stressed rats than in controls, and associated with stress-related physiological and behavioural outcomes. This significant increase suggests a potential mechanism for resilience to stress-related replicative senescence.

1. INTRODUCTION

Stressful life experience alters health, life expectancy, and markers of cellular senescence, including the length of leucocyte telomeres [1,2]. Telomeres— repetitive DNA sequences at the ends of eukaryotic chromosomes—shorten with each cell division and typically decrease in length across the vertebrate life-course [2–4]. Telomere length is affected by life experience [1–3,5–7] and may reflect shortening after cell division, oxidative damage to DNA [8] and variable lengthening.

The primary mechanism of telomere length maintenance and lengthening is via the enzyme telomerase, which is active at high levels in germline and cancer cells, and at low levels in peripheral blood mononucleocytes (PBMCs) [9–11]. Telomerase deficiency is associated with premature immune and whole-body ageing, and enhanced telomerase activity prevents cellular senescence and promotes longevity [12–14], demonstrating a role for telomerase in the regulation of ageing.

The effects of life experience on telomerase expression are unclear. Telomerase regulation occurs at several molecular levels, and observational studies have yielded varying findings. In healthy women, PBMC telomerase expression was inversely associated with duration and perception of stress [1]. Data from other cohorts suggest that telomerase activity may increase as a compensatory mechanism for acute stress, chronic stress and/or depression [15,16]. Contradictory findings may be partly attributable to cohort differences, doses/durations of stress or comorbid conditions. Studies of wild-derived house mice indicate potential for telomere length change in rodents in response to reproductive, social and immunological stressors [2,5], and studies of laboratory mice have documented transient enhancement in telomerase expression following immune challenge [17].

Many organisms initiate physiological adaptations to stress that may be protective [18,19]. In the first experimental study of the effects of chronic stress on telomerase activity, we investigated whether leucocyte telomerase activity was enhanced or suppressed following exposure to chronic unpredictable stress in laboratory rats. Stressed rats exhibited reduced body weight, increased anxiety-like behaviour in three tests, and altered corticosterone secretion consistent with the experience of stress. These expected changes were concomitant with the observation of elevated telomerase activity.

2. MATERIAL AND METHODS

(a) Animals

Long–Evans rats (Charles River, Inc.) were bred locally. Maternal observations were conducted 5 h per day for 6 days as in Sakhai et al. [20]. Because maternal grooming frequency influences anxiety behaviour [21], young experiencing grooming ± 1 s.d. from mean were excluded. From eight to 20 weeks of age, male rats were exposed to randomized stressors 5 days per week (stress group, $n = 16$), or housed in standard conditions (control group, $n = 14$). Stressors consisted of: 5 min swim, 5 min fox urine odour exposure, 3 h water bottle removal, 1 h cage tilt (30°), less than or equal to 5 min weak tail pinch, 0.1 cc saline injection or 15 min restraint. Rats were weighed weekly. Blood (7 ml) was extracted at sacrifice and processed for telomerase activity. Corticosterone concentrations were assayed in separate 'cort-controls' to avoid stress exposure in the main controls.

(b) Behavioural tests

In order to examine the extent to which stress treatment induced expected changes related to anxiety [22], we examined behaviour in three contexts. Light–dark box (LDbox): rats were placed in the dark portion of a black Plexiglas box ($38 \times 38 \times 20$ cm) connected to a clear lidless chamber ($38 \times 38 \times 38$ cm). Measures included time in the light chamber, latency to exit the dark box, and activity. Open-field (OF): exploratory behaviour was assessed in a circular open arena (diameter = 152 cm). Time spent within 15 cm of the wall, latency to enter the centre and activity were scored. Elevated plus-maze (EPM): the plus-maze was constructed of black Plexiglas with two open and two enclosed arms (10×112 cm), elevated to 50 cm. Latency to enter the light arm, time in light : dark arms and activity were scored. Behavioural tests lasted 5 min and were video-recorded. Three tests were excluded: two recordings less than 5 min (1 OF, 1 EPM), and one rat who fell from the EPM.

