Silent Spring – the lost call

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The "silence" in Rachel Carson's *Silent Spring*[1] alludes to the demise of bird populations through reproductive problems and death resulting from exposure to the pesticides of that time, many of which are endocrine active. Endocrine disrupting chemicals (EDCs) are compounds which may interfere with the endocrine system, producing effects that may disrupt the physiologic function of hormones. Early research on EDC exposure in humans and wildlife has focused mainly on reproductive effects of estrogenic chemicals, however recent studies have revealed that effects of estrogenic as well as non-estrogens active chemicals are often more far reaching than the reproductive system, and even mild exposures experienced early in development may have detrimental effects that are maintained throughout adulthood. Here we show trenbolone acetate, an androgen active environmental contaminant used as a growth promoter for cattle, to cause a literal silence in Japanese quail (*Coturnix japonica*) chicks following a one time embryonic exposure. Vocalizations were not merely lessened; this is the first study to demonstrate an environmental contaminant to cause a complete abolishment of the ability to vocalize. Since many reasons for vocalization in birds are directly linked to survival of the individual and species, the potential for detrimental population effects is a grave possibility for many avian species that may encounter androgen active chemicals in the environment. Many androgen active EDCs are persistent and ubiquitous in distribution, therefore chances for exposure to these

chemicals in birds may be high. We hope that powerful, yet subtle effects like the ones presented here will encourage further research with EDCs to expand beyond the traditional focus of reproductive effects of estrogenic chemicals.

Although a variety of tests are used to study motor function in adult birds[2,3], few exist for chicks. Here, we performed a novel test of motor behaviour in chicks that assesses an important survival skill: locating and returning to its brood when separated. Japanese quail are precocial birds, being able to leave the nest and find their own food at day of hatch. It is necessary for chicks to remain with the brood after hatch for protection from predators and for thermoregulation. If a chick becomes separated from its brood, it calls to its conspecifics. Siblings vocally respond to the chick's separation call, which helps the separated individual to locate and return to the rest of the brood.

Quail were exposed at day four of incubation by yolk injections to sesame oil (control), or 0.05, 5, 50, or 125 μ g of trenbolone. After hatch, individuals were separated from ten randomly selected conspecifics and allowed to call and return to the group at the opposite end of a runway (182 cm long) within three minutes. The runway was divided into five lanes that were each 12 cm wide. Motor behaviour was assessed as the amount of time it took individuals to reach conspecifics, distance travelled, and the number of lanes crossed. Assumptions for parametric statistics were examined prior to analysis. Chi-square tests were performed to assess results expressed as percentages; all other data were analyzed by two-way analysis of variance with Tukey tests being used for post hoc pairwise comparisons. Isolated trenbolone-treated chicks reached their siblings faster than controls at week 1 (Fig. 1; $p<0.05$), however no difference was observed at week 2. Although androgens have been shown to induce locomotor activity in adults[4] (Wada 1984), this is the first study to examine similar effects in chicks. The distance travelled and number of lanes crossed did not differ among treatments.

While the main purpose of this study was to assess the chicks' abilities to locate and return to their siblings, the most interesting (and unexpected) result was the complete absence of vocalization from the 50 µg treatment group. In the first trial, vocalization was measured by observing whether or not individuals called to their separated conspecifics or not. Therefore, vocalization for the first trial is expressed as the percentage of individuals per treatment that performed stress vocalizations (Fig. 2a). These unexpected results prompted a second confirming trial to be made. Calling behaviour in the second trial was quantified at both weeks 1 and 2 of age by measuring the number of calls produced by individuals (Fig 2b). Vocalization was completely abolished at 50 μ g for both weeks in both trials (Fig. 2 [$X^2(4, N=50) = 19.6$], Fig. 2b ($p<0.05$). The majority of the birds that were unable to produce sound were straining and moving their beaks when isolated. Thus, the behavioural response remained intact; separated individuals were able to respond to the stress of being separated from their conspecifics, but were unable to effectively vocalize.

