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Author for correspondence:
Jenny Q. Ouyang
e-mail: jouyang@unr.edu

†Present address: Department of Biological Sciences, Quinnipiac University, Hamden, CT, USA.

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Physiology

Stressful city sounds: glucocorticoid responses to experimental traffic noise are environmentally dependent

Scott Davies†, Nicole Haddad and Jenny Q. Ouyang

Department of Biology, University of Nevada, Reno, NV 89557, USA

SD, 0000-0003-3890-7372; JQO, 0000-0002-5111-5145

A major challenge in urban ecology is to identify the environmental factors responsible for phenotypic differences between urban and rural individuals. However, the intercorrelation between the factors that characterize urban environments, combined with a lack of experimental manipulations of these factors in both urban and rural areas, hinder efforts to identify which aspects of urban environments are responsible for phenotypic differences. Among the factors modified by urbanization, anthropogenic sound, particularly traffic noise, is especially detrimental to animals. The mechanisms by which anthropogenic sound affects animals are unclear, but one potential mechanism is through changes in glucocorticoid hormone levels. We exposed adult house wrens, *Troglodytes aedon*, to either traffic noise or pink noise (a non-traffic noise control). We found that urban wrens had higher initial (pre-restraint) corticosterone than rural wrens before treatment, and that traffic noise elevated initial corticosterone of rural, but not urban, wrens. By contrast, restraint stress-induced corticosterone was not affected by noise treatment. Our results indicate that traffic noise specifically contributes to determining the glucocorticoid phenotype, and suggest that glucocorticoids are a mechanism by which anthropogenic sound causes phenotypic differences between urban and rural animals.

1. Introduction

Urban animals differ phenotypically from their rural conspecifics, probably as a result of the modified environment created by urbanization [1,2]. However, the environmental factors modified by urbanization, e.g. light, temperature and noise, are intercorrelated [3]. We are unaware of any experimental manipulations of these factors in both urban and rural areas to evaluate phenotypic responses in birds. Consequently, it is difficult to identify which aspects of the urban environment are responsible for the observed phenotypic differences and which factors determine whether a species can adjust to urban areas.

Anthropogenic sound, which in urban areas comes primarily from motor vehicles, is ubiquitous in urban environments, differs acoustically from most other types of noise, and has spread at an evolutionarily unprecedented rate [4]. It also hinders acoustic communication and predator detection [4,5], and reduces fitness and species diversity [6,7]. The mechanisms by which anthropogenic sound affects animals are unclear, but one potential mechanism is through changes in circulating levels of corticosterone, which is the primary glucocorticoid hormone in birds and a key part of the vertebrate endocrine stress response [8]. Because corticosterone secretion is sensitive to environmental stimuli, its receptors are found in most vertebrate cell types, and binding regulates target cell gene expression, this hormone links environmental change and phenotypic responses [9].

Noisy human activities are generally associated with elevated glucocorticoid levels [10–13], but the results of traffic noise exposure experiments are

Table 1. Model estimates of the effects of noise treatment and site on initial corticosterone levels pre- and post noise treatment (time). *p*-values <0.05 are indicated in bold.

variable	estimate	s.e.	*t*-value	*p*-value
intercept	−2.9	12.0	−0.2	0.81
treatment × site × time	−12.7	5.3	−2.4	**0.016**
treatment × site	−0.18	5.0	−0.04	0.97
treatment × time	15.9	3.7	4.3	**<0.0001**
site × time	3.2	4.1	0.8	0.43
treatment	−2.9	3.6	−0.8	0.42
site	9.6	3.7	2.6	**0.009**
time	−0.7	2.6	−0.3	0.78
body condition	0.9	1.2	0.7	0.48
sex	0.2	1.7	0.1	0.90

inconsistent [7,14,15]. Moreover, previous experiments exposed only rural free-ranging animals to noise, and used natural background noise as the control. Therefore, it is unclear, first, whether it is traffic noise *per se* or elevated noise in general that affects glucocorticoids and, second, whether traffic noise contributes to determining phenotypic differences between urban and rural individuals. To address these issues, we experimentally tested the effects of traffic noise versus pink noise (a non-traffic noise control) on the corticosterone levels of adult house wrens, *Troglodytes aedon*, in both urban and rural areas. We predicted that exposure to traffic noise or pink noise would elevate initial and stress-induced corticosterone of rural wrens, because they inhabit an environment with little anthropogenic noise. Furthermore, we predicted that traffic noise would have a stronger effect on corticosterone levels of rural wrens than pink noise, potentially because pink noise is less variable than traffic noise. By contrast, because urban wrens inhabit an environment that already has high levels of traffic noise, we predicted that neither traffic noise nor pink noise exposure would affect initial or stress-induced corticosterone levels.

