Bearing with noise: the effects of highway noise on behaviour and development in zebra finches

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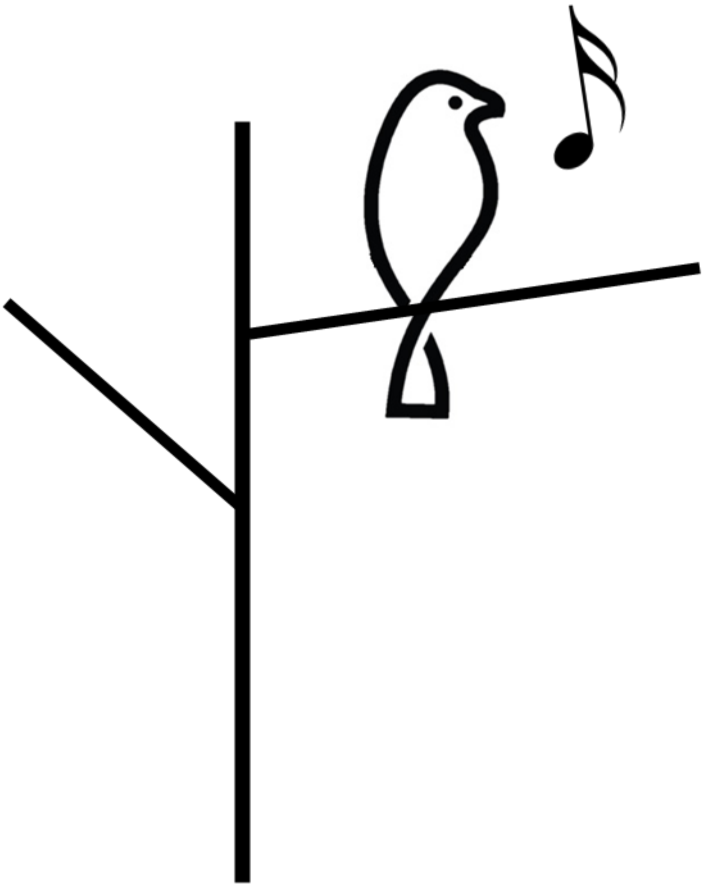
The effect of rearing noise conditions on singing effort and exploration tendency in adult zebra finches



Chapter 5

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Abstract

Birds are increasingly exposed to anthropogenic noise worldwide. Traffic noise is particularly pervasive and birds in populations in noisy areas show behavioural differences in contexts such as foraging, breeding, singing and responsiveness to threats when compared to those in more quiet areas. However, it is as yet unclear whether these behavioural changes arise from noise and if so whether it is current, recent or chronic exposure to noise. A particular vulnerable period could be juvenile development but there is little experimental work on the question as to which extent noisy rearing conditions shape adult behavioural phenotypes. Relatively high ambient noise levels could affect development directly or indirectly (via parental effects) and result in phenotypic differences in adult behaviour. We here investigated if rearing noise levels affect singing effort (number of song bouts, song bout duration and total singing duration) and exploration tendency, two well studied behaviours known to show phenotypic plasticity in songbirds. Zebra finch (*Taeniopygia guttata*) siblings raised under different types of traffic noise exposure were compared in their singing effort (males) and their explorative tendencies (both sexes). Noise treatment had no significant effect on the singing efforts of males nor on the variation in performance in the exploration test. We discuss these result in view of findings in the literature and discuss future avenues to explore potentially long-term effects of chronic noise rearing conditions on a broad range of adult behaviours.

Introduction

Bird populations in urban areas are often reported to behave differently from birds in rural areas (Evans et al., 2010; Lowry et al., 2013; Papp et al., 2015). The difference in anthropogenic noise levels between the noisy urban areas and the more quiet rural areas may potentially be a causal factor explaining or contributing to the changes in behaviour (Slabbekoorn, 2013). Birds living in high level noise habitats have been found to be more aggressive towards same-sex territorial competitors (Phillips & Derryberry, 2018; Wolfenden et al., 2019), sing with different acoustic structures (Ríos-Chelén et al., 2012; Guo et al., 2016; Luther et al., 2016; Hill et al., 2018), or at different times (Dominoni et al., 2016; Bermúdez-Cuamatzin et al., 2020) than individuals from the same species living in more quiet habitats. Such population differences could be the outcome of selection for particular geno-phenotypes in noisy environments. However, if chronic noise exposure is also a form of development stress that can induce phenotypic behavioural differences in birds (Buchanan, 2011; Farrell et al., 2015), population differences may also arise from being exposed to chronic noise during development.

Individuals could also differ in their behaviour because of shorter-term behavioural adjustments. A number of studies using a variety of methods have investigated whether short-term noise exposure ranging from minutes to hours affects birds' behaviour. Using audio playbacks of traffic noise, birds have been reported to avoid traffic noise in the field (McClure et al., 2013) and in the laboratory (Liu et al., 2020). Noise playback has also been shown to increase vigilance (Owens et al., 2012) and, if played back during foraging, to reduce food intake in birds (Quinn et al., 2006; Evans et al., 2018). Playbacks of noise can advance the dawn chorus (Arroyo-Solís et al., 2013), cause an immediate increase in song amplitude (Cynx et al., 1998; Brumm & Todt, 2002; Templeton et al., 2016), or lead to change in song structure (Bermúdez-Cuamatzin et al., 2011; Potvin & Mulder, 2013). During breeding, artificially elevated noise levels in the nest can hamper the detection of a returning feeding parent (Leonard & Horn, 2012; Lucass et al., 2016) and make the breeding birds more vigilant (Meillère et al., 2015). These studies focused primarily on behavioural changes in birds' responses to experimental, temporary noise exposure which was higher than they normally experience in their environment. It is unclear whether birds that have been familiar with such noise levels for long periods, or even grew up under such noisy conditions will show the same type of behavioural reaction to the experimental noise exposure.

While still relatively little is known about long-term effects of noise on behaviour, several studies have investigated the long-term effects of rearing noise conditions on stress physiology. The baseline corticosterone of nestlings in three songbird species was negatively correlated with noise levels near their nestboxes. However, the level of acute corticosterone response among the same birds was positively correlated with their rearing noise levels (Kleist et al., 2018). When exposed to traffic noise playbacks until fledging, tree swallow (*Tachycineta bicolor*) chicks had higher baseline corticosterone levels and stronger stress-induced response levels (Injaian et al., 2018, 2019). Changes in baseline corticosterone levels can indicate developmental stress, which may lead to consistent phenotypic differences later in life (Buchanan, 2011; Boogert et al., 2014; Farrell et al., 2015; Brandl et al., 2019), including song development (Spencer et al., 2003). As there is increasing evidence that chronic noise is a potential stressor, we can expect long-term behavioural effects of chronical noise exposure during development (Potvin, 2017).