(c) Corticosterone assay

Plasma corticosterone was assessed by enzyme immunoassay (Assay Designs Corticosterone EIA; sensitivity = 27 pg ml^{-1}). Blood was collected by tail nick within 1 min of capture prior to restraint, following 20 min restraint, and at four additional 30 min intervals. Samples were centrifuged (4°C, 20 min), and plasma was stored at −80°C. Samples were thawed, centrifuged, diluted 1 : 20 with assay buffer and aliquoted into 96-well plates in duplicate with six standards (32–20 000 pg ml^{-1}) and reference samples. Mean variation was 4.2 per cent intra-assay, 8 per cent inter-assay.

(d) *Telomerase activity*

Blood samples were mixed 1 : 1 with phosphate-buffered saline and separated by Ficoll gradient. One extraction yielded insufficient PBMCs and was excluded (final $n = 14$ control, 15 stress). Counts were standardized to 1×10^6 cells by haemocytometer and stored at $-80°C$ in 1X CHAPS lysis buffer. Telomerase assays were conducted as reported in Lin *et al.* [23], briefly: Gel-TRAP reactions were performed by telomerase repeat amplification protocol (TRAPeze telomerase detection kit, Upstate/CHEMICON) using 2000–10 000 cells and run on 10 per cent polyacrylamide–8M urea sequencing gels. Gels were exposed to a phosphorimager plate overnight and scanned. Telomerase activity is expressed as equivalent of 293T cancer cells. 1 unit = amount of product from one 293T cell/10 000 immune cells. Values did not deviate significantly from normal (Shapiro–Wilk), and are reported untransformed.

3. RESULTS

Stressed rats exhibited 54 per cent greater telomerase activity after 12-week exposure than same-age control rats ($t_{27} = 2.3$, $p = 0.029$; figure 1a). Higher telomerase activity was accompanied by physiological and behavioural responses to stress. Stressed rats weighed less than control rats within two weeks of onset ($t_{28} = 6.2$, $p < 0.0001$; figure 1b). However, inclusion of terminal mass in a generalized linear model did not improve model fit ($p = 0.99$, electronic supplementary material), indicating body mass did not drive telomerase differences.

After 12 weeks, corticosterone levels in stressed rats, while matching baseline levels in the cort-control population (0.64 ± 0.24 versus 0.57 ± 0.14, mean $\pm$ s.e.m.), increased and cleared faster in response to a standardized stressor (figure 1c; $t_{33} = 3.0$ $p < 0.005$ peak timing; $t_{33} = 2.1$ $p < 0.05$ concentration 90 min post-restraint). Area under the curve did not differ ($t_{33} = 0.87$, $p = 0.18$). The relationship between telomerase activity and corticosterone secretion could not be assessed (because of distinct control groups).

Stress and control rats differed on nearly all behavioural test attributes. Stressed rats spent less time in the centre of the open field ($t_{27} = 4.7$, $p < 0.0001$; figure 2a), with fewer zone crossings ($p < 0.0001$). Stressed rats emerged to the open arm of the EPM later ($t_{26} = 4.4$, $p < 0.005$), with less time in the open : closed arm ($t_{26} = 3.0$, $p = 0.006$; figure 2b), and fewer zone crossings ($t_{26} = 3.5$, $p < 0.005$). Finally, stressed rats exhibited higher latency to exit the LD box ($t_{28} = 4.85$, $p < 0.0001$) with less time in the light ($t_{28} = 3.5$, $p = 0.017$; figure 2c).

Correlation of telomerase activity with behaviour across treatments revealed more anxiety-like behaviours in rats with higher telomerase expression, including less time in the centre of the open field ($r = 0.39$, $p = 0.038$; figure 1d), later initial exit from the light–dark box ($r = 0.46$, $p = 0.05$ for rats who exited), and a trend towards greater latency to move on the EPM ($r = 0.36$, $p = 0.06$).