The intercollicular nucleus of the mesencephalon has been identified as the vocal neural system responsible for producing the distress call in Japanese quail chicks[5]. Adult male neurons from the intercollicular nucleus have many more dendrites than those of females. This difference suggests that the development of these neurons may be influenced by differences in sex steroids between the sexes, although it has not yet been determined if this difference is indeed caused by testosterone[6]. Neural projections connect the intercollicular nucleus with the hypoglossal nucleus, the control centre of the syrinx[7], which is the avian vocal cord located at the junction of the trachea into the primary bronchi. Syrinx mass is greater in male zebra finches (*Poephila guttata*) than females[8]. Although the mechanisms behind this dimorphism are unknown, it is suggestive of a possible role of sex steroids during development. Indeed, most of the research investigating the effects of gonadal steroids on syrinx development has used this species. The zebra finch syrinx has been found to contain androgen receptor

mRNA by embryonic day 10, in order to prepare the chick for post-hatch food begging behavior[9]. One study has shown that silastic implants of testosterone in adult zebra finches caused significant increases in syrinx mass and the size of the ventralis and dorsalis syrinx muscles, and implants of flutamide, an anti-androgenic chemical, decreased syrinx weight[10]. However, results from the few studies that investigate the effects of gonadal steroids on syrinx development are inconsistent. It is clear that the effects of these hormones on syrinx development are complicated, and it is generally agreed that although they do not appear to be directly responsible for the stimulation and control of the overall process of sexual differentiation of the syrinx, areas of the forebrain to the syrinx responsible for vocalization can be altered by exogenous administration of them[11].

Regardless of the mechanisms behind the observed trenbolone-induced suppression of vocalization, one must consider the ecological implications of this effect. The vocalization behaviour measured in this experiment is a survival behaviour for Japanese quail chicks. Suppression of this vocalization, therefore, could increase individual mortality and affect viability at a population level. Other avian species vocalize for a number of additional critical behaviours to ensure survival and fitness: food begging, mate attraction, sexual selection, and territory defence. Risk assessment of EDC-exposure in field birds is difficult because environmental levels of EDCs are generally not toxic and often weakly hormonal in action. When considering the significance of the myriad purposes of avian vocalization, the importance of assessing non-lethal and subtle effects of EDC exposure becomes clear. A relatively incidental effect, namely absence of vocalization, in an otherwise apparently healthy individual can have long-term implications for individual survival and population fitness. The unexpected effect of trenbolone on vocalization in this study is a compelling call for reassessment of androgen active EDCs and adds further support for the use of often more subtle, yet extremely vital behavioural endpoints.

References

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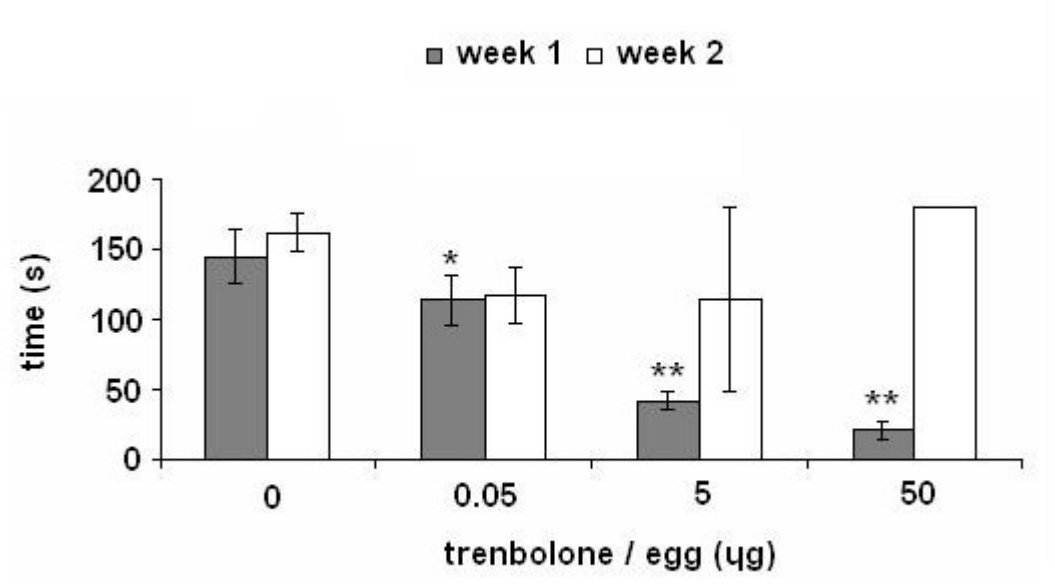

Figure 1. The mean amount of time (s) for separated Japanese quail (*Coturnix japonica*) chicks that had been exposed to trenbolone acetate *in ovo* to return to conspecifics with standard errors of the mean. Significant differences are indicated by asterisks.