Several studies have touched upon the long-term effects of developmental noise exposure on singing behaviour in bids. Extremely loud noise can mask the auditory feedback needed for song learning in birds (Marler et al., 1973; Funabiki & Konishi, 2003; Funabiki & Funabiki, 2009). Zebra finches and white-crowned sparrows (*Zonotrichia leucophyrs*) chicks exposed to anthropogenic noise at more ecologically relevant levels during song development were found to change the spectral components of their songs (Potvin et al., 2016; Moseley et al., 2018) but a comparable study (Zollinger et al., 2017) in great tits (*Parus major*) did not find any effect on song spectral parameters. These experimental studies focused on structural aspects of bird songs but other, more performance related aspects of singing can also be affected. For example, blackbirds (*Turdus merula*) near Madrid Airport sung more and earlier than birds at a nearby control site (Sierro et al., 2017) and great tits at urban versus rural sites differed in the timing and amount of singing (Bermúdez-Cuamatzin et al., 2020).

Variation in singing effort has not been investigated experimentally in the context of rearing noise levels in songbirds, but singing effort has been found to be associated with other phenotypic traits known to contribute to avian behavioural syndromes like exploration (Naguib et al., 2010, 2016). Interestingly, exploration is often tested as a part of the shy-bold continuum in birds and may differ systematically between populations of the same species occupying noisy and quiet habitats (Lowry et al., 2013; Tryjanowski et al., 2016; Thompson et al., 2018; Biondi et al., 2020). Consistent habitat-dependent differences in both singing behaviour (Slabbekoorn & den Boer-Visser, 2006; Slabbekoorn & Ripmeester, 2008) and exploration could result from developmental plasticity or selection for different song and behavioural types (Slabbekoorn & Smith, 2002; Patricelli & Blickley, 2006; Slabbekoorn, 2013). Experimental studies specifically testing whether chronic noise exposure during development is a causal factor contributing to behavioural differences in adult phenotypes could help understand how such population differences could arise.

Zebra finches are a suitable model system to test for long-term behavioural effects of noise exposure on singing and exploration. Zebra finches have been tested experimentally for impact of short-term noise exposure on intra-pair communication, foraging and spatial preferences (Villain et al., 2016; Evans et al., 2018; Liu et al., 2020). How early developmental stress affects singing behaviour of this species has been studied extensively (e.g. Spencer et al., 2003; Buchanan et al., 2004; Gil et al., 2006; Brumm et al., 2009, Holveck et al 2008). Zebra finches show interindividual differences in explorative behaviour that can be easily measured using already validated exploration tests (Schuett & Dall, 2009; David et al., 2011). In a previous study, we have shown that birds raised in different levels of traffic noise may behave differently in terms of noise avoidance (Chapter 4). This suggests noisy rearing conditions potentially have other behavioural effects in zebra finches. Good candidate behaviours known to show phenotypic plasticity, namely singing and explorative behaviour, seem good starting points to investigate this further.

In the current study, we studied the effect of high level noise exposure during rearing conditions on adult behaviour focusing on singing effort and explorative behaviour. We first examined the repeatability of our exploration test in our laboratory population and then used these birds as parental generation to raise offspring under two noise exposure treatments: continuous highway playback recorded at either near (15 m) or far (200 m) distance from a highway. In the offspring generation, we tested the explorative behaviour of both sexes in a novel environment test and measured the singing effort of males when moved to a recording chamber away from the colony.

Material and Methods

Subjects and housing

All subjects from this study originated from the breeding colony at Leiden University (The Netherlands). The individuals from the parental generation were the birds reported in Liu et al. (2020) and their offspring (see chapters 2 & 3). Figure 1 gives a schematic overview of all procedures: subjects of the parental generation (N = 48; 24 males and 24 females) were tested for behavioural syndromes among several traits (Liu, Amin et al. in prep) and noise avoidance (Liu et al., 2020). Birds had been raised in family groups and from 65 days old

