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

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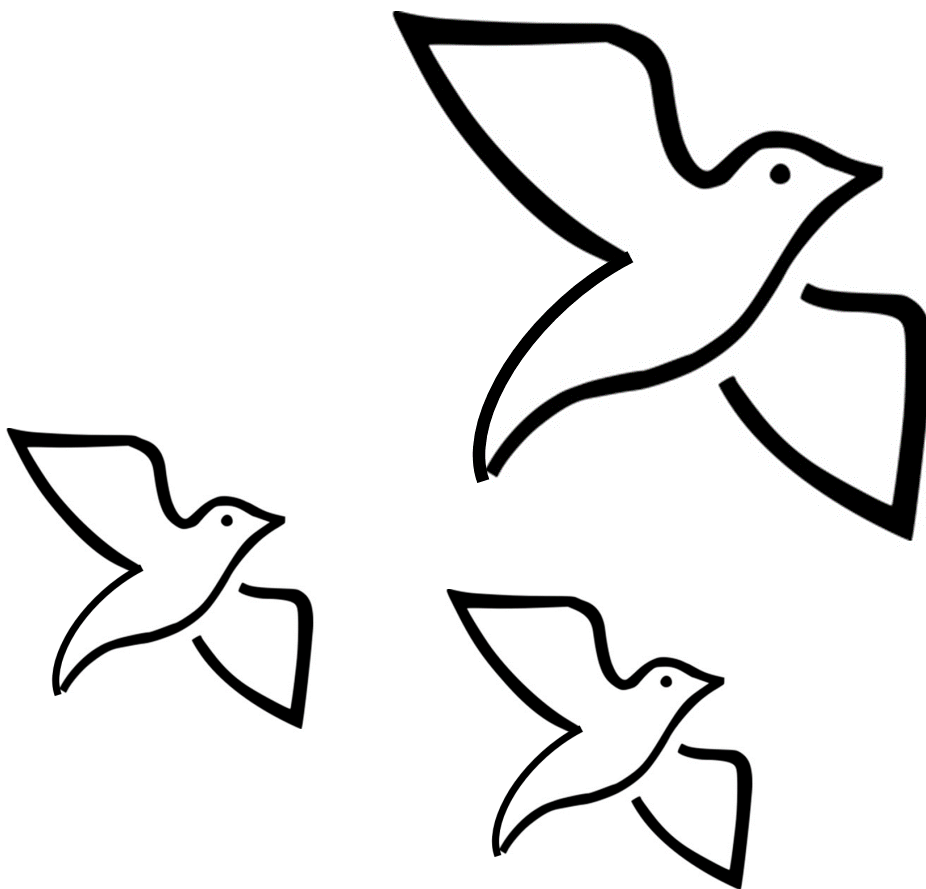
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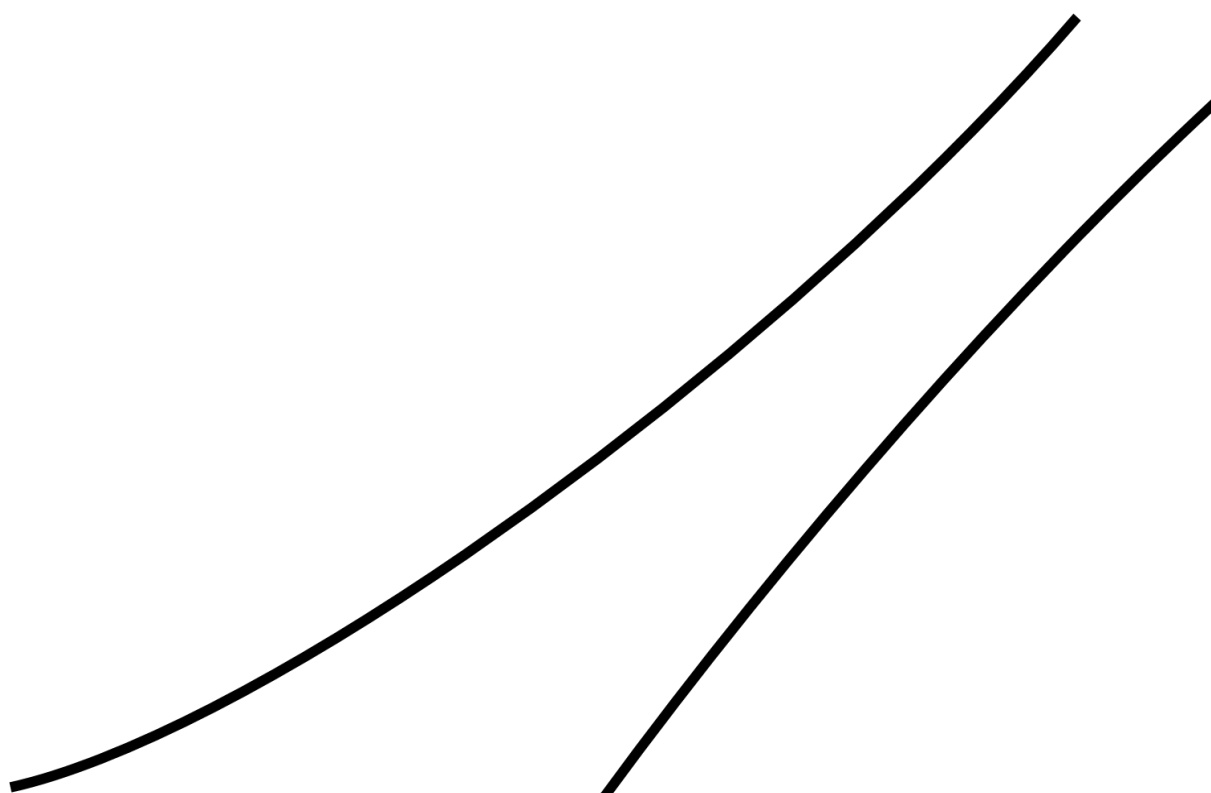
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Chapter 4

The effects of rearing noise condition on spatial avoidance of traffic noise in juvenile and adult zebra finches