4. DISCUSSION

This is the first experimental demonstration that prolonged stress upregulates telomerase activity in leucocytes *in vivo*. This increase was accompanied by changes in body mass, corticosterone secretion and anxiety-like behaviours, indicating that stressors were effective. Tests such as the open-field measure fear of
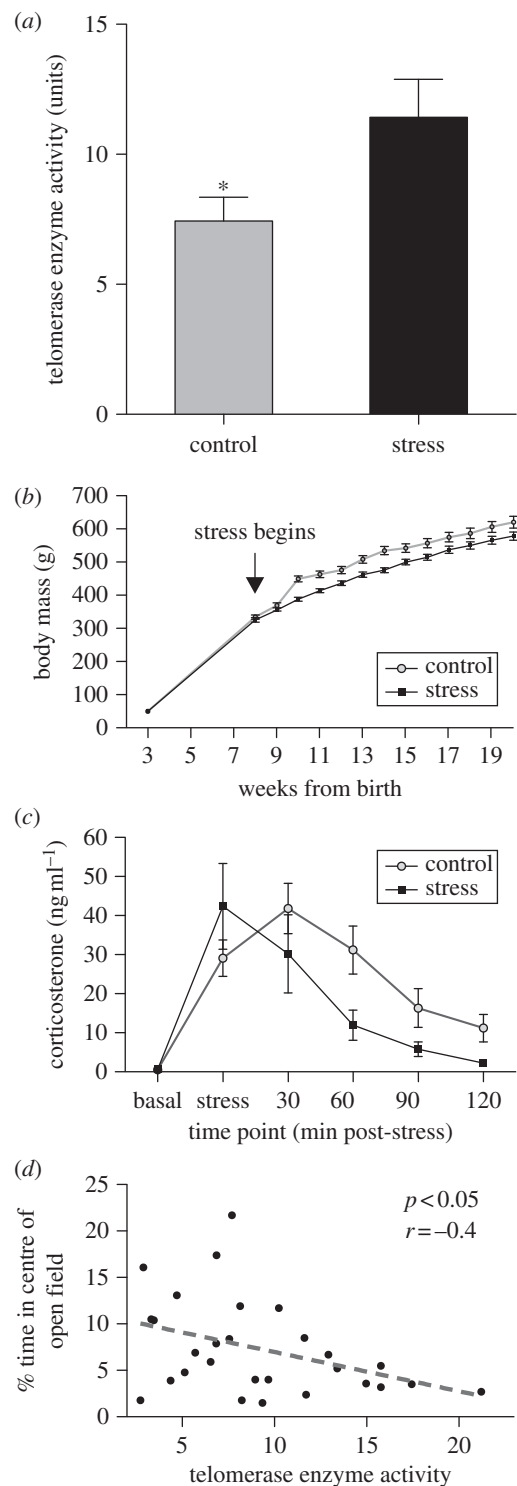
Figure 1. Physiological differences between control and stressed rats (mean $\pm$ s.e.m.). (*a*) Individuals exposed to stress exhibited higher leucocyte telomerase activity in standardized cell counts ($p < 0.05$). (*b*) Growth was reduced within two weeks of stress onset ($p < 0.05$ all subsequent timepoints). (*c*) Stressed rats downregulated corticosterone more efficiently than cort-controls following restraint, showing earlier peak timing ($p < 0.005$). (*d*) Rats with higher telomerase activities also displayed greater behavioural anxiety in open-field tests.