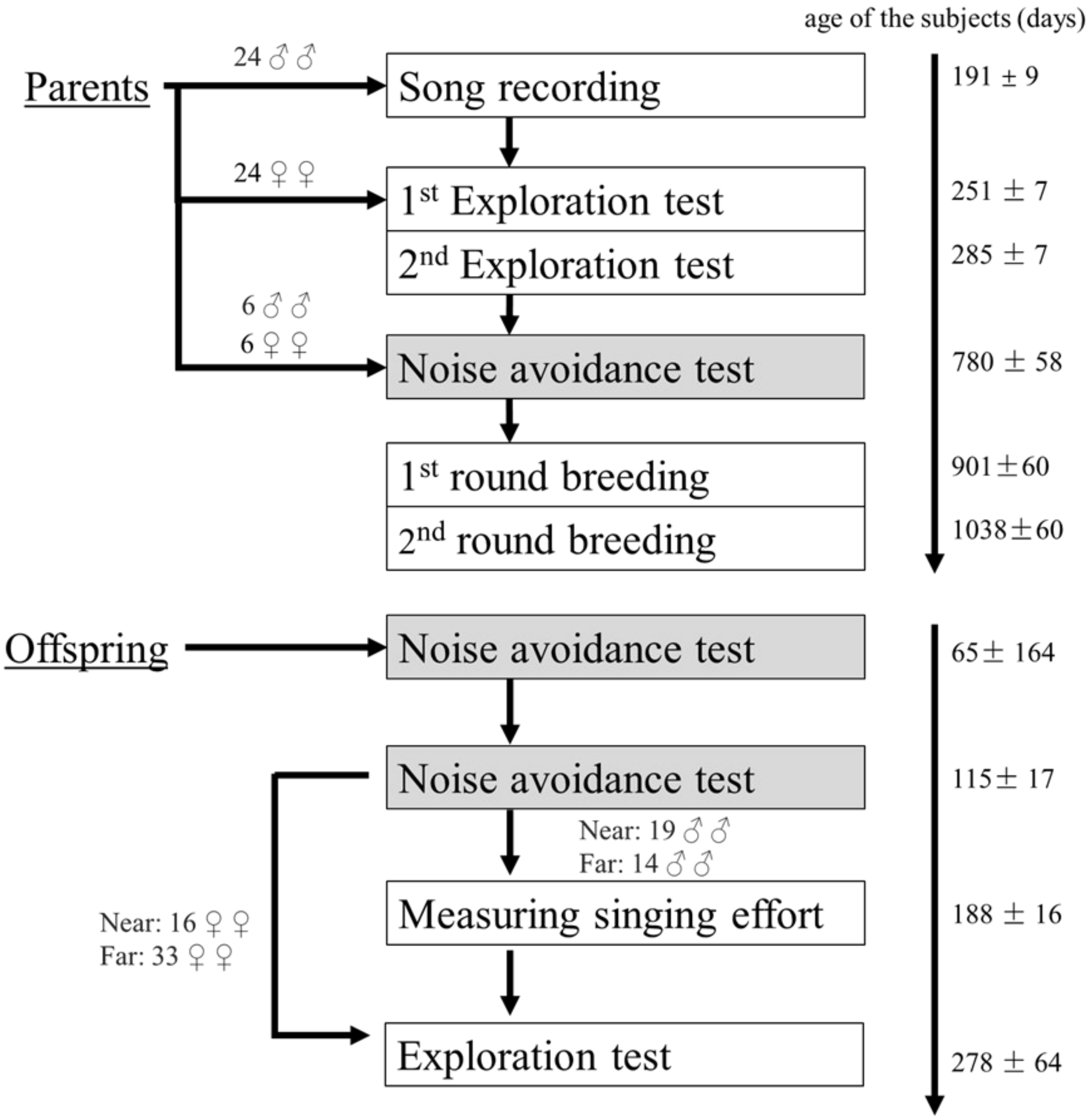

Figure 1: Timeline and procedures of the study. In the parental generation, males were recorded for their songs first. Males and females were then tested in a novel environment for explorative behaviour. Subsequently, all tested birds plus an additional 12 new birds (6 males, 6 females) were randomly paired to breed while exposed to one of two types of traffic noise. All male offspring's singing effort was recorded at about 4 months of age, and at around 10 months all offspring were tested in an exploration test.

onwards subjects were housed in same-sex aviaries (L x W x H: 180 x 80 x 200 cm, 10 - 20 birds). Before testing, birds were moved from the aviaries to smaller group cages (80 x 40 x 40 cm) holding four individuals each. From these cages, individual males were taken to sound proof chambers for song recordings (see below). At this stage males were on average 191 ± 9 days old. One month later, all birds had been recorded and four weeks after the recording of the last bird, the exploration test with all males and females started (the average of subjects of both sexes was now 251 ± 7 days). The exploration tests were all conducted within the course of two weeks. The subjects were tested a second time about a month later (at 285 ± 7 days old). Between and after the tests, birds continued to be housed in their group cage. All subjects, plus an additional 6 males and 6 females, were tested at 901 ± 60 days old to establish preference between two types of traffic noise recordings in an unconstrained two -way choice test (Liu et al., 2020). Using the insights from this experiment, tree months later, birds were then allowed to breed with continuous exposure to 24 hour traffic noise recordings, one level of which had been avoided in the aforementioned experiment and another level that had not been avoided (details in Liu et al 2020, see chapter 2). Using these types of noise stimuli for the breeding experiment, would allow us later to compare explorative behaviour of birds raised in two different noise conditions: one that was aversive to their parents (noise experimental) and another one that was not (noise control).

To initiate breeding, males and females were randomly paired and moved to individual breeding cages (100 x 50 x 40 cm). Each cage had a nestbox that could be attached or removed any time during the experiment via a removable door of metal bars. All breeding cages were placed in one of two breeding rooms of the same size (365 x 305 x 300 cm) and made from the same materials. The breeding experiment is described in detail by Liu et al. (see Chapter 3). Briefly, in each breeding room, 15 cages were stacked along two of the walls forming an L-shape of three and two cages wide and three cages high. In the corner of the room, opposite the L-shaped cage wall, two loudspeakers (Blaupunkt CB 4500) were positioned to continuously play back traffic noise. In one room this was the near-distance highway noise, in the other the far-distance highway noise. The loudspeakers were positioned and calibrated such that during playback sound pressure levels were almost the same in all the cages and within the nestboxes (reference level: SPL re 20 μPa, far-distance: cages 51.5 ± 0.4dB(A), empty nestbox 51.4 ± 0.6dB(A), nestbox with nest: 52.1 ± 0.4dB(A); near-distance: cages 70.2 ± 0.5dB(A), empty nestboxes: 68.5 ± 0.9dB(A), nestbox with nest: 69.1 ± 1.1dB(A)). The levels in the room and cages were equivalent to the levels at the original recording sites in the field (for more information on stimuli, see Liu et al. 2020). Before starting the noise exposure playback, pairs were given 14 days to acclimate to the room at ambient sound levels. Playbacks were then started and were linearly increased over the next two weeks until the full levels of the original recordings were reached. At this stage nest boxes were introduced. Pairs were then allowed to breed and the noise continued until chicks in both rooms were moved out at 65 dph. Nestboxes and nesting material were also removed now and the amplitude of the playbacks was gradually faded out over the next seven days returning the rooms to their baseline level of ambient sound (< 40 dB(A) when birds were quiet). After a break of 14 days without playback (and a total of 146 days after the start of the first breeding), playback treatments were resumed but reversed between rooms. For the next 14 days playback levels were thus gradually raised again to the respective full treatment levels when birds were given nestboxes for a second breeding round. After the second breeding round, each pair had thus bred once with continuous playback of near- and once with far-distance traffic noise. Because parental quality and brood size are both factors known to affect nestling development and song learning, chicks were cross-fostered with non-siblings whenever possible keeping brood sizes between 1-6 chicks, the range observed in our colony (for all details see Chapter 3). Chicks stayed with their parents until 65 dph. Then they were tested for noise avoidance behaviour for 2 days (for details see Chapter 4), after which the birds were moved into aviaries (100 x 200 x 200 cm), with birds of the same sex. At 188 ± 16 dph, male singing effort was recorded. At 278 ± 64 dph, offspring of both sexes could voluntarily participate in an exploration test (see below).

Throughout the experiments, all birds were kept at a temperature of 20 - 22 degree Celsius with humidity levels ranging from 45 - 63%. The light:dark regime at all rooms was 14:10 with a daily 30 minute transition period between light and dark where the lights were gradually in- or decreased until on or off. Birds had *ad libitum* access to water and food throughout. This was supplemented with egg food, fruits and vegetables, every other day, and germinated seeds twice per week.

Song recordings and singing effort measurements

To record songs, males were caught from their home cage or aviary between 0800 and 1200h and moved individually to a recording cage (100 x 60 x 30 cm), placed into one of two identical sound attenuated recording chambers (300 x 125 x 240 cm). The cage had a solid floor but the sidewalls and ceiling were made from mesh wire and thereby acoustically transparent. An omnidirectional microphone (Sennheiser MKH50-P48) hung above the cage (50 cm above the perches in the cage) and recorded directly onto the hard disk of a computer placed outside the chamber (sound card: TASCAM UH-7000, Ishmael software: version 1.0.2.0 with sampling frequency of 44.1 kHz, accuracy of 16 bits, file format: .wav). As soon as a bird had been placed into the setup, the experimenter started the software that automatically recorded sounds in the cage. The next day, after 24 hours of recordings, the experimenter stopped the recording software and returned the bird to its home aviary.