Quanxiao Liu, Hans Slabbekoorn & Katharina Riebel



Abstract

Traffic noise is increasingly recognized to affect the distribution of wildlife and this is especially well documented in birds. Globally, the diversity and the abundance of birds are declining along roads. Noise avoidance has been suggested to be one of the factors contributing to this decline. However, the causality between noise and spatial avoidance is not well established as studies are either observational or, if experimental, only tested adult birds known to be naïve to noise or of unknown origin. To investigate whether early experience with noise would affect noise avoidance behaviour, we here tested experimentally whether chronic exposure to different types of traffic noise during development affected noise avoidance behaviour in zebra finches (*Taeniopygia guttata*). At the age of 65 days, all offspring were tested for noise avoidance in a two-choice set-up allowing free movement between two interconnected aviaries offering different noise conditions. After this first test, experimental birds were housed without noise playbacks and were re-tested two months later when the birds had matured into adults. When the offspring were juveniles, birds from both noise treatment groups did not avoid either near- or far-distance traffic noise but both types of noise conditions were avoided by same-age juveniles raised without noise playbacks (controls). In the second test, all offspring from both noise treatments now avoided both near- and far-distance traffic noise. The results demonstrate behavioural flexibility in noise avoidance, but provide no evidence for early noise exposure permanently increasing tolerance to noise. We interpret the increased tolerance in the noise-reared (but not the control) juveniles as a transient effect of noise experience because the birds reared in noise did not develop into noise tolerant adult phenotypes. The results suggest that noise tolerance is a behavioural trait of behavioural flexibility. Such flexibility might be an adaptation to cope with short-term fluctuations in ambient noise levels across time and space. However, rearing in chronic noise does not necessarily lead to noise tolerant adult phenotypes.

Introduction

Traffic noise is increasingly associated with negative effects on wildlife (Barber et al., 2011; Iglesias Merchan et al., 2014). A number of reports have now revealed noise-related declines in diversity and abundance, particularly for birds (Reijnen et al., 1996; Bayne et al., 2008; Francis et al., 2009; Parris & Schneider, 2009; Arévalo & Newhard, 2011; Goodwin & Shriver, 2011; Herrera-Montes & Aide, 2011; Proppe et al., 2013). Birds may fare less well in noisy conditions because their acoustic signals are masked (Brumm & Slabbekoorn, 2005; Grade & Sieving, 2016; Villain et al., 2016), or because noise makes them forage less efficiently due to distraction or increased vigilance (Quinn et al., 2006; Ware et al., 2015). The above have been hypothesized to contribute to less successful breeding observed at noisy compared to quiet breeding sites (Halfwerk et al., 2011; Kight & Swaddle, 2011; Schroeder et al., 2012). Bird numbers may also decline because birds simply move away to avoid noisy but otherwise suitable habitat (Brown, 1990; Blickley et al., 2012; McClure et al., 2013; Liu et al., 2020). There is as yet little knowledge on transgenerational versus ontogenetic effects of noise on birds, and whether behavioural phenotypes of birds in different habitats arise from selective or from ontogenetic effects from being raised in quiet versus noisy conditions (Dooling & Popper, 2007; Potvin, 2017).

Noise exposure could affect animals' development resulting in phenotypic differences among individuals and populations, but the degree of impact will likely be influenced by the level, frequency, duration and context of exposure. The effects of these noise characteristics are difficult to systematically assess in the field. In controlled experimental laboratory studies, high level sounds near hearing damage levels can cause permanent or temporary hearing threshold shifts (Dooling & Saunders, 1974; Ryals et al., 1999; Valero et al., 2017). Rats (*Rattus norvegicus domestica*) normally avoid low- and high-frequency noise, but experimental long-term exposure to loud high-frequency sound, resulted in permanently shifted hearing thresholds: the rats stopped avoiding high-frequency noise, but started showing stronger avoidances of low-frequency noise (Manohar et al., 2017). Moderate level sound exposure during development could change sensory processing. For example, grey squirrels (*Sciurus carolinensis*) raised in noisy urban area responded more often to visual than acoustic warning signals from a robot squirrel than their rural counterparts (Partan et al., 2010).

Differences such as the one reported between urban and rural squirrel populations (Partan et al., 2010) could be the result of selection for particular pheno- or genotypes that are more

easily settling or surviving in either habitat, but they could also be the result of a chronic noise induced developmental effect. In rats, for example, continuous exposure to moderate level of white noise during development made the primary auditory cortex develop more slowly (Chang & Merzenich, 2003). In guppies (*Poecilia reticulata*), noisy rearing conditions can hamper the development of multisensory integration (Xu et al., 2014). However, some of these shifts might also be a case of adaptive developmental plasticity and sensory switching to other modalities; a way to optimally adapt to environmental conditions, as has been reported for dark-reared guppies switching from vision to olfaction to forage (Chapman et al., 2010; Kimbell et al., 2019). Noise can also affect cognition: juvenile lizard fish (*Synodus dermatogenys*) and moonwrasses (*Thalassoma lunare*) exposed to boat noise during training failed to learn predator odour and survived less well after being released to the sea, indicating the impact of noise on learning about predation risk (Ferrari et al., 2018).

We do not know the underlying mechanisms for most of the above reported changes in the sensory systems and processing. However, at least for birds, there is some evidence that noise exposure during development can affect the pituitary-hippocampus-adrenal axis (Kleist et al., 2018; Beaugeard et al., 2019, but also see Angelier et al., 2016), which can affect many aspects of an individuals' behavioural phenotype (Spencer, 2017). However, any of these physiological changes could arise from the noise exposure directly, or be an indirect effect of the noise on the parents and the quality of parental care they provide. Several studies have reported noise-level dependent variation in parental behaviour (Schroeder et al., 2012; Naguib et al., 2013; McIntyre et al., 2014; Leonard et al., 2015; Meillère et al., 2015; Injaian et al., 2018a), highlighting the complexity of these issues and the difficulties to understand the effect of long-term noise exposure on birds directly and on their distribution patterns along roads.

An area that has seen quite some experimental work into the effect of long-term noise exposure during development on adult behaviour concerns vocal development. For example, when male zebra finches were exposed to very loud sound masking part or all of their song during vocal development, the timing and results of song development and crystallization were severely affected (Marler et al., 1973; Funabiki & Konishi, 2003; Zevin et al., 2004; Funabiki & Funabiki, 2009). Even less severe masking and less intense sound were reported to affect vocal learning. Developing white-crowned sparrows (*Zonotrichia leucophrys*) learned preferentially high-frequency songs when exposed to city-like low-frequency noise (Moseley et al., 2018). This result could be explained by selective masking of low-frequency songs leading the birds to only copy the songs they could hear better. This phenomenon may not apply to all species. In great tits (*Parus major*), chicks exposed to noise, masking either

high (3 - 6 kHz) or low frequencies (0 – 3 kHz), did not show any masking dependent learning of song types (Zollinger et al., 2017).