novelty/open spaces, with less time in the centre indicating a greater threat response [24]. Avoidance of the centre was associated with a higher telomerase activity, which we interpret to mean that rats perceiving greater stress had greater compensatory telomerase responses.
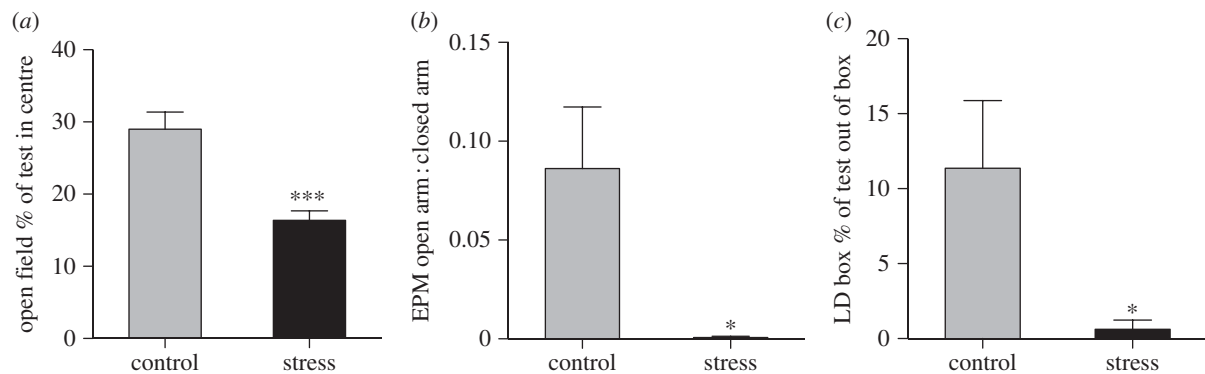
Figure 2. Stressed rats showed greater anxiety-like behaviour than controls. (*a*) Stressed rats spent less of a 5 min open-field test in the centre, (*b*) less time in the open : closed arm of an elevated plus maze, and (*c*) less time in the light portion of a light–dark box. $*p < 0.05$, $***p < 0.005$.

This is consistent with human findings that degree of perceived threat correlates with telomerase activity immediately after acute stress [15].

While prolonged stress is ultimately deleterious, acute or intermediate stress durations may lead to counter-regulatory coping mechanisms, including upregulation of telomerase. We propose this may be part of a cyto-protective response that reduces oxidative stress and DNA damage. This is the opposite of the finding that telomerase activity was reduced in chronically stressed carers [1], potentially reflecting differences in extent or duration of stress, and exhaustion of coping reserves in the carers.

High doses of cortisol reduce telomerase activity *in vitro* [25], and exposure to oxidative stress shortens telomeres [8]. Telomerase activity may nonetheless reduce the impact of oxidative stress, as it confers resistance to physical and chemical stressors in cells with short telomeres [26]. Despite matching baselines, stressed rats downregulated corticosterone responses faster than controls, another potential sign of beneficial physiological adaptation to stress.

Changes in leucocyte subtype distributions in peripheral blood do not appear to account for increases in PBMC telomerase after short-term stress in humans [15]. Nonetheless, telomerase increases in the present study could be due to lymphocyte activation, and/or redistribution of cell subtypes towards those with higher telomerase, e.g. B-lymphocytes [23]. Previous studies of stress effects on immune response in rats have found suppression of B-lymphocytes is only temporary after cold stress or social stress, with return to baseline by 48 h and no change after chronic restraint [27–29]. Future studies should confirm that leucocyte subtype redistribution is not the cause of elevated telomerase activity following chronic stress. Females should also be studied, as sex differences in telomerase activity have been detected in somatic tissues [30].

It is well established that mild exposure to stress activates anti-ageing mechanisms–a phenomenon sometimes described as hormesis (reviewed in Epel [31]). This study demonstrates that PBMC telomerase activity increases in response to three-month chronic stress, potentially indicating long-term hormesis. Healthy young individuals may more easily mount telomerase responses, which in turn should protect their telomeres. Given known functions of telomerase in maintaining telomere length and other aspects of cellular health, this finding provides a potential cellular mechanism for understanding resilience to mild chronic stress and possibly longevity.