To measure the singing effort, all audio recordings from the 24 hours after the software started were subsequently screened for song utterances with a self-written script in R that used validated amplitude interval thresholds to automatically find and then save songs as .wav files (for script, see https://github.com/TempestMars/Songdection). The extracted songs were inspected (by QL) using spectrogram displays (Audacity 2.2.0, window size: 1024, window type: Hanning, zero padding factor: 1, gain (dB): 20, range (dB): 80, frequency Gain (dB/dec): 0, Audacity Team, 2019) with concurrent audio playbacks to remove the false positives (this was very rare, and these were mostly long series of calls). From these files, the number of song bouts, song bout duration and the total singing duration within the recorded 24 hours were obtained as aspects of singing effort.

Exploration tests: parental generation

In the parental generation, 24 males and 24 females were tested twice (1st: 251 ± 7 days of age, 2nd: 304 ± 7 days of age) for explorative behaviour in one of two novel aviaries (200 x 150 x 200 cm) with an identical interior but mirrored in the arrangement of features. Each aviary had two artificial trees (2 m high wooden poles) placed at ca. 73 cm distance on the diagonal of the aviary. Each tree had two wooden rods (100 cm long, 18 mm diameter) pushed through a hole in the pole at 80 or 100 cm creating two perpendicular branches. In sum, the novel aviary had two trees and eight branches and one water and one food dispenser placed on the ground. To start a test, a subject was carefully caught in the home cage, put in a transportation box (20 x 30 x 20 cm) and placed within this box onto the floor in the novel aviary by the experimenter (BA) who would immediately leave the room afterwards. The door of the box could be opened remotely from outside of the aviary by pulling on a string that opened the bolt of the door which marked the start of the trial. The door was always opened within a minute after the bird in the transport box had been moved into the aviary. All trials lasted one hour and were video recorded (webcam Logitech Pro Webcam C920). After one hour, the trial ended and the experimenter entered the aviary, caught the bird and returned it to its home cage. All trials took place between 0800 and 1600 h. Each bird's starting time during the first trial was also used again as starting time when the bird was tested for the second time 34 days later.

Exploration tests: offspring generation

All offspring were tested for participation in a voluntary exploration test at 278 ± 64 dph. Birds of the same sex from the same breeding round were housed in one large aviary (200 x 200 x 200 cm), connected to a second 'novel' aviary of the same size through a mesh wire

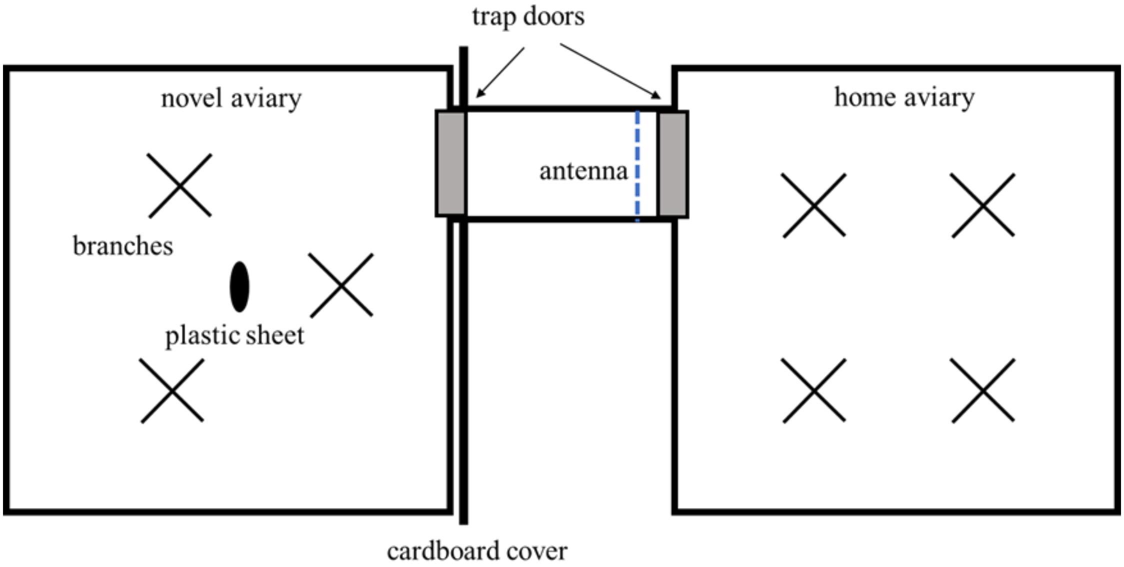

Figure 2: Top view of the voluntary exploration test setup. The novel aviary was connected to birds' home aviary through a tunnel with a trap door at either end which could be remotely opened and closed. An antenna near the entrance to the novel aviary identified passing birds ID tags and send a signal with this information on identification(s) to a PC placed outside the room.

tunnel (see Figure 2, 100 x 50 x 50 cm). At both ends of the tunnel, there was a trap door that could be opened or closed from outside the room via a simple string-pulling mechanism. The entrance to the tunnel and the novel aviary were filmed via webcams (webcam Logitech Pro Webcam C920) and also connected to a PC and monitor outside the room. An antenna was placed right behind the trap door at the end to the home aviary for automatic, real-time identification of passing birds through a leg-mounted micro transponder tag (antenna ANT-SER300, tags ID100A, Dorset, Aalten, The Netherlands) allowing immediate identification of a passing bird so that the experimenter outside the room could decide to allow or deny the bird to proceed to the novel aviary.

The outer walls of the novel aviary facing the subjects' home aviary were fully covered by cardboard pieces so that birds could not see this aviary from their home aviary and vice versa. In the novel aviary, three artificial trees were evenly placed, each with one top branch and one bottom branch, positioned in the same way on the trees as had been done for the trees in the parents' novel environment tests. For the offspring, other than for the parents, all the "branches" of the artificial trees were wrapped with white paper to make the novel aviary look different from the home aviary where subjects were housed with the same type of artificial trees and branches. Moreover, a black plastic sheet (80 x 60 cm of surface) was suspended from the top of the aviary such that it hung among the three trees and partially blocked the view from the tunnel, so that birds could land on the first tree, but had to fly around the sheet to get into the aviary to find additional branches to perch on. One food and one water feeder were placed on the ground in the middle of the aviary. A webcam (Logitech HD Pro Webcam) was positioned at one wall so that all tree branches were visible in the recording.

To start a trial, the experimenter sat outside the room in front of a PC monitor showing the webcam's recording of the entrance to the tunnel and waited until a bird entered the tunnel. The monitor also showed the read-outs of the identification tags. If the passing bird had not been tested before, the experimenter would instantly close the trap door to the home aviary and open the door to the novel aviary. If the bird passing the antenna had been tested already, the door to the novel environment would not open and the experimenter would wait for at least ten minutes. If the bird was still in the tunnel, the experimenter would operate the door and gently encourage the bird to move back into the home aviary. If there were multiple birds in the tunnel, the experimenter would also operate the door in the same way to keep only one bird in the tunnel. When the trap door to the novel aviary opened, the webcam started to record for 90 minutes. After the recordings, the trap door to the home aviary opened again to allow the birds to fly back.