Although noise exposure during development has been shown to cause adjustments in physiology and song, we know little to nothing about the avoidance behaviour of birds raised in noisy conditions. Observational studies in the wild documenting settlement tendencies to vary across areas with different ambient noise levels (Halfwerk et al., 2011; Kight et al., 2012; Schroeder et al., 2012) cannot rule out confounding effects of other factors varying among territories. Moreover, these studies have no information of the rearing noise levels the settling individuals may have experienced. It is important to stress that while sound level dependent settlement patterns in adult birds can be a direct effect of some birds avoiding settling under aversive noise levels, differential settlement can also arise from condition-dependent success in getting a high-quality territory (Reijnen & Foppen, 1991). Playback experiments in the field (Blickley et al., 2012; McClure et al., 2013) and the laboratory (Evans et al., 2018; Liu et al., 2020) are useful to gain insight into noise avoidance tendencies, but such studies have typically used adult birds that were naïve to noise or were of unknown rearing background. Furthermore, birds may show experience-dependent changes in their noise avoidance tendencies in the course of their development, while most exposure experiments only tested birds once at a specific stage in life. Therefore, to examine if chronic exposure to traffic noise affects noise avoidance behaviour in birds, a controlled experiment with tests at different stages during development is required.

Zebra finches are an excellent model to test how sound exposure during development affects noise avoidance. Zebra finches have a relatively short generation time and breed easily in captivity, allowing to breed the same pairs under different acoustic conditions in brief succession. The hearing curve and thresholds for auditory damage are also known for this species (Okanoya & Dooling, 1987; Ryals et al., 1999; Noirot et al., 2011), which allows working with sound levels of potential behavioural consequences, but that do not accidentally lead to physiological hearing impairment. Zebra finches have been shown to be affected by sound levels elevated above regular ambient noise with respect to song learning, communication and foraging (Cynx et al., 1998; Tumer & Brainard, 2007; Villain et al., 2016; Tachibana et al., 2017; Evans et al., 2018). Furthermore, in an earlier experiment, we have shown with a two-choice paradigm that domesticated adult zebra finches naïve to noise, avoided traffic noise recorded at near but not at far distances from highways (Liu et al., 2020). This setup thus provides an excellent approach to test noise reared offspring, of the previously (naïve to noise) tested adults, to investigate whether noisy rearing conditions affect noise avoidance behaviour as well as whether this behaviour changes over time.

In the current study, we tested whether birds, that grew up under constant exposure to two types of traffic noise, showed noise avoidance behaviour that differed from their parents, and whether it was dependent on the type of rearing noise. To this end, one group was raised at a level that had been avoided by their parents (high intensity traffic noise, recorded near a Dutch highway), while another group was raised with a noise level that was shown not to be aversive to their parents (recorded far from the same highway, Liu et al. 2020). The birds were tested twice, once as juveniles and once as adults. If early noise exposure has an effect on noise avoidance, the offspring should behave differently than control (no noise playback) birds of the same age when tested as juveniles. If there is a long-term effect, the offspring raised in noisy conditions should also behave differently from their parents when tested as adults.

Material and Methods

Subjects and housing

All subjects originated from the breeding colony at Leiden University. To set up breeding pairs, 30 males and females were selected from the experimental subjects that had been tested by Liu et al. (2020) for noise avoidance (details see below). Preceding the experimental breeding, the birds (age: 900.9 ± 60.3 days old) had been housed in one all-male and one all-female aviary of identical make and size (L x W x H: 200 x 200 x 200 cm). To start breeding, birds were randomly paired and each pair was assigned to a breeding cage (100 x 50 x 40 cm) in one of two identical breeding rooms (365 x 305 x 300 cm, see Figure 1 in Chapter 3). In each room, 15 identical breeding cages were stacked in 5 (3+2) columns along two adjacent walls with 3 cage layers, so that they formed an L-shape (with the lowest cages 60 cm above the floor). For each cage, a nest box (11 x 9 x 9 cm) could be inserted via a small trap door at the top right corner of each cage.

Playback stimuli for continuous noise exposure

Traffic noise of a Dutch highway was recorded for 24 hours, from 10 am - 10 am on 27 - 28 July 2017, using two song meters (Wildlife Acoustics, model SM1), at two locations, near (52.098504N, 4.439159E, 50 meters from highway) and far (52.103469N, 4.441135E, 300 meters from highway) from the highway A4 (main route for cars between Amsterdam and Rotterdam/The Hague) near Park Vlietland (details in Liu et al. 2020). During the first three minutes of each recording, a sound pressure meter (Model 30, Pulsar Instruments Plc, Filey,

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UK, settings: A-weighted, reading LAT at a 10 s interval) was positioned at the microphone of the song meters to measure absolute sound pressure levels at the recording locations. The sound pressure levels (reference level throughout this study: SPL re 20 μ Pa) were 68.7 ± 3.2 and 52.8 ± 5.2 dB(A), for near- and far-distance traffic noise respectively. These levels were used as reference for later playback levels.

Experimental procedure for breeding with chronic noise exposure

Two loudspeakers (Blaupunkt CB 4500) were positioned in each room so that during playback of the highway recordings, noise levels were almost the same in all cages (near-distance traffic noise treatment: cages: 70.2 ± 0.5 dB(A), nest boxes: 68.5 ± 0.9 dB(A), far-distance traffic noise treatment: cages 51.5 ± 0.4 dB(A), nest boxes 51.4 ± 0.6 dB(A), measured with a sound pressure level meter (Model 30, Pulsar Instruments Plc, Filey, UK, settings: A-weighted, reading LAT at a 10 s interval).

