**Electronic supplementary material**

**Traffic noise exposure affects telomere length in nestling house sparrows** Alizée Meillère, François Brischoux, Cécile Ribout, Frédéric Angelier

**Supplementary Methods**

1. ***Experimental noise exposure***

We recorded the traffic noise used for playback 5 meters from a 4-lane highway near Paris (48°44’52N, 2°11’49E) with a Zoom H4n recorder (Zoom Corporation, Tokyo, Japan). The traffic noise recording used in the experiment and all the sound analyses and experimental procedures are described in detail in a related article [1]. Briefly, we started sound treatment several weeks before sparrows began laying eggs. To broadcast the traffic noise recording for the “disturbed treatment”, we used iPod shuffles (Apple Inc., Cupertino, CA) connected to Logitech LS11 stereo speakers (Logitech, Fremont, CA; frequency response: 70-20000 Hz) that were hidden approximately 3-4 meters from the nest-boxes (2 speakers for 4-5 neighbouring nest-boxes). The traffic noise recording was played in a loop, six hours a day (from 9 to 12 am and from 2 to 5 pm), seven days a week. As a result, disturbed nest-boxes were exposed to traffic noise that produced noise levels similar to those experienced by birds breeding in urban environments (disturbed nest-boxes: 63.32 ± 1.65 dB(A) at the entrance hole, urban areas: 61.35 ± 1.21 dB(A)), but higher than those experienced by birds breeding in control nest-boxes (43.04 ± 0.47 dB(A) at the entrance hole, see [1] for details). Moreover, because disturbed and control nest-boxes were located in the same site, this experimental design allowed us to separate the effects of noise from other confounding environmental factors (e.g. food availability, predation pressure). Over the course of the study, we checked all nest-boxes every two days to determine laying dates, clutch sizes, hatching dates and brood sizes. Occupancy rates (Pearson’s Chi-squared test: *Χ²1* = 0.02, *p* = 0.881; disturbed: 52.4 %, control: 54.3 %), laying dates (*t*-test: *t* = 0.12, *p* = 0.908) and clutch sizes (Wilcoxon-Mann-Whitney test: *Z* = -0.28, *p* = 0.779; disturbed: 5.0 ± 0.3 eggs, control: 4.9 ± 0.4 eggs) did not differ between control and disturbed nests.

1. ***Nestling body size and condition***

When nestlings were 9-days old, we measured their wing (steel rule: ± 0.5 mm), tarsus, and bill lengths (caliper: ± 0.1 mm), and their body mass (electronic balance: ± 0.1 g). To assess nestling size and condition [2], a body size index was calculated using the first factor from a principal component analysis on the three body size measurements. The first factor explained 91.1 % of the size variation in nestlings. Body condition was then expressed as the residual mass from a linear regression relating body mass to body size (r² = 0.81, n = 37, *p* < 0.001).

1. ***Molecular sexing, corticosterone and telomere assays***

All laboratory analyses were performed at the Centre d’Etudes Biologique de Chizé (CEBC). Plasma corticosterone levels were measured in duplicate by radio-immunoassay, as previously described [3]. The minimum detectable corticosterone level was 0.83 ng.mL-1, and the intra- and inter-assay coefficients of variation were 7.07% and 9.99% respectively. Because two nestlings were not blood sampled within 3 minutes of capture, their corticosterone levels could not be considered to reflect baseline levels [4], and were thus removed from corticosterone analyses.