Scoring of video tapes

In the parental generation, the videos were scored using the VLC-Media Player software (Version 2.2.1, with default settings, at normal speed). All events were manually written down by the experimenter (BA). The latency for the bird to leave the transportation box (defined to be the moment when both feet were outside the box) was scored as emergence time. The total number of different branches visited by each bird were also scored. A branch visit was defined as any event where the bird landed with both feet on the branch at least once. Interobserver reliability was very high: the scores for 10 recordings were consistent between two observers (BA, SV, correlation test, intraclass correlation coefficient = 1). For the offspring generation, the first 80 minutes of all video recordings were scored in 'BORIS' (Friard & Gamba, 2016). The scoring method was the same as for the parents except that we used the 'point event' function in BORIS to record the birds' visiting to different branches instead of writing it down. To score the exploration behaviour in the voluntary test, birds were given a score of 1 for entering the novel aviary and for each newly visited branch another 1 was added to the score. Once a branch had been visited, repeat visits to a previously visited branch received no extra scores. After the testing in the novel environment, birds scores could thus range from 1 (bird had entered but not visited any branches) to 7 (birds had entered the new aviary and visited all branches). In addition, birds that did not participate (i.e. did not enter the novel environment) were given a score of 0. The experimenters who scored these videos (KF, CD, QL, intraclass correlation coefficient = 1) were blind to the treatment of the birds.

Statistical analyses

All statistical analyses were conducted using R (R Core Team, 2019) statistical software (v. 3.5.3). We first tested if total number of song bouts and bout duration of the offspring were affected by rearing noise conditions using mixed linear models. In these models, rearing noise (far/near) and breeding round (first/second) were fixed factors, brood size was a covariate, (experimental) and brood IDs were random intercepts. The model included the interaction between rearing noise and brood size. For the bout duration, bird IDs were also treated as random intercepts. To test if the total singing duration was affected by rearing noise conditions and whether total singing duration was correlated with explorative behaviour, a linear mixed model was created with the logarithmically transformed total singing duration as response variable; rearing noise and breeding round as fixed factors. The model also included the interaction between rearing noise and brood size. Exploration score and brood size were covariates. Brood IDs were random intercepts.

The repeatability of the main response variable (number of different branches visited) in the exploration test of the parental generation was calculated with a generalised linear mixed model (lmer package), with the number of different branches visited as response variable (Poisson distribution); sex (female/male) and test round as fixed factors; emergence time, age, test date and time as covariates (linearly and quadratically, numeric values were scaled to mean = 0, sd = 1); and bird ID as random intercepts. The purpose of this full model was to find the best fitting model to calculate the repeatability of the birds (random intercepts) in this test through model selection (MuMIN package). The model with the most degrees of freedom within 2 ΔAICc was selected as the best model. Finally, the repeatability of the exploration test was estimated (using rptR package) in R(epeatablity) value of the random intercepts (bird IDs) in the best model (Nakagawa & Schielzeth, 2010), following the method used in several other studies (Patrick & Weimerskirch, 2014; McCowan et al., 2015; Wuerz & Krüger, 2015; Thompson et al., 2018).

To test if rearing noise conditions affected offspring's explorative behaviour in a novel environment, a generalised linear mixed model was created: exploration score as response variable (Poisson distribution); rearing noise, breeding round, sex and the housing group ID (a/b/c/d, birds were housed as two male and two female cohorts) as fixed factors; brood size was a covariate; brood ID was random intercept to account for the variance caused by the social parents and the siblings. The model was built through the glmmTMB function from the package 'glmmTMB'.

Results

Singing effort

During the recorded 24 hours, male birds raised in near-distance traffic noise (n=26 males) sung on average 307 ± 18 song bouts with an average duration of 1.79 ± 0.80 s and total duration of 533 ± 788 s. Males raised in far-distance traffic noise (N=19 males) sung on average 360 ± 19 song bouts with an average duration of 1.55 ± 0.70 s and a total duration of 564 ± 717s. Rearing noise conditions did not affect any of the aforementioned parameters (Figure 3, Table 1).

Table 1: Results of (generalised) linear mixed model analyses of singing activity: a) number of song bouts, b) song bout duration and c) singing duration were not affected by the rearing noise.

Fixed factors	**Level**	**Estimate**	**S.E.**	χ^2	**P value**
a) Number of song bouts: noise + noise x brood size + breeding round + brood size					
Noise				0.05	0.83
	Far	*2.02*	*1.26*		
Breeding round				1.69	0.34
	Second	*0.82*	*0.63*		
Brood size	*Covariate*	*0.02*	*0.26*	0.91	0.19
Noise x brood size				1.75	0.19
b) Song bout duration: noise + noise x brood size + breeding round + brood size					
Noise				1.04	0.31
	Far	*-0.05*	*0.48*		
Breeding round				0.11	0.86
	Second	*0.07*	*0.20*		
Brood size	*Covariate*	*0.03*	*0.08*	0.03	0.73
Noise x brood size				0.09	0.77
c) Singing duration: noise + noise x brood size + exploration score + breeding round + brood size					
Noise				0.00	0.95
	Far	*2.23*	*1.71*		
number of different branches visited	*Covariate*	*-0.02*	*0.14*	0.02	0.88
Breeding round				1.76	0.19
	Second	*0.91*	*0.69*		
Brood size	*Covariate*	*0.02*	*0.28*	0.88	0.35
Noise x brood size				1.90	0.17

Marginal and conditional R2 for the models are a) 0.13 and 0.13, b) 0.01 and 0.22 and c) 0.13 and 0.13. Brood IDs were random intercepts. For the song bout duration, bird IDs were also treated as random intercepts.