Each pair participated in two rounds of breeding: once with continuous playback of the near-distance noise and once with continuous playback of the far-distance noise. In the first round, Room A was assigned to the near-distance traffic noise exposure and Room B to the far-distance traffic noise exposure treatment. The experiment started with introducing the breeding pairs to the breeding cages and leaving them for 3 days to acclimate before the noise playbacks started. For the next 14 days, the volume of the playbacks was gradually increased daily, starting from zero, by 5 dB(A) for near-distance traffic noise, and by 4 dB(A) for far-distance traffic, until the sound level inside the breeding cages reached the amplitude levels that had been measured at the recording site. Once the final levels had been reached the breeding pairs were given another week to acclimate before we introduced nest boxes (11 x 9 x 9 cm, attached to the top right front corner of each cage) and nesting material (hay and coconut fibre).

From then on, all nest boxes were checked daily for new eggs and hatched chicks. Chicks were individually marked on the day of hatching, or the day after, by cutting down feathers at one or two places of six specific body parts (head, back, left or right leg, or left or right wing, see Adam et al. 2014). Chicks (within a treatment) were cross-fostered around 3.6 ± 1.7 dph to break any correlation between parental quality and brood size, and to ensure also having a number of larger brood sizes, as we expected potential interactions between noise treatment and brood size dependent body condition of the chicks (see chapter 3). Full sibling groups were split up whenever possible and the age composition was aimed to emulate those found in natural broods. Final brood sizes ranged from 1 to 6. At 11 days post hatching (dph), just before the down feather cuts were no longer recognisable, chicks were marked by attaching an orange plastic ring with a unique identity number to their left leg.

At 65 ± 4 dph, chicks were caught and weighed and they received an additional ring on the right leg with a micro transponder tag (ID100A, Dorset, Aalten, The Netherlands). After tagging, they were moved to the noise avoidance test aviaries (see below). After all chicks from both breeding rooms had been moved out, the traffic noise playbacks were gradually faded out in the course of one week until levels were back to base line ambient levels (10 dB(A)/day for near-distance traffic noise and 8 dB(A)/day for far-distance traffic noise). Breeding pairs were given a two week break with these ambient levels, before the second breeding round started. Pairs remained in their cages. The noise treatment was now reversed between rooms but all other procedures were an exact replicate of the first breeding round. Figure 1 gives a schematic overview of the procedures and the timeline. Most pairs bred in both conditions (details see chapter 3) and all offspring (near-distance: 37 females and 22 males; far-distance: 45 females and 18 males) were subsequently tested in the noise avoidance test. In addition, another 28 control subjects (14 females and 14 males) were recruited from the la-

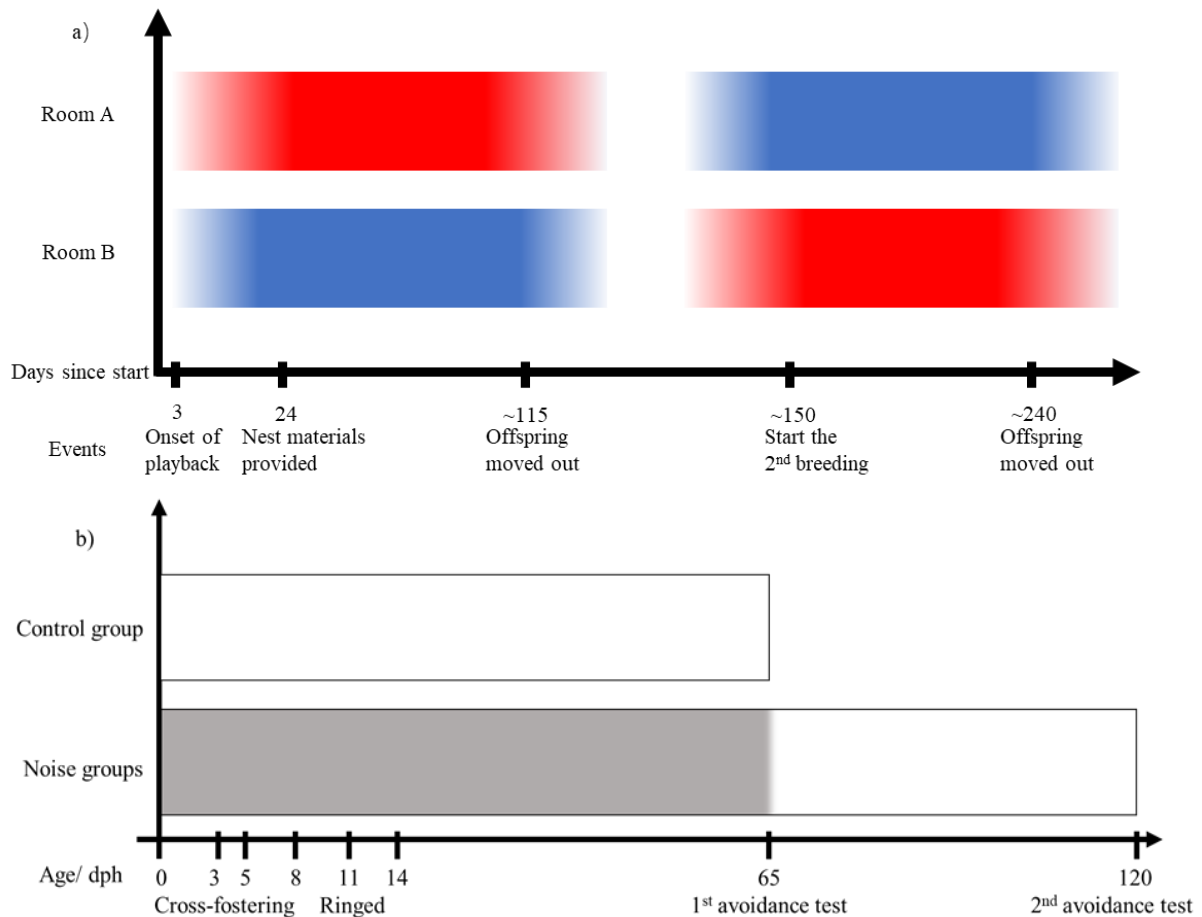


Figure 1: a) Overview of the treatment schedule for the breeding pairs in the two experimental rooms. Red and blue colours indicate exposure to noise recorded at a near or far distance from the highway. The intensity of the colours stands for the relative noise levels to their peak level. b) Experimental procedure for the offspring. The grey bar symbolises the noise exposure (either level), the white bar the absence of playback (= “lab ambient”).

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boratory breeding stock after the second breeding round. These young birds had been raised without any noise playback (ambient levels in their breeding room had always been lower than 40 dB(A) when the birds were quiet).