1 Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D. & Cawthon, R. M. 2004 Accelerated telomere shortening in response to life stress. *Proc. Natl Acad. Sci. USA* **101**, 17 312–17 315. (doi:10.1073/pnas.0407162101)

2 Kotrschal, A., Ilmonen, P. & Penn, D. J. 2007 Stress impacts telomere dynamics. *Biol. Lett.* **3**, 128–130. (doi:10.1098/rsbl.2006.0594)

3 Epel, E. S. 2009 Telomeres in a life-span perspective: a new 'psychobiomarker'. *Curr. Dir. Psychol. Sci.* **18**, 6–10. (doi:10.1111/j.1467-8721.2009.01596.x)

4 Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B. & Monaghan, P. 2012 Telomere length in early life predicts lifespan. *Proc. Natl Acad. Sci. USA* **109**, 1743–1748. (doi:10.1073/pnas.1113306109)

5 Ilmonen, P., Kotrschal, A. & Penn, D. J. 2008 Telomere attrition due to infection. *PLoS ONE* **3**, e2143. (doi:10.1371/journal.pone.0002143)

6 Shlush, L. I. *et al.* 2011 Telomere elongation followed by telomere length reduction, in leukocytes from divers exposed to intense oxidative stress-implications for tissue and organismal aging. *Mech. Ageing Dev.* **132**, 123–130. (doi:10.1016/j.mad.2011.01.005)

7 Turbill, C., Smith, S., Deimel, C. & Ruf, T. 2012 Daily torpor is associated with telomere length change over winter in Djungarian hamsters. *Biol. Lett.* **8**, 304–307. (doi:10.1098/rsbl.2011.0758)

8 Cattan, V. *et al.* 2008 Chronic oxidative stress induces a tissue-specific reduction in telomere length in CAST/ei mice. *Free Radic. Biol. Med.* **44**, 1592–1598. (doi:10.1016/j.freeradbiomed.2008.01.007)

9 Armanios, M. & Greider, C. W. 2005 Telomerase and cancer stem cells. *Cold Spring Harb. Symp. Quant. Biol.* **70**, 205–208. (doi:10.1101/sqb.2005.70.030)

10 Blackburn, E. H. 2005 Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett.* **579**, 859–862. (doi:10.1016/j.febslet.2004.11.036)

11 Marión, R. M. & Blasco, M. A. 2010 Telomeres and telomerase in adult stem cells and pluripotent embryonic stem cells. *Adv. Exp. Med. Biol.* **695**, 118–131. (doi:10.1007/978-1-4419-7037-4-9)

12 Yamashita, S., Ogawa, K., Ikei, T., Udono, M., Fujiki, T. & Katakura, Y. 2012 SIRT1 prevents replicative senescence of normal human umbilical cord fibroblast through potentiating the transcription of human telomerase reverse transcriptase gene. *Biochem. Biophys. Res. Commun.* **417**, 630–634. (doi:10.1016/j.bbrc.2011.12.021)

13 Bernardes de Jesus, B., Vera, E., Schneeberger, K., Tejera, A. M., Ayuso, E., Bosch, F. & Blasco, M. A. 2012 Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol. Med.* **4**, 691–704. (doi:10.1002/emmm.201200245)

14 Jaskelioff, M. *et al.* 2011 Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature* **469**, 102–106. (doi:10.1038/nature09603)

15 Epel, E. S., Lin, J., Dhabhar, F. S., Wolkowitz, O. M., Puterman, E., Karan, L. & Blackburn, E. H. 2010 Dynamics of telomerase activity in response to acute psychological stress. *Brain Behav. Immun.* **24**, 531–539. (doi:10.1016/j.bbi.2009.11.018)

16 Damjanovic, A. K., Yang, Y., Glaser, R., Kiecolt-Glaser, J. K., Nguyen, H., Laskowski, B., Zou, Y., Beversdorf, D. Q. & Weng, N. P. 2007 Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J. Immunol.* **179**, 4249–4254.