2. Material and methods

We conducted our study from May to July 2016 at one urban location in Reno, NV, USA and one rural location in Sparks, NV, USA (see the electronic supplementary material for details of the study sites and urbanization estimates). We monitored house wren nests to determine the hatch date (day 0). On day 8, we caught the parents at the nest, collected a blood sample (for details of capture and blood collection, see the electronic supplementary material) within 3 min of capture to determine initial corticosterone, then placed each bird in a cloth bag for 30 min to determine stress-induced corticosterone [16]. We also measured body mass (0.1 g) and tarsus length (0.01 mm) to calculate body condition [17]. On day 9, we randomly assigned all nests to a traffic noise ($n = 7$ rural; 10 urban) or pink noise treatment ($n = 7$ rural; 5 urban). For details of the traffic and pink noise tracks, see the electronic supplementary material. On day 9, we placed an Arespark AS200 speaker 5 m from each nest-box and broadcast traffic or pink noise from 08.00 to 17.00. On day 10, we caught both parents and collected initial and stress-induced blood samples, as described above. We measured plasma corticosterone using enzyme-linked immunosorbent assays (Enzo Life Sciences; Farmingdale, NY, USA), validated for the house wren (see the electronic supplementary material for details).

(a) Statistical analyses

We conducted statistical analyses using R (v. 3.1.2). All final models met assumptions, and $\alpha = 0.05$. We used a repeated measures linear mixed model (LMM) with random intercepts to test whether initial and stress-induced corticosterone levels were different within individuals before and after noise treatment, with the interaction of treatment (traffic or pink noise), site (urban or rural) and time (pre- or post noise treatment). If the interaction was significant, we broke down our analyses to LMMs examining whether pre-treatment initial and stress-induced corticosterone levels differed between sites. We included individual and nest as random effects, and sex and body condition as covariates in all models. We also initially included fledgling number as a covariate, but due to lack of variation, we removed it from all models.

3. Results

The three-way interaction between treatment, site and time was significant for initial corticosterone, but not stress-induced corticosterone (table 1). Before noise treatment, rural wrens had lower initial corticosterone than urban wrens (coeff = 9.5, s.e. = 1.8, $t = 5.29$, $p < 0.0001$). Traffic noise caused initial corticosterone to increase in rural, but not urban wrens, and pink noise had no effect on initial corticosterone of either urban or rural wrens (figure 1*a*). Stress-induced corticosterone did not differ between habitats or noise treatment either before or after noise exposure (figure 1*b*). Sex and body condition were not significant in any model (table 1). The number of fledglings did not differ between habitats (mean ± s.e.m.: urban = 7.1 ± 0.6, rural = 7.3 ± 0.9).

4. Discussion

We found that traffic noise has environmentally dependent effects on glucocorticoid levels of breeding adult house wrens, whereby exposure to traffic noise elevated initial corticosterone of rural, but not urban wrens. By contrast, no corticosterone measure changed in response to pink noise exposure. Our study did not include a 'no noise' control, so it is unclear whether the response to pink noise differs from the response to no noise. Nevertheless, our findings indicate that an aspect specific to traffic noise, not noise in general, elevated initial corticosterone levels in rural wrens. The traffic noise-induced increased in initial corticosterone of rural wrens