Behavioural testing: noise avoidance test

Experimental subjects were tested twice: as juveniles (65 ± 4 dph) and as young adults (115 ± 17 dph). Control birds were only tested as juveniles, but not again as adults as testing all the experimental offspring at a comparable age was given priority and because a large number of naïve adult birds (the parents of the subjects) had already been tested in the setup previously (Liu et al. 2020). Birds were tested in groups of four, and with subject originating from at least 2 different nests (number of groups, 1st round = 26, 2nd round = 19) with the exception of a total of eight groups that were tested in smaller groups because no same age peers were available (seven groups of three: 1st round = 2, 2nd round = 5; and one group of two birds in the 1st round). Juveniles were tested as they reached 65 days, and groups could be all-male or all-female or mixed, but adults were tested in all-male or all-female groups only to avoid males and females engaging in courtship. To start a test, a test group was moved into the experimental setup to acclimate 18 hours before the test started. During this phase, there was continuous playback of the test group's rearing background noise.

The two-choice set-up consisted of aviaries (200 x 200 x 200 cm) that were connected by a wire mesh tunnel (100 x 50 x 50 cm). All side walls of the aviaries and the tunnel were covered by 3 cm thick sound attenuating foam. Inside each aviary, we placed one loudspeaker (CB4500, Blaupunkt, Hildesheim, Germany) at 1 m height in the corner furthest away from the tunnel. A webcam (HD Pro C920, Logitech, Lausanne, Switzerland) was installed at the top of the corner facing the tunnel. Four parallel perches, perpendicular to the tunnel, were hung from the top of the aviary and a fifth perch was placed in the tunnel. Food and water dispensers were suspended from the two outermost perches, granting birds *ad libitum* access to food and water. The connecting tunnel had an opening (50 x 50 cm) at each end that could be opened and closed remotely. The middle of the tunnel could be passed only through a 30 x 30 cm black metal antenna (ANTSER300, Dorset, Aalten, The Netherlands) connected to a PC outside the room to register the IDs of the tags of passing birds.

On test days, a noise avoidance test always started between 9 am and 10 am. After a pre-playback phase of 15-minute rearing noise, the groups were exposed to playback 1 and playback 2, which included presentation (in a fully balanced design) of the following playback stimuli: 1) quiet vs far-distance traffic noise (~55 dB(A)) or 2) quiet vs near-distance traffic noise (~70 dB(A)), with a 15-minute break in between. For each of these first two playback trials, the loudspeaker in one aviary started to play a 30-minute-long noise stimulus with a 2

min fade-in and 2 min fade-out time while the loudspeaker in the other aviary was turned on but not playing back any sound. Once the first 30 min playback was finished, the previously silent speaker started to play the same stimulus (for another 30 min) and the previously noisy speaker became silent. This playback was followed by a 15 min break, then birds were exposed to playback 3. The procedure of playback 3 was the same as for the two previous playbacks, but now the loudspeakers in the two aviaries played near- and far-distance traffic noise simultaneously for 30 minutes, and then after stimulus reversal for another 30 minutes. All playbacks were balanced with respect to which type of noise was played back in which aviary first. This test procedure was identical to the procedure used by Liu et al (2020), except for two minor modifications: 1) during the acclimation and pre-playback phase, the speakers in both aviaries were playing back birds' rearing noise and 2) the simultaneous playback phase in Liu et al (2020) consisted of one 30-minute simultaneous playback, now this phase was extended for another 30 minutes to also have a stimulus reversal during playback 3.

Each group that had completed their first preference test was then moved and from then onwards housed with other groups in single-sex aviaries (100 x 200 x 200 cm, quiet laboratory ambient conditions (< 40 dB(A))). Two months later, as adults, these birds were tested for a second time, but now always in male or female only groups to avoid interest in potential partners affecting spatial behaviour (group size four birds: $n = 20$ groups; three birds: $n = 5$). The protocol of the second test was identical to the first, except that birds now received no noise playback during the acclimation and pre-playback phases, because the acclimation phase was set to match housing noise levels (which were without noise playbacks at this stage). After completing the second test, subjects were returned to their home aviaries.

All tests were filmed with the webcam in the aviary and behaviours were scored using BORIS video analysis software (v. 6.1.6; Friard & Gamba, 2016). One of us (QL) conducted all video analyses blindly with respect to the noise playback (sound was turned off during scoring) and subjects' rearing conditions (videos were coded by random numbers). The main response variable for the tests was the cumulative time birds spent in either aviary during each phase of the experiment (pre-playback, playback 1, 2 and 3). The cumulative score for an aviary could range from 0 min (not a single bird visited) to 240 minutes (4×60 minutes, 4 all birds stayed for the whole time).

Analyses

Prior to statistical analyses, the proportion of time spent in the relatively more quiet aviary was calculated for all groups and all playbacks (for pre-playback: quiet vs. quiet, we used the time spent in aviary A). These values were arcsine square root transformed for normality.

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All statistical analyses were performed in R 3.5.2 (R Core Team, 2019). To test whether proportion of time spent in the quiet room was deviating from a 0.5 chance level, we subtracted the (arcsine square root transformed) chance level of 0.5 from each of these values and then tested for a significant deviation from zero.

We then analysed if juvenile birds from all rearing conditions used both aviaries equally during the pre-playback phase using one-way ANOVA, with rearing noise condition (near/far/control) as the factor. To test whether rearing noise levels affected time spent in the aviary during playbacks 1 and 2, we used a mixed linear model with ‘time spent in the quiet aviary’ (without noise playback) as response variable, ‘group ID’ as random intercept, rearing condition (near/far/control), noise playback type (near/far) and their interaction as fixed factors (Table 1, Model A, lmer function from the ‘lme4’ package in R). To further analyse the differences among treatments, the least-squares means (EMMs) for three rearing conditions were computed and compared with the ‘emmeans’ function in the R package ‘emmeans’ (Table 1, Model A, EMMs).

For Playback 3, a linear regression model was created with time spent in the aviary with far-distance traffic noise as response variable and rearing condition (near/far/control) as the fixed factor (Table 1, Model B, ‘lm’ function from package ‘stats’ in R). Similar to playback 1 & 2, EMMs were computed to analyse the differences among all three rearing conditions (Table 1, Model B, EMMs). We applied the same statistical methods to the data from the second test round with the adults, but then with two, instead of three, rearing conditions (near/far).