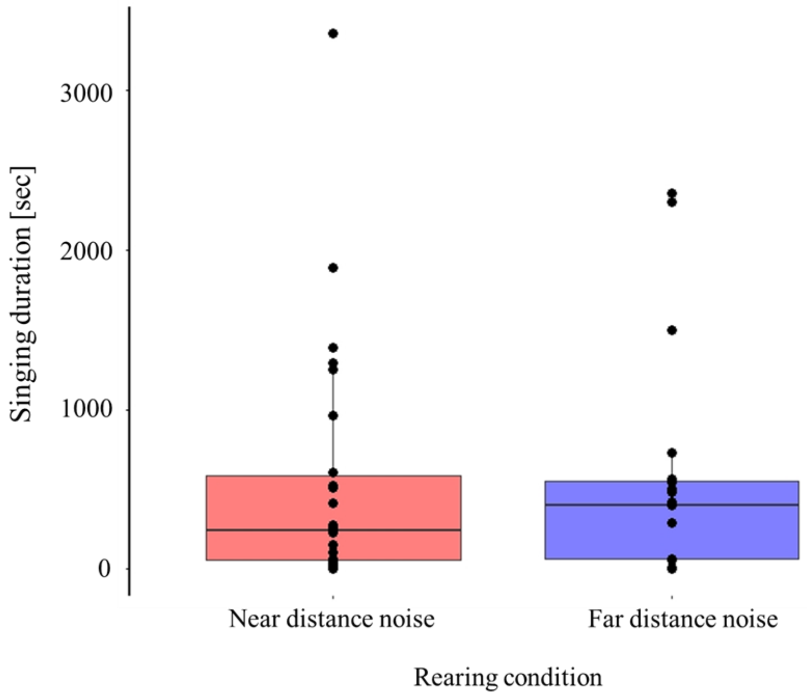

Figure 3: Singing duration of male offspring raised in two types of traffic noise exposure conditions. The Y-axis shows the singing duration in seconds. Each dot represents a single bird. Rearing conditions had no effects on the three quantitative measures: number of song bouts, song bout duration and total singing duration (see Table 1).

Exploration tests: parents

The number of different branches visited by individual birds from the parental generation in the exploration test was repeatable ($R = 0.49$, 95% CI [0.14, 0.63], $n = 48$, $p < 0.001$, see Figure 4) in the best model (Exploration: Emergence$^{2}_{\mathrm{scaled}}$) which only includes quadratically scaled emergence time.

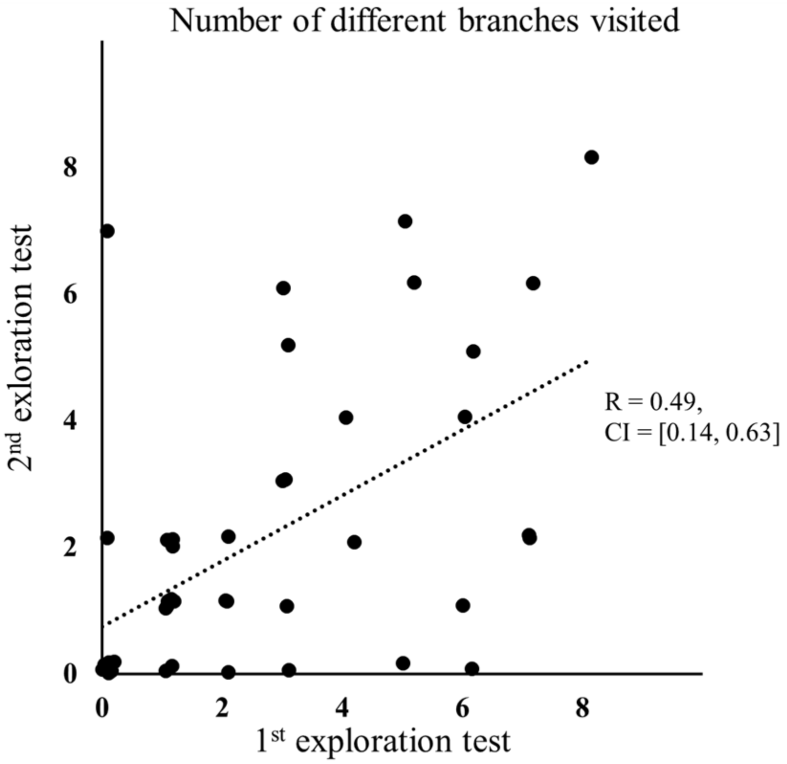

Figure 4: Repeatability of the number of different branches visited by the parents. Every dot represents a single bird. X and Y axes are the number of different branches visited (randomly jittered by 0 - 0.2) during the 1st and the 2nd exploration test.

In the offspring generation, 19 (out of 26) males and 16 (out of 28) females raised from near-distance traffic noise and 14 (out of 20) males and 33 (out of 46) females raised from far-distance traffic noise entered the novel environment. There was no significant difference in terms of participation between birds raised from the two treatment groups (chi-squared test, p = 0.41), and between the two sexes (chi-squared test, p = 0.48). Comparing the explorative behaviour of birds showed no systematic difference among the birds from the different rearing environment, suggesting that the early rearing noise conditions did not affect the explorative behaviour in the offspring (see Figure 5, Table 2). The brood size, breeding round, sex and the housing group ID also did not affect the exploration score of the offspring in a novel environment.

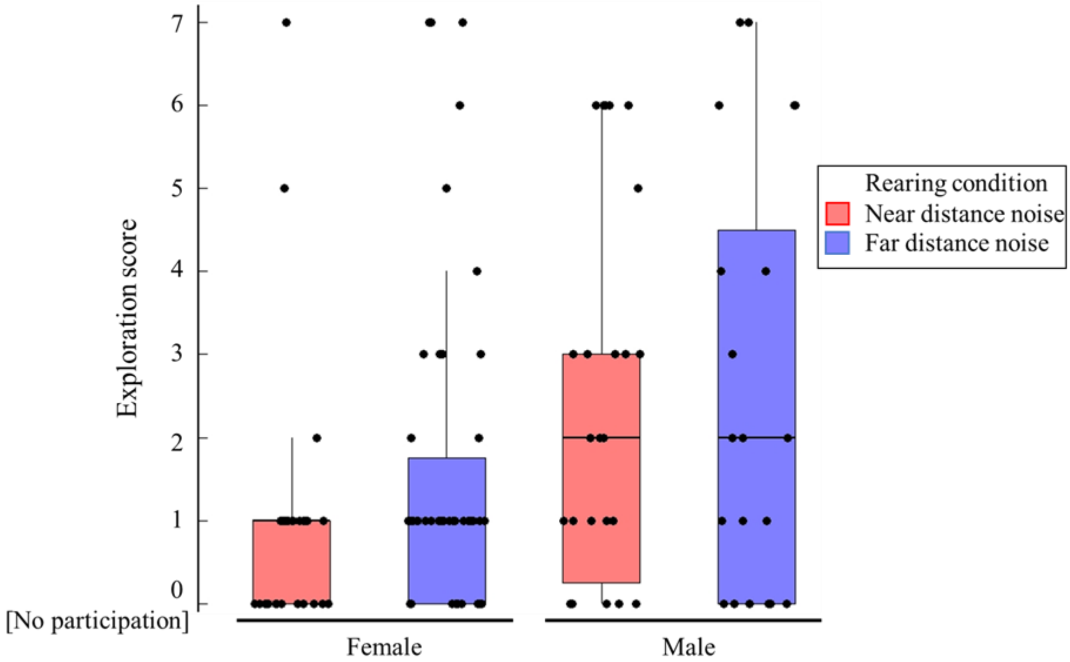

Figure 5: Boxplots showing the exploration score of the offspring generation in the voluntary exploration test plotted by rearing condition and sex. The y-axis shows the exploration score. Zero scores indicate birds that did not participate in the voluntary exploration test. Each dot shows the exploration score of a single individual.