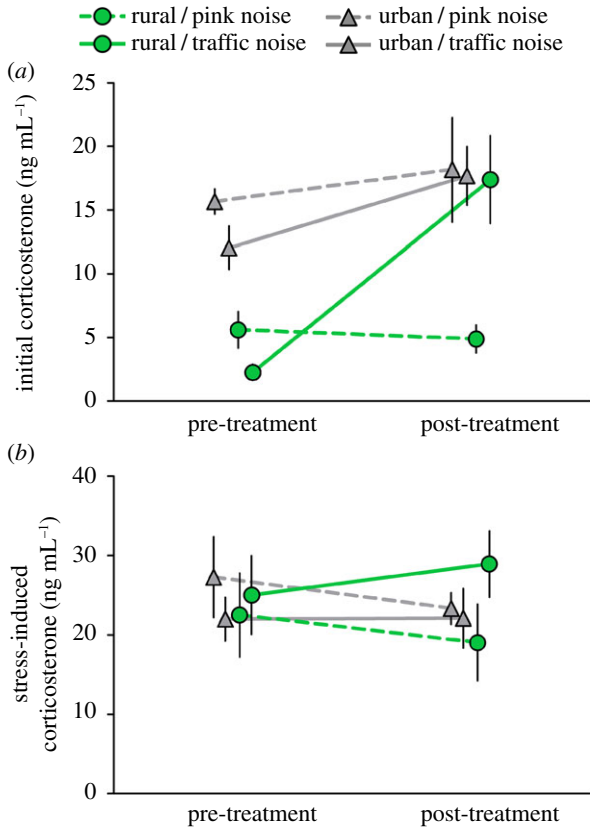

Figure 1. (*a*) Traffic noise exposure increased initial corticosterone in rural, but not urban, adult house wrens, whereas exposure to pink noise had no effect on initial corticosterone of either rural or urban wrens. (*b*) Stress-induced corticosterone did not differ between any of the groups. Points depict means (±s.e.m.).

contrasts to the findings of previous studies on free-ranging rural birds [14,15], which found no effect of noise exposure on initial corticosterone. These contrasting findings could be due to differences in the duration of noise exposure. Alternatively, the previous studies investigated nestlings, whereas our study investigated adults, which suggests that nestlings and adults may respond differently to noise exposure.

The specific aspect of traffic noise that caused initial corticosterone to increase in rural, but not urban wrens is unclear. It is possible that, compared to pink noise, the variable nature of traffic noise (i.e., unpredictable changes in frequency and amplitude associated with sirens and other especially loud traffic noises) acted as a stressor for rural wrens. Alternatively or in addition, traffic noise may have been less effective than pink noise at masking other sounds in the environment that elevate corticosterone. Compared to pink noise, traffic noise may have reduced the ability of rural wrens to hear predators and rival neighbours, to communicate acoustically with their mate, and/or to hear the begging calls of their chicks [4,5]. A lack of similar response to experimental noise exposure in urban wrens may be because they have adjusted to the already high levels of traffic noise in urban areas by changing aspects of their anti-predator behaviour and/or vocal communication [5,18]. It is notable that traffic noise exposure elevated initial corticosterone of rural wrens to levels similar to those of urban wrens, which is consistent with the idea that the elevated initial corticosterone levels of urban wrens, relative to rural wrens, is determined, at least partly, by higher levels of traffic noise in urban areas. Unequivocally demonstrating whether higher levels of traffic noise in urban areas elevates initial corticosterone levels would entail a comparison before and after either reducing traffic noise in urban areas or translocating urban individuals to areas lacking traffic noise.

In contrast to initial corticosterone, stress-induced corticosterone of urban and rural wrens was not significantly affected by either noise treatment. This finding suggests that traffic noise specifically affects initial corticosterone levels, which could be related to the different roles of initial versus stress-induced corticosterone. Initial corticosterone is thought to regulate energy availability and daily processes, such as feeding and activity cycles, whereas stress-induced corticosterone is thought to redirect behaviour and energy stores solely towards immediate survival [19].

Our study suggests that traffic noise, in particular, contributes to determining the glucocorticoid phenotype, and is consistent with the proposition that glucocorticoids are a mechanism by which anthropogenic sound causes phenotypic differences between urban and rural animals. As glucocorticoids have wide-ranging effects on physiology and behaviour, this traffic noise-induced difference in glucocorticoids potentially also underlies differences in other phenotypic traits between urban and rural animals. More broadly, our findings demonstrate that phenotypic responses are environmentally dependent, and highlight that evaluating phenotypic responses across environmental gradients, such as urban to rural, can improve our understanding of physiological adaptations to challenging environments.

Ethics. Our methods were approved by the UNR institutional animal care and use committee and conducted under relevant state and federal permits.

Data accessibility. Data from the manuscript are archived in Dryad (http://dx.doi.org/10.5061/dryad.qb08c) [20].

Authors' contributions. J.Q.O. and N.H. conceived of and designed the study, collected and analysed the data and edited the manuscript. S.D. carried out the hormone assay, contributed to data analysis and wrote the manuscript. All authors gave final approval for publication and agree to be accountable for the content therein.

Competing interests. We have no competing interests.

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