Results

As juveniles, birds from all rearing conditions spent equal amounts of time in either aviary during the pre-playback phase (one-way ANOVA, $F_{2,46} = 0.16$, $p = 0.85$, Figure 2a). During playback 1 & 2, zebra finches from both noise treatments did not avoid either type of traffic noise while the control birds, raised in quiet ambient conditions, always avoided noise (Figure 2b-c). In addition, the avoidance was stronger towards near- than far-distance traffic noise (EMM contrast test: playback near vs. far, $p = 0.006$). During playback 3, when exposed to simultaneous playback of near- and far-distance traffic noise, control birds and birds raised in far-distance traffic noise avoided near-distance traffic noise. In contrast, the

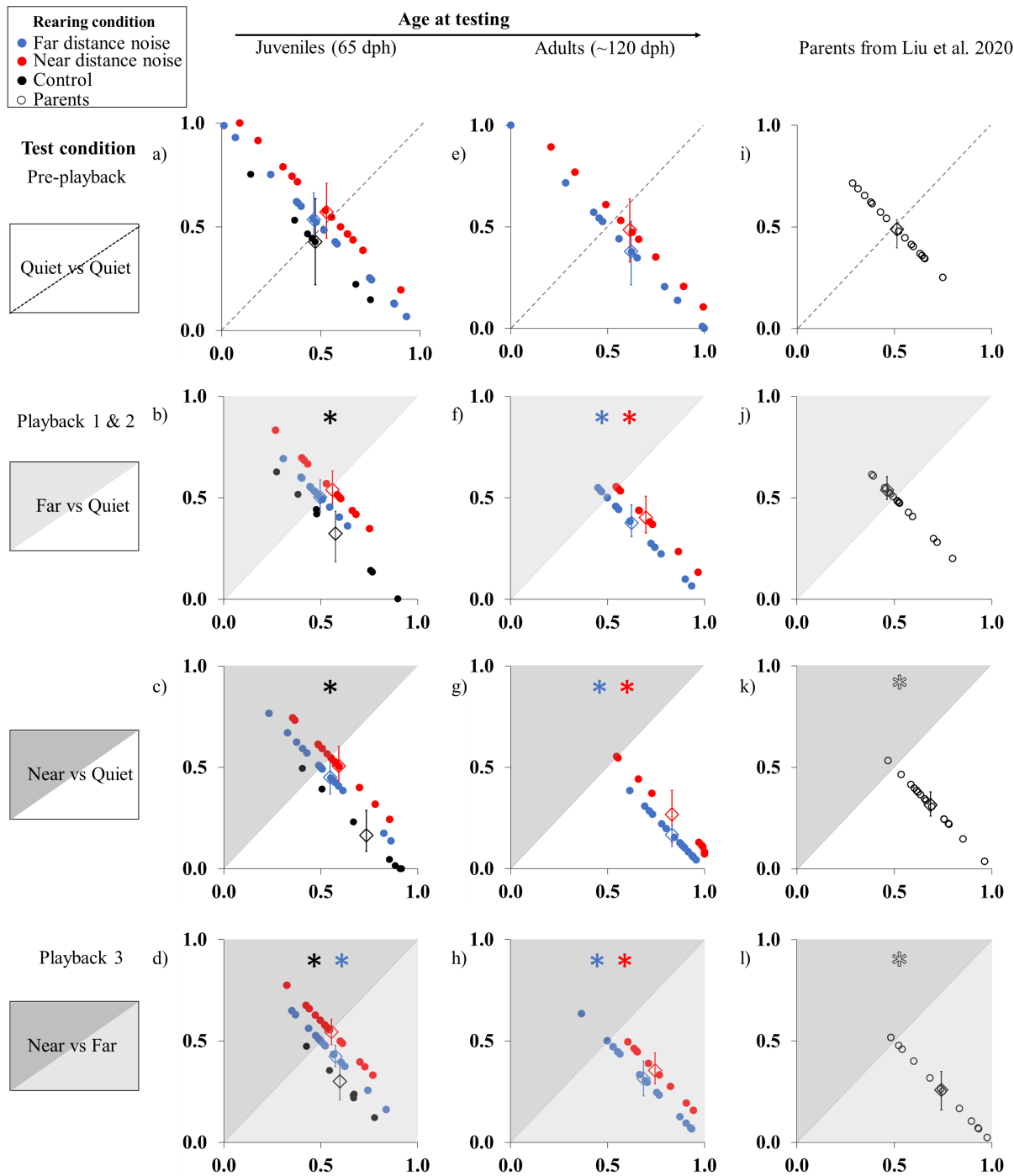


Figure 2: Percentage of time spent by each group in either aviary during the noise avoidance tests. Each dot represents one tested bird group (blue/red/black indicate near-distance noise/far-distance noise/control rearing conditions). Open diamonds with error bars represent mean \pm se per treatment. The data of the parental generation (from Liu et al., 2020) are provided for reference. To avoid dots overlapping, dots were slightly shifted diagonally away from the ' $y = 1 - x$ ' line by adding 0.05. Significances are indicated by * in the colours corresponding to the rearing conditions.

Table 1: Models and least-squares means (EMMs) comparisons of time spent in the more quiet aviary of the tested juvenile zebra finch groups from the three different rearing conditions in playback 1, 2 & 3.

Rearing conditions	estimate	Std. Error	p value	lower limit	upper limit
<i>Model A: Far/Near vs Quiet: Rearing condition (R)* + Playback type (P)* + R:P¹, as juveniles</i>					
<i>Model A – EMMs1, Playback type = Near</i>					
Control birds	0.35	0.07		0.21	0.48
Far	0.03	0.05		-0.06	0.12
Near	0.03	0.05		-0.08	0.12
Control birds – Far	0.32	0.08	0.0005		
Control birds – Near	0.33	0.09	0.0004		
Near – Far	-0.01	0.07	0.99		
<i>Model A – EMMs2, Playback type = Far</i>					
Control birds	0.15	0.07		0.01	0.29
Far	-0.01	0.05		-0.06	0.14
Near	0.04	0.05		-0.10	0.08
Control birds – Far	0.16	0.09	0.42		
Control birds – Near	0.11	0.08	0.13		
Near – Far	0.05	0.07	0.73		
<i>Model B: Far vs Near: Rearing condition*, as juveniles</i>					
<i>Model B – EMMs1, Playback type = Near vs Far</i>					
Control birds	0.16	0.05		0.06	0.26
Far	0.04	0.03		0.03	0.13
Near	0.00	0.04		-0.06	0.07
Control birds – Far	0.11	0.06	0.37		
Control birds – Near	0.15	0.06	0.04		
Near – Far	-0.04	0.05	0.18		

¹ R:P stands for the interaction between rearing conditions and playback types; * the factor is significant in the model, $p < 0.05$

Table 2: Models and least-squares means (EMMs) comparisons of time spent in the more quiet aviary of the adult zebra finch groups from two rearing conditions in playback 1, 2 & 3.