Table 2: Results of the generalised linear mixed model analysis testing the effect of rearing conditions on offspring exploration score. None of the tested factors were statistically significant.

Fixed factors	**Level**	**Estimate**	**S.E.**	**χ^2**	**P value**
offspring exploration score: noise + brood size + sex + housing group ID					
Noise				1.56	0.87
	Far	*0.35*	*0.23*		
Brood size	*Covariate*	*-0.01*	*0.08*	-0.24	0.81
Sex				0.00	1.00
	Male	*0.34*	*859.71*		
Housing group				3.19	0.36
	Group b	*-0.05*	*859.71*		
	Group c	*-0.10*	*7.61*		
	Group d	*-0.39*	*859.71*		

Brood IDs were random intercepts. Marginal and conditional R^2 for this model are 0.13 and 0.40.

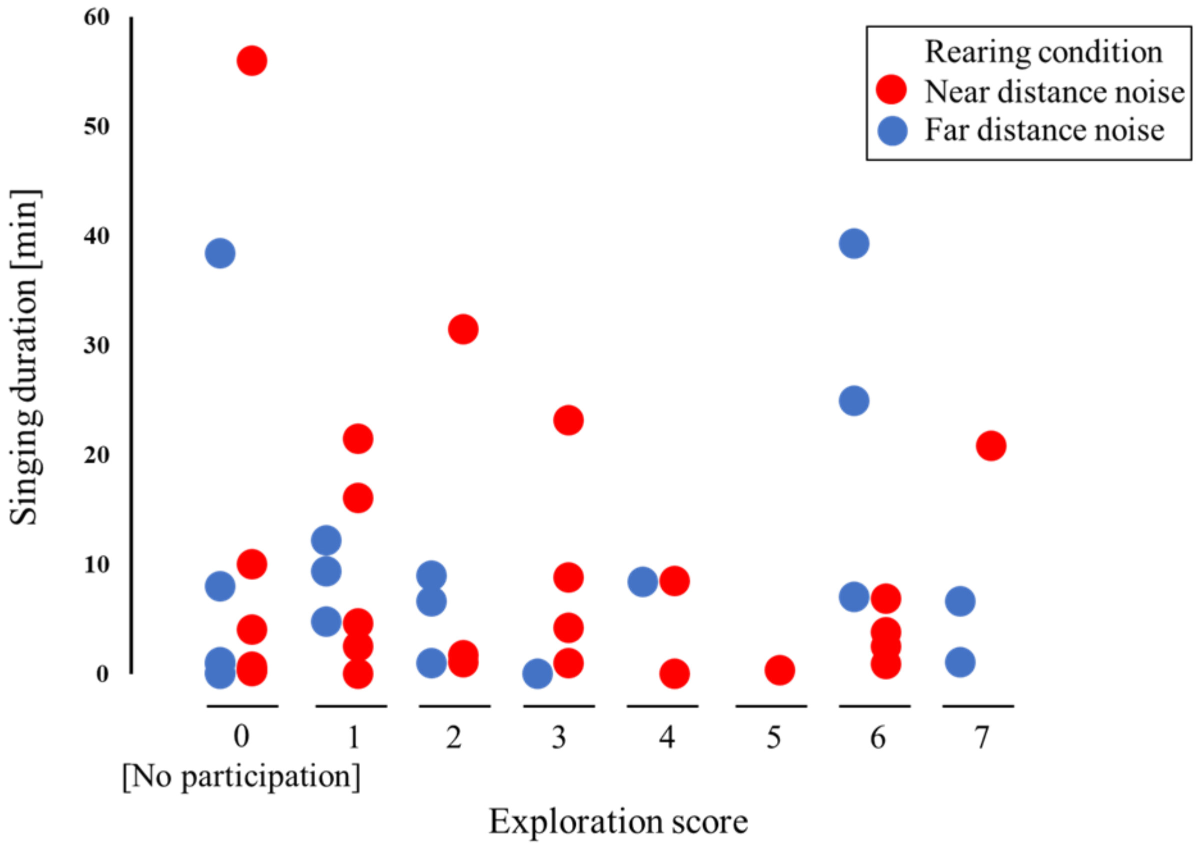

Figure 6: Total singing duration of male offspring plotted against their exploration scores in the novel environment. The y-axis shows the singing duration in minutes. The x-axis shows the novel environment exploration score. Each dot represents a single bird. Treatment had no effect on the plotted variables (statistical analyses in Table 1).

In the tested males, the total singing duration and the exploration tendency did not covary (see Figure 6, Table 1c). Similarly, rearing noise, breeding round, brood size and the interaction between rearing noise and the brood size all did not affect the exploration tendency.

Discussion

We aimed to test if chronic exposure to traffic noise during rearing would affect adult phenotypes by focusing on two behaviours known to show developmental plasticity in the zebra finch: singing effort and explorative behaviour. There was no significant effect of noise treatment on male singing effort, measured as total time singing or number of song bouts, nor on the performance in the exploration test. Exploration behaviour has been shown to be consistent in zebra finches. The parental birds were tested on two different occasions one month apart and they behaved consistently in our exploration test. Such consistency in exploration has also been found in several other zebra finch studies, indicating the robustness of the exploration test (David et al., 2011; McCowan et al., 2015; Wuerz & Krüger, 2015). We did not test for consistency in singing effort, but we also did not find any correlation between the total singing duration and the explorative behaviour.

Singing effort: body condition and social context

Across songbird species singing effort does show individual differences but is also highly condition dependent (Gil & Gahr, 2002) and this also holds for zebra finches (Riebel, 2009). Developmental stress is known to affect the HPA-stress axis which has been implicated both in individual differences in personality traits (Groothuis & Trillmich, 2011; Spencer, 2017), and song learning (Podos et al., 2009; Spencer & MacDougall-Shackleton, 2011). We had hypothesised that if the chronic noise exposure affected the parents or offspring, singing and exploration might differ between the birds raised in high level, behaviourally aversive noise (see chapter 2 and 3) versus those reared in the control, lower noise level condition. The absence of a difference on singing effort between the two treatment groups could indicate a true absence of an effect, or that our method is not suitable to detect it.

The absence of an effect may also be due to the fact that there were no differences between the birds in both treatments in body condition, which has been most convincingly linked to singing effort. Singing effort shows substantial variation among individuals in the field during breeding seasons (Dunn & Zann, 1996) and this variation has been related to both internal and external factors. Body condition, for example, has been shown to affect singing effort in zebra finches. Food restriction has been found to reduce the rate of song bouts (Ritschard & Brumm, 2012) while supplementary lutein and cholesterol can help zebra finches maintain song output with decreased photoperiod (Casagrande et al., 2016). The birds tested in the present study, however, did not differ in body mass (see Chapter 3), which may be one explanation for the lack of difference in singing effort.