The sex of nestlings was determined by molecular sexing as detailed in [5]. Telomere lengths were measured using a real-time quantitative PCR (qPCR) technique previously validated for birds (see [6] for details). We selected the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene as our reference gene (‘single-copy gene’) because it has already been successfully used for telomere measurements by qPCR in birds, and more specifically, in passerine species [6–9]. Moreover, this was confirmed by the presence of only one peak in the dissociation curve. This qPCR method has been successfully used in several vertebrate species and gives similar results to the Telomere Restriction Fragment method (TRF, [6–10]). Samples were first digested with proteinase K, and DNA was then extracted from red blood cells using DNeasy Blood and Tissue Kit (Qiagen). DNA quality was checked by gel electrophoresis and optical density spectrophotometry [11], and there was no sign of DNA degradation. qPCR for both GAPDH and telomeres was performed using 5 ng of DNA per reaction. The telomere primers (Tel1b and Tel2b) were used at a concentration of 800 nM. The GAPDH primers (GAPH\_F and GAPDH\_R) were used at a concentration of 200 nM. The 96-well plate included a standard curve, which consisted of various concentrations of pooled house sparrow DNA (10-2.5 ng.mL-1). This standard curve was used to generate a reference curve to control for the amplifying efficiency of the qPCR [6]. The efficiency of our qPCR was within the acceptable range for both GAPDH and telomere qPCR (GAPDH: 106 %, r² = 0.987, Telomere: 104%, r² = 0.958). For both GAPDH and telomeres, all samples were run on a single plate and the intra-plate CVs were respectively 0.47% for GAPDH and 1.27 % for telomere qPCR.

1. ***Statistical analysis***

All statistical analyses were performed in R.3.1.0 [12]. We analysed “body size”, “body condition”, baseline corticosterone levels, and “telomere length” data using linear mixed models (LMMs, normal error distribution, identity link function). We used “sound treatment” (disturbed vs. control), “sex” (male vs. female) and “brood size” as independent variables/factors, and nest identity as a random factor to control for the non-independence of siblings. Each full model also included all 2-way interactions between “sound treatment” and other independent variables. For telomere and corticosterone analyses, we also included “body condition” as an independent variable because condition could possibly affect both telomere length and baseline corticosterone level (the results were similar when using body size or body mass in the model). Telomere lengths and corticosterone levels were log-transformed to ensure the normality of model residuals, but we present non-transformed values in Figure 1 to facilitate interpretation. LMMs were fitted using restricted maximum likelihood (REML) estimation. Finally, because all 9-days old nestlings of a given nest were either all successful at fledging or all failed, we could not analyse fledging success using mixed models with nest identity as random factor. Thus, we analysed the data on fledging success on a per brood basis (proportion of nestlings that fledged; binary response variable 0/1), using generalized linear models (GLMs, binomial error distribution, logit link function) with “sound treatment”, “brood size” and their interaction as independent variables/factors.

**Supplementary Analysis**

To better test whether parent quality could have differed across the two sound treatments, we performed supplementary analyses using body size and condition of parents as proxies for individual quality.

1. ***Parent body size and condition***

13 parents (out of the 16 studied nests) were captured at their nest-box during the chick-rearing period. Captured adults were measured (wing, tarsus, and bill length), weighed and released at their nest. As for nestlings, a body size index was calculated using the first factor from a principal component analysis on the three body size measurements. The first factor explained 50.8 % of the size variation. Body condition was then expressed as the residual mass from a linear regression relating body mass to body size (r² = 0.58, n = 13, p = 0.0014).

1. ***Statistical analysis***

To test whether body size and body condition of parents differed across the two sound treatments, we used two-way ANOVAs with “sound treatment” (disturbed vs. control), “sex” (male vs. female) and interaction as independent factors.

1. ***Results***

Parent’s body size and condition did not differ between sound treatments (ANOVA: body size: *F*1,11 = 0.31, *p* = 0.591; body condition: *F*1,11 = 1.22, *p* = 0.293). Moreover, there were no significant effects of sex or the “sound treatment × sex” interaction (all p > 0.127). We obtained the same results when analyses were done using the body size measurements or body mass (all *p* > 0.577).