Rearing conditions	estimate	Std. Error	p value	lower limit	upper limit
<i>Model C: Near/Far vs Quiet: Rearing condition (R) + Playback type (P)* + R:P¹, as adults</i>					
<i>EMMs, Playback type = Near</i>					
Far	0.38	0.05		0.28	0.48
Near	0.35	0.06		0.22	0.47
Near – Far	-0.03	0.08	0.67		
<i>EMMs, Playback type = Far</i>					
Far	0.14	0.05		0.04	0.23
Near	0.16	0.06		0.03	0.29
Near – Far	0.02	0.08	0.76		
<i>Model D: Far vs Near: Rearing condition, as adults</i>					
Far	0.21	0.05		0.11	0.31
Near	0.21	0.06		0.08	0.34
Near – Far	0.00	0.09	0.98		

¹ R:P stands for the interaction between rearing conditions and playback types; * the factor is significant in the model, $p < 0.05$

birds raised in near-distance traffic noise did not avoid the near-distance noise and spent similar amounts of time in both aviaries (Figure 2d).

In the second test round, which took place two months after the first test (the juvenile birds had now matured into adults), we only tested the birds from the two noise rearing groups. They used both aviaries equally during the pre-playback phase (one-way ANOVA, $F_{1,22} = 0.21$, $p = 0.65$, Figure 2e). During playbacks 1 & 2, birds from both treatments avoided either noise playback and more strongly so the louder, near-distance traffic noise playbacks (Table 2, Figure 2f-g). During playback 3, birds from both treatments also avoided near-distance traffic noise (Figure 2h).

Discussion

We tested whether chronic exposure to traffic noise during development affected noise avoidance behaviour in zebra finches. To this end, breeding pairs were given the opportunity to breed in two different noise conditions. Noise avoidance behaviour in their offspring was tested at both the juvenile and adult stage. Additionally, a control group of zebra finches was tested as juveniles. Experimental rearing noise affected the noise avoidance behaviour in juveniles. Juveniles from both chronic noise exposure treatments did not avoid noise recorded at near or far distance from a highway. In contrast, same-age juveniles from the control group, reared in quiet conditions, avoided both the near- and far-distance traffic noise and preferred the quiet space. When simultaneously presented with both near- and far-distance traffic noise, juveniles raised in near-distance traffic noise behaved indifferently and showed no preference or avoidance of either type of noise. In contrast, both the controls and the birds raised in far-distance traffic noise significantly avoided the near-distance traffic noise and preferentially stayed in the aviary with the playback of the far-distance traffic noise. In the second test round, as adults, birds from both treatment groups now avoided both near- and far-distance traffic noise (see Figure 2f and 2g). Birds from both noise treatments equally strongly avoided near-distance traffic noise and moved into the aviary with far-distance traffic noise during the simultaneous playbacks (see Figure 2h).

The experimental birds that were reared with chronic highway noise exposure, when tested as juveniles, did not avoid traffic noise in favour of quiet and thus behaved strikingly different from controls that did move away from noise in favour of quiet. The treatments seemed to make juvenile birds more tolerant to the noise, a phenomenon that could have been in-

duced by changes in hearing physiology, such as hearing damage or temporary threshold shift. Hearing damage is however an unlikely cause of the behaviour of the birds in this experiment. The birds were exposed to noises around 55 and 70 dB(A) and these two levels are considerably below the estimated threshold of around 93 dB(A), identified as the level to cause temporary threshold shifts in zebra finches (Dooling, 2011). Zebra finches exposed to continuous noise at 110 dB(A) from 35 days old for more than hundred days (Funabiki & Konishi, 2003), were reported to have some hair cell damage, but the hearing of these birds started to recover within two days after the exposure and was fully recovered after four days. Since 110 dB(A) contains around 10000 times more energy than the 70 dB(A) we used, and earlier work has already demonstrated that exposure up to 93dB(A) does not cause hearing damage, hearing damage seems no likely explanation for the behaviour during the preference test. It is also unlikely that the lack of avoidance was caused by the lack of familiarity to the set-up in the first test because the control groups were also naïve to the set-up, but showed clear avoidance of the noise.

Although hearing damage is thus most unlikely, the lack of avoidance in our juvenile zebra finches might still originate from other effects of the chronic noise on the development of receiving and processing sounds. Noise can induce delayed song maturation (Funabiki & Konishi, 2003). Exposure to loud noise after 35 days of age caused juvenile males to crystallize their songs much later than the normal age. The delay was associated with a lack of structural and physiological changes in song nuclei during the noise exposure, probably caused by the absence of auditory feedback. Among these subjects, birds exposed to noise for more than 80 days had lower resemblance to their tutor in terms of temporal order of syllables. Similarly, noise exposure during early development may have delayed some aspects of auditory processing involved in the noise avoidance response. This could have changed during the subsequent two months without noise and this might have led to recovery or catch up of a noise avoidance response which the birds did exhibit at the later test as adults.

We lack sufficient insight into the potential impact of noise exposure on brain development as an explanation for our results. Nevertheless, exposure to traffic noise has been shown to impact developing birds in several ways including body condition (Schroeder et al., 2012; Injaian et al., 2018b), growth rate, oxidative stress (Injaian et al., 2018a), telomere degradation (Dorado-Correa et al., 2018; Injaian et al., 2019), immune system (Crino et al., 2013) and corticosterone levels (Kight & Swaddle, 2011; Kleist et al., 2018; Beaugeard et al., 2019, but see Angelier et al., 2016). Only one study to date has measured two brain areas related to song learning (Area X and HVC, Potvin et al., 2016) in birds exposed to recorded traffic urban noise during development. The authors report effects of noise on brain development, but the results are difficult to interpret, as only the traffic noise of moderate level but

not the other (including the high levels) had an effect on the structure volume of HVC. Even less is known regarding the effects of noise exposure on the processing of non-song learning related sounds. Therefore, physiological and neurological studies are required to confirm whether noise exposure delays the development of the auditory system and/or the processing of sounds.