Singing effort in zebra finches may also vary with variation in their social environment. One study compared singing in males housed with one unfamiliar female or a group of 40 - 45 unfamiliar birds of both sexes and found more singing in the more simple social environment, but also a gradual change towards an increase in singing effort in both groups after the change to a new environment (Adar et al., 2008). Males also differ in how much they sing when alone or in company (Jesse & Riebel, 2012; Bruno & Tchernichovski, 2019). In our study, the birds were all housed in a similar social environment, which may have led to a homogenous singing effort, but this seems not very likely as other studies also reported systematic individual differences in song output when monitoring males' singing effort during comparable durations of solo housing (Jesse & Riebel, 2012; Bruno & Tchernichovski, 2019).

In summary, singing effort has been used in most studies to date to assess current condition, or effects of social context, making it more difficult to link this literature to the studies that report differences in singing effort between birds in noisy and quiet habitats in the field. It is currently discussed whether these differences arise from the current or the recent exposure of noise versus long-term effect of being reared in a noisy condition (Potvin, 2017). Bermúdez-Cuamatzin et al. (2020) recorded great tit (*Parus major*) singing at 11 paired urban-rural sites and found the urban birds sang fewer songs outside the dawn chorus compared to their rural counterparts. Also urban and rural red-winged blackbirds (*Agelaius phoeniceus*) distributed their singing effort differently over the day (Cartwright et al., 2014). As our negative results could arise from a true absence of an effect but also from using an unsuitable method, currently our result cannot support or dismiss that such differences in singing effort are long-term effects of rearing traffic noise exposure.

Explorative behaviour: developmental stress, parental effects and social environment

Explorative behaviour in the offspring was not significantly affected by the rearing noise conditions in our study. We had picked this parameter as several studies had already shown that this behaviour can systematically covary with manipulations of the rearing environment and in particular in the context of developmental stress. Martins et al. (2007) scored how many feeders in a novel cage were visited by zebra finches from three lineages of different baseline corticosterone levels and found that the birds from higher baseline corticosterone lineages visited more feeders, and among the birds from lower baseline corticosterone lineages, the more plasma corticosterone they had, the more feeders they visited. Spencer & Verhulst (2007) experimentally administrated exogenous corticosterone to developing zebra finches and observed the birds' behaviour with either a toy moose or a furry ball at 30 and 50 days old. They found males in the treatment groups had shorter latency to approach the perch with the object. We did not measure the cortitestorne levels in the subjects of our study, but did weigh the offspring at 11, 65 and 120 days old but found no differences between the two groups (see Chapter 3). Although this is only an indirect way to measure stress, lack of a difference in weight between groups could be in line with no difference in stress, although we suggested that parental behaviour may also have compensated for offspring stress (see Chapter 3). Interestingly, the studies exposing developing birds to anthropogenic noise report very different outcomes, ranging from increased (Crino et al., 2011) to decreased baseline corticosterone (Kleist et al., 2018). However, the majority of studies to date have not found noise exposure affecting baseline corticosterone (Crino et al., 2013; Angelier et al., 2016; Casasole et al., 2017; Injaian et al., 2019; Flores et al., 2020).

Group housing and a voluntary test can affect the exploration behaviour of zebra finches. In our voluntary test, not all birds participated but this might change by opening more entrances in the future or reducing the total number of birds in the aviary as some birds started to monopolize the access to the tunnel by chasing other birds away (personal observations). This highlights social influences on exploration of an area (Templeton et al., 2014), which we need to understand better as most studies on individual differences and behavioural syndromes test birds individually for exploration. In zebra finches, the association between exploration and other personality traits in individual versus paired situations vary (Schuett & Dall, 2009) or can be absent. Emmerson & Spencer (2017) reported that their corticosterone treatment affected young zebra finches' behaviour in a novel environment test, featuring several novel objects. However, in social testing conditions, birds from all treatments spent similar time perching near novel objects. This indicates the importance of considering social environment in behavioural tests.

Link between singing effort and explorative behaviour

We also tested for a potential covariance between singing behaviour and behavioural traits related to exploration that have been reported in several bird species. In wild collared flycatchers (*Ficedula albicollis*) the males that explored more in a novel environment were more likely to sing at lower song posts (Garamszegi et al., 2008) than their less explorative conspecifics. In great tits, exploration score, normally measured as the number of movements between different features in an exploration room, has been found to correlate with the number of songs in different stages of breeding (prior to egg laying, Naguib et al. 2010; during main periods of fertility and maternal investment, Naguib et al. 2016) and territory defence (Amy et al., 2010; Snijders et al., 2015). However, we did not find any correlation between exploration and singing effort in our subjects. This may be due to species and/or methodical differences (Carter et al., 2013; Jacobs et al., 2014).

The function(s) and context of singing differ among species and this may also affect the correlation between singing effort and exploration. The most prominent findings on correlation between singing effort and exploration so far have been reported in great tits (e.g. Amy et al., 2010; Jacobs et al., 2014; Snijders et al., 2015) as this is also one of the species where these questions have been most intensively studied in the field. Great tits sing seasonally and an important function of their song is territorial defence and higher exploration is associated with more aggressiveness (Verbeek et al., 1996; Carere et al., 2005). In great tits, the relationship between exploration and singing effort could be mediated by other personality traits (e.g. aggressiveness). In zebra finches, singing is year-long and has the function of pair bond maintenance (Dunn & Zann, 1996). Singing probably also has a social function in the large flocks (Elie et al., 2011). In this context, singing effort should perhaps be expected to be positively correlated with sociality, as what has been demonstrated in a few studies (Adar et al., 2008; Jesse & Riebel, 2012).

Conclusions

For the experimental subjects raised with chronic noise exposure of near-distance (actively avoided by birds) and far-distance traffic noise, singing efforts did not differ during a 24h assessment of song output in a standardised situation in a (novel) recording chamber. The birds' explorative behaviour, did not show measurable covariance with the two noise rearing treatments. The total singing duration in an unfamiliar recording chamber was not correlated with the birds' explorative behaviour in a novel environment. However, as a negative result is not proof of absence of an effect this is difficult to interpret: there might have been no effect, but given that the birds were tested several months after the end of the treatment, the observed behavioural responses in singing and exploration might also have been influenced by current or relatively recent exposure to noise or social factors. With just two types of behaviour investigated we cannot draw firm conclusions on the effects of noise on behavioural development. This is an understudied area, and we hope the insights here can contribute to the design of future studies to further choose and develop methods to address the important questions of potential long-term effects of noisy rearing environments on behavioural phenotypes.

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