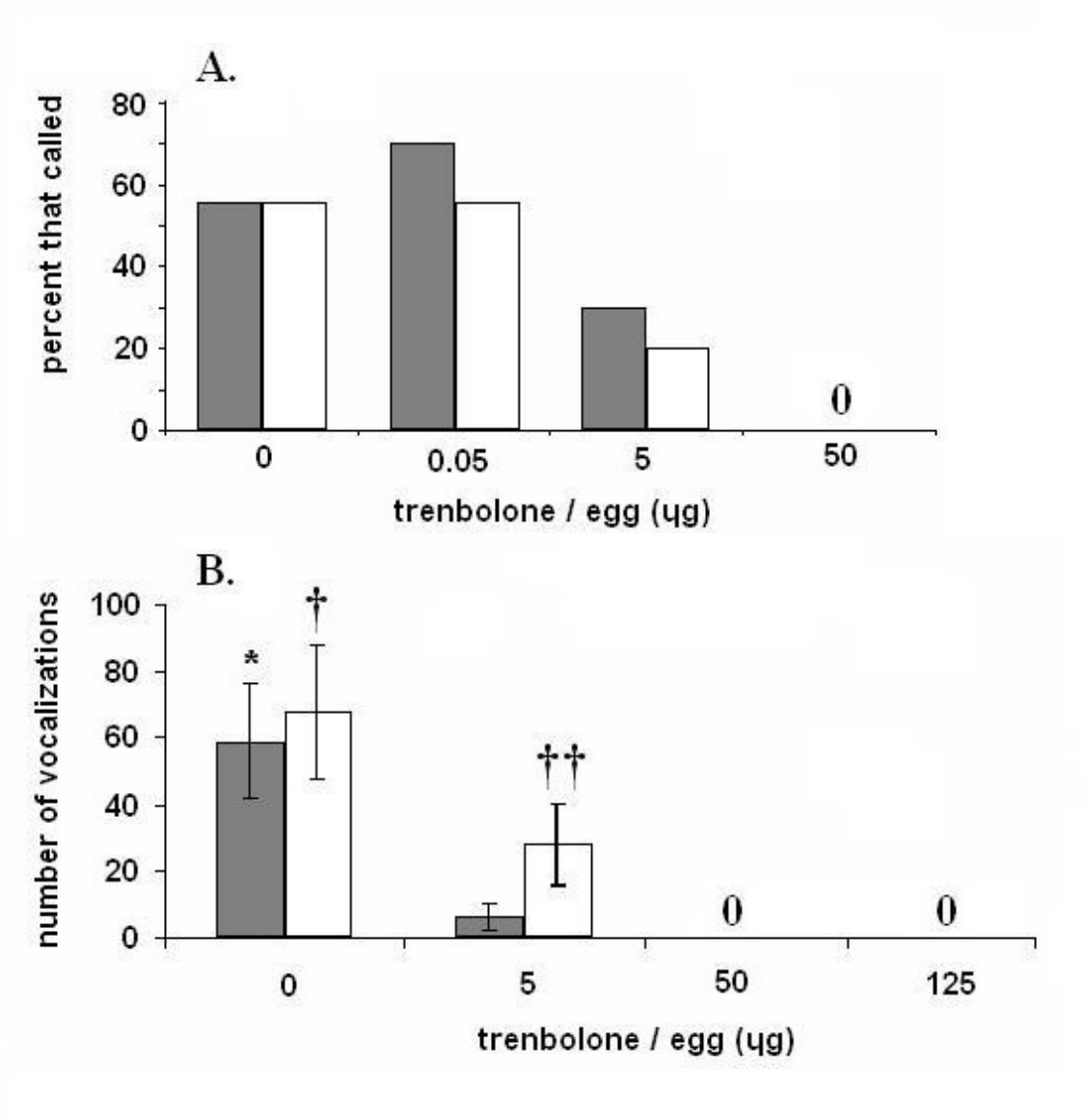

Figure 2. The percentages of Japanese quail (*Coturnix japonica*) chicks that called to their conspecifics at weeks one and two of age when isolated post *in ovo* exposure to trenbolone acetate in trial 1 (Fig. 2a). The number of vocalizations made by isolated Japanese quail chicks exposed to trenbolone acetate *in ovo* when separated from their conspecifics in trial 2 (Fig. 2b).