Another explanation for our results may be a compensatory sensory switch. Birds have been observed to temporarily switch from using auditory to visual cues in noisy areas while foraging. Zebra finches and chaffinches (*Fringilla coelebs*) have been shown to tilt their heads up more often for scanning, when foraging in noisier conditions (Quinn et al., 2006; Evans et al., 2018). Similarly, the juveniles in our study, raised in noisy conditions, may also have been relying more on visual signals and less on acoustic signals to orientate in their environment, therefore showing no avoidance to the noise. Such sensory switches have been documented in several species when one of multiple sensory modalities is compromised by the environment (reviewed by Partan, 2017). In guppies, a sensory switch has been linked to rearing environment. Guppies raised in low-light conditions tended to respond more to chemical instead of visual cues (Chapman et al., 2010) and turbid water rearing conditions may also make guppies to develop opsins that are more sensitive to long wave lengths that could help motion detection in turbid waters (Ehlman et al., 2015). This particular type of compensatory plasticity normally restricts the animals to the developed phenotype, which was clearly not the case in our birds. However, a less restricted type of sensory plasticity may still explain our results.

Habituation, which can act on a much shorter time scale, and for a much shorter period than sensory plasticity, could be another mechanism (Rankin et al., 2009) that can explain the behaviour of the birds in our test. In experimental tests, exposing previously naïve birds to high levels of noise, depending on the species and the characteristics of the ambient noise, adult animals were observed to habituate to noisy environments from within a day to tens of days (Conomy et al., 1998; Brumm & Slabbekoorn, 2005; Masini et al., 2008; Linley et al., 2018; Neo et al., 2018). In the experiment described here, the juveniles are likely to have habituated to the noise after having been continuously exposed to the same noise stimuli for around 65 days. It is also possible that not the upbringing for the whole period in the noise was important, but that only the exposure in the days just before the tests mattered. The second test took place two months after the first test and after two months housing in the more quiet conditions, any habituation that might have been present would have been lost and that would explain why the adult noise reared birds now started avoiding the noise. That the experimental birds were habituated to the noise when tested as juveniles, also seems a more likely explanation than that the experimental birds lacked the skills to manoeuvre the large

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aviaries because they had been reared in cages. This is unlikely, because the control birds, like the experimental birds, had also been raised in cages and not experienced the aviaries before the test but showed clear noise avoidance and moved into the more quiet aviaries. Since the aim of this study was to test how noise exposure during development would affect birds rather than how long it takes for juveniles to habituate to noise, our experimental design did not include sufficient experimental noise rearing groups to vary the duration of the noise exposure which would have been necessary to titrate how long it would take the birds to habituate and to dishabituate. We therefore cannot draw any further conclusions now as to whether habituation or a temporary sensory threshold shift was most likely responsible for the difference between experimental and control birds.

It is also possible that the birds from the experimental treatments did not avoid the noise, because they had not yet experienced that they could actively move away from a noise source because the noise in their rearing environment was ubiquitous and showed no gradients. The position of the loudspeakers had specifically been arranged such that the noise levels were constant in the rearing cages. The birds had thus never experienced that they could actively fly away from noise before they participated in the noise avoidance tests in the aviaries. This may explain why the birds did not avoid the noise during their first test, as exposure to non-avoidable negative stimuli has been shown to affect the acquisition of appropriate performance in an avoidance task (Seligman & Beagley, 1975; Peterson, 2010; Swanson et al., 2012). This could also explain why as adults, the same birds that now had experienced quiet environments, avoided the noisy aviary.

Variation in physiological state arising from developmental stress has been shown to affect information processing in zebra finches in the contexts of mate choice, social learning and exploration (e.g. Holveck & Riebel, 2009; Krause & Naguib, 2011; Riebel et al., 2012). It is of interest to establish whether chronic noise exposure during development would have similar effects (for discussion, see Potvin, 2017). As yet, little is known about effects of noise exposure on animal decision making and cognition although it might have far reaching fitness consequences. For example, juvenile lizard fish and moonwrasses, failed to associate an odour with a predator when the odour was presented simultaneously with boat noise during training sessions in the laboratory (Ferrari et al., 2018). In the following experiment, a new batch of fishes underwent the same procedure. Fishes exposed to noise during training survived less well after being released to the sea than the control groups experienced no noise during training. This result indicates the potential impact of noise on learning about predation risk and suggests that it might also affect learning in other contexts.

To summarise, from our results, it does not seem likely that noisy rearing conditions could

potentially create a more noise resistant next generation. While it remains to be further investigated which mechanisms underlie the differences in behaviour between the control and the experimental groups at day 65, we can infer for now that the effects of the noise treatment had different consequences in the short and in the long term. The traffic noise exposure until 65 days affected the birds' behaviour in the first (juveniles) but not in the second test (adults). This suggests that the rearing noise did not induce adult noise tolerance. Instead, the noise tolerance of our test birds decreased after the birds had also experienced quiet conditions. If rearing noise does not induce more noise tolerant phenotypes, noise avoidance behaviour will be likely to continue to contribute to diminishing bird numbers in noisy habitats.

Our results are not only in line with but also providing a possible explanation for field observations showing that noisy but otherwise suitable habitats often have lower bird numbers than comparable quiet control habitats, although the presence of the noise source has spanned several generations and despite ongoing breeding activities in the noisy areas. Noise avoidance of otherwise suitable habitat may not be unique to birds. Mammals, reptiles, fish and insects also show reduced abundances in noisy versus quiet habitats (Shannon et al., 2016; Bunkley et al., 2017). Future studies of the developmental effects of noise exposure are required to fully understand the mechanism(s) underlying transient, but apparently reversible effects of experience-dependent noise tolerance as found in the current experiments. What can be concluded for now is that the increased noise tolerance in the juveniles is not long lasting: rearing in noise did not lead to a noise resistant phenotype. Our observations may have implications for further investigations into declines in bird diversity and breeding densities along noisy roads.

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