17 Hathcock, K. S., Jeffrey Chiang, Y. & Hodes, R. J. 2005 *In vivo* regulation of telomerase activity and telomere length. *Immunol. Rev.* **205**, 104–113. (doi:10.1111/j.0105-2896.2005.00267.x)

18 Costantini, D., Metcalfe, N. B. & Monaghan, P. 2010 Ecological processes in a hormetic framework. *Ecol. Lett.* **13**, 1435–1447. (doi:10.1111/j.1461-0248.2010.01531.x)

19 Karatsoreos, I. N. & McEwen, B. S. 2011 Psychobiological allostasis: resistance, resilience and vulnerability. *Trends Cogn. Sci.* **15**, 576–584. (doi:10.1016/j.tics.2011.10.005)

20 Sakhai, S. A., Kriegsfeld, L. J. & Francis, D. D. 2011 Maternal programming of sexual attractivity in female long Evans rats. *Psychoneuroendocrinology* **36**, 1217–1225. (doi:10.1016/j.psyneuen.2011.02.016)

21 Beery, A. K. & Francis, D. D. 2011 Adaptive significance of natural variations in maternal care in rats: a translational perspective. *Neurosci. Biobehav. Rev.* **35**, 1552–1561. (doi:10.1016/j.neubiorev.2011.03.012)

22 Walf, A. A. & Frye, C. A. 2007 The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc.* **2**, 322–328. (doi:10.1038/nprot.2007.44)

23 Lin, J., Epel, E., Cheon, J., Kroenke, C., Sinclair, E., Bigos, M., Wolkowitz, O., Mellon, S. & Blackburn, E. 2010 Analyses and comparisons of telomerase activity and telomere length in human T and B cells: insights for epidemiology of telomere maintenance. *J. Immunol. Methods* **352**, 71–80. (doi:10.1016/j.jim.2009.09.012)

24 Belzung, C. 1999 Measuring rodent exploratory behavior. In *Techniques in the behavioral and neural sciences* (eds W. E. Crusio & R. T. Gerlai), pp. 738–749. Amsterdam, The Netherlands: Elsevier.

25 Choi, J., Fauce, S. R. & Effros, R. B. 2008 Reduced telomerase activity in human T lymphocytes exposed to cortisol. *Brain Behav. Immun.* **22**, 600–605. (doi:10.1016/j.bbi.2007.12.004)

26 Rubio, M. A., Davalos, A. R. & Campisi, J. 2004 Telomere length mediates the effects of telomerase on the cellular response to genotoxic stress. *Exp. Cell Res.* **298**, 17–27. (doi:10.1016/j.yexcr.2004.04.004)

27 Goundasheva, D., Andonova, M. & Ivanov, V. 1994 Changes in some parameters of the immune response in rats after cold stress. *Zentralbl. Veterinarmed. B* **41**, 670–674.

28 Stefanski, V. & Engler, H. 1998 Effects of acute and chronic social stress on blood cellular immunity in rats. *Physiol. Behav.* **64**, 733–741. (doi:10.1016/S0031-9384(98)00127-9)

29 Basso, A. M., Depiante-Depaoli, M., Cancela, L. & Molina, V. A. 1994 Chronic restraint attenuates the immunosuppressive response induced by novel aversive stimuli. *Physiol. Behav.* **55**, 1151–1155. (doi:10.1016/0031-9384(94)90403-0)

30 Leri, A., Malhotra, A., Liew, C. C., Kajstura, J. & Anversa, P. 2000 Telomerase activity in rat cardiac myocytes is age and gender dependent. *J. Mol. Cell Cardiol.* **32**, 385–390. (doi:10.1006/jmcc.1999.1084)

31 Epel, E. S. 2009 Psychological and metabolic stress: a recipe for accelerated cellular aging? *Hormones (Athens)* **8**, 7–22.