**Data used for analysis**

1. ***Nestlings***

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **nest** | **treatment** | **sex** | **brood size** | **size** | **condition** | **cort** | **telomere length** | **fledging** |
| A | disturbed | M | 4 | -3.550 | -1.486 | 7.38 | 0.829 | no |
| A | disturbed | F | 4 | -1.721 | 0.520 | 0.95 | 1.155 | no |
| A | disturbed | F | 4 | -1.158 | 0.568 | 1.72 | 0.986 | no |
| B | disturbed | F | 5 | -1.263 | 1.246 | 0.85 | 1.121 | no |
| C | control | F | 4 | -0.281 | 0.985 | 4.31 | 1.102 | yes |
| C | control | F | 4 | -0.642 | -1.304 | 5.93 | 1.202 | yes |
| D | disturbed | M | 4 | 0.561 | -1.979 | 3.86 | 1.072 | yes |
| D | disturbed | M | 4 | 1.239 | -2.227 | 4.76 | 1.128 | yes |
| D | disturbed | M | 4 | 1.838 | -0.631 | 6.92 | 1.237 | yes |
| E | control | F | 5 | -1.567 | 1.630 | NA | 1.549 | no |
| E | control | F | 5 | -2.043 | 0.145 | NA | 1.485 | no |
| E | control | F | 5 | -2.553 | 0.648 | 8.61 | 1.067 | no |
| E | control | M | 5 | -2.895 | 0.686 | 10.45 | 1.135 | no |
| F | control | F | 2 | 1.853 | 2.456 | 1.84 | 1.116 | yes |
| F | control | F | 2 | 2.019 | 1.135 | 4.86 | 1.081 | yes |
| G | disturbed | F | 5 | 0.513 | -0.558 | 12.52 | 1.054 | no |
| G | disturbed | M | 5 | 1.047 | 0.638 | 7.26 | 1.002 | no |
| G | disturbed | M | 5 | 2.245 | 3.211 | 0.95 | 0.960 | no |
| G | disturbed | M | 5 | 1.162 | -0.557 | 5.39 | 0.843 | no |
| H | control | M | 5 | -1.677 | -0.254 | 8.89 | 1.339 | no |
| H | control | F | 5 | -2.043 | -0.835 | 4.57 | 1.187 | no |
| H | control | M | 5 | 1.329 | -0.138 | 1.16 | 1.454 | no |
| H | control | F | 5 | -1.126 | -1.087 | 3.32 | 1.090 | no |
| I | control | F | 5 | -2.019 | 2.424 | 6.32 | 0.948 | yes |
| J | disturbed | F | 5 | -1.009 | 1.115 | 5.39 | 0.866 | no |
| K | disturbed | M | 5 | -0.623 | -0.537 | 1.36 | 1.087 | no |
| L | control | M | 6 | 1.290 | 0.328 | 1.56 | 0.961 | yes |
| L | control | M | 6 | 0.844 | -2.319 | 1.95 | 1.194 | yes |
| M | disturbed | M | 4 | -0.565 | -1.135 | 2.16 | 1.410 | no |
| M | disturbed | F | 4 | -0.099 | 0.177 | 18.35 | 0.935 | no |
| N | disturbed | F | 5 | 2.780 | -0.893 | 1.38 | 1.014 | yes |
| N | disturbed | F | 5 | 1.110 | -1.568 | 5.73 | 0.929 | yes |
| N | disturbed | F | 5 | 0.154 | -2.451 | 7.52 | 1.017 | yes |
| N | disturbed | M | 5 | 2.140 | -0.510 | 2.26 | 1.086 | yes |
| O | control | F | 4 | 1.379 | 0.977 | 1.47 | 1.179 | yes |
| P | disturbed | M | 3 | 1.034 | 0.659 | 4.22 | 1.012 | yes |
| P | disturbed | F | 3 | 2.300 | 0.918 | 3.12 | 1.078 | yes |

1. ***Adults***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **nest** | **treatment** | **sex** | **size** | **condition** |
| A | disturbed | F | 0.900 | 1.370 |
| C | control | M | 2.423 | 0.890 |
| D | disturbed | F | -0.110 | -0.116 |
| E | control | F | -1.152 | 0.128 |
| F | control | M | 0.554 | -1.417 |
| G | disturbed | F | -1.929 | 0.931 |
| H | control | M | -1.856 | 0.134 |
| I | control | F | -0.557 | -1.011 |
| L | control | F | 1.424 | 0.195 |
| M | disturbed | F | -0.168 | 1.037 |
| N | disturbed | M | -0.935 | 0.031 |
| O | control | F | 0.480 | -0.450 |
| P | disturbed | M | 0.925 | -1.453 |

**Supplementary References**

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