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Housing conditions and sacrifice protocol affect neural activity and vocal behavior in a songbird species, the zebra finch (*Taeniopygia guttata*)

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Abbreviations :
AH, anterior hypothalamus
VT, (arginine) vasotocin
VT-ir, VT-immunoreactive
BSTm, medial bed nucleus of the stria terminalis
IEG, immediate early gene
POA, preoptic area
POM, medial preoptic area
PVN, paraventricular nucleus of the hypothalamus
SBN, social behavior network
Sl, lateral septum
VMH, ventromedial hypothalamus
Zenk-ir, Zenk-immunoreactive

ABSTRACT

Individual cages represent a widely used housing condition in laboratories. This isolation represents an impoverished physical and social environment in gregarious animals. It prevents animals from socializing, even when auditory and visual contact is maintained. Zebra finches are colonial songbirds that are widely used as laboratory animals for the study of vocal communication from brain to behavior. In this study, we investigated the effect of single housing on the vocal behavior and the brain activity of male zebra finches (*Taeniopygia guttata*): male birds housed in individual cages were compared to freely interacting male birds housed as a social group in a communal cage. We focused on the activity of septo-hypothalamic regions of the "social behavior network" (SBN), a set of limbic regions involved in several social behaviors in vertebrates. The activity of four structures of the SBN (BSTm, medial bed nucleus of the stria terminalis; POM, medial preoptic area; lateral septum; ventromedial hypothalamus) and one associated region (paraventricular nucleus of the hypothalamus) was assessed using immunoreactive nuclei density of the immediate early gene Zenk (egr-1). We further assessed the identity of active cell populations by labeling vasotocin (VT). Brain activity was related to behavioral activities of birds like physical and vocal interactions. We showed that individual housing modifies vocal exchanges between birds compared to communal housing. This is of particular importance in the zebra finch, a model species for the study of vocal communication. In addition, a protocol that daily removes one or two birds from the group affects differently male zebra finches depending of their housing conditions: while communally-housed males changed their vocal output, brains of individually housed males show increased Zenk labeling in non-VT cells of the BSTm and enhanced correlation of Zenk-revealed activity between the studied structures. These results show that housing conditions must gain some attention in behavioral neuroscience protocols.

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1. Introduction

Individual housing represents an impoverished physical and social environment for gregarious animals, which may have deleterious effect on behavior and brain [1–4]. For instance, social deprivation has deleterious effects on the mammalian pre-frontal cortex or its avian homolog, the nidopallium caudolaterale [2]. In songbirds, individual housing also leads to the development of stereotypies (repetitive, unvarying, and apparently functionless behaviors) that correlate with signs of altered brain striatal functioning [5]. However, individual housing is commonly used in behavioral neuroscience protocols because it facilitates the acquisition of individual data [6–10]. The zebra finch (*Taeniopygia guttata*) is a songbird widely used as a model system for the study of vocal learning, vocal production, and auditory perception [11–13]. Although the zebra finch is a highly social species, male zebra finches are commonly housed in individual cages or soundproof booths for weeks in order to record their songs or undergo neurophysiological experiments in laboratories [14–17]. In their native environment, the sub-arid zones of Australia, zebra finches form year round groups of dozens to hundreds of birds and establish life-long pair-bonds [18]. In this species, social interactions with pair-mate and group-mates occur through intense vocal communication [18–24], but also largely through physical interactions that can be both affiliative [e.g., grooming (allopreening), perching in close contact (clumping), rubbing each other beaks (beak fencing)] or aggressive (e.g., pecking, chasing, aggressive beak fencing) [18,25–28]. Such physical interactions are essential to pair-bond establishment and socialization [18,28,29]. Zebra finches that experience physical separation from their social partners with or without maintained acoustic contact exhibit an increased plasmatic corticosterone level [14,27]. This increase in physiological stress hormone suggests that suppression of physical social contact is a potent stressor in this species and confirms that cage-mates are social stimuli that normally elicit affiliation [30]. The zebra finch is a model of choice to study the effect of housing conditions resulting in long-term deprivation of socialization on vocal behavior and brain activity.

In birds that learn their vocalizations by imitation of a conspecific tutor, song ontogeny and auditory sensitivity are highly influenced by the acoustic environment experienced by the bird [13,31]. Young birds would for instance develop hybrid songs if they are given two successive tutors during the sensory period of learning [32]. The acoustic environment of adult birds also influences their perception of sounds. More precisely, it changes the tuning properties of neurons in the caudomedial nidopallium, a telencephalic auditory area that selectively responds to conspecific vocalizations [4]. Although the importance of the characteristics of the acoustic environment has been described in both young and adult birds, housing condition variations (single versus group housing) have never been considered as potential parameters of the acoustic environment. Here, we tested whether group or individual housing influences the quantity and quality of vocalizations emitted by male zebra finches.

Evidence across vertebrates shows that evolutionarily conserved brain circuits in limbic regions are implicated in the motivational processes of social behaviors [33–35]. Because of its role in animal social behavior, the so-called "social behavior network" (SBN) could be deeply affected by a lack of social interactions due to poor housing conditions. The SBN is mainly constituted of midbrain areas (ventral tegmental area and periaqueductal gray, substantia grisea centralis in birds) and, in the forebrain, of the medial extended amygdala (medial amygdala—formerly nucleus taeniae [36,37]—and medial bed nucleus of the stria terminalis [BSTm] in birds), the preoptic area (POA), the anterior hypothalamus (AH), the ventromedial hypothalamus (VMH), and the lateral septum (Sl). Studies of the functional activity of these regions have identified several overlapping subnetworks implicated in agonistic, reproductive, or maternal behaviors [34]. No particular region is involved in a specific behavior, but the balance of activity within each area and across all the areas of the network seems to determine the behavioral output [34]. In birds, studies of immediate early gene (IEG) expression revealed that distinct but overlapping neural networks of the SBN are active during aggressive encounters [38,39] and during reproductive behaviors [37,40].

Functional activity of the SBN has mainly been described in reproductive and agonistic contexts during short encounters implicating a small number (two to three) of individuals, like resident-intruder confrontation in territorial species, male–male competition for access to females or male–female courtship [30,33,37–47]. Little is known about the effect on SBN activity of repeated exposure to social stimuli due to social living, or lack thereof [30,33,46,48].

In this article, we hypothesize that physical restraint and lack of social interactions derived from individual housing conditions should change the activity across the network. To analyze the impact of physical and social stress due to individual housing on vocal behavior and brain activity, we compared the quality and quantity of the vocal output, and the basal activity of septo-hypothalamic regions of the SBN of zebra finches in two housing conditions commonly used in behavioral neuroscience protocols:

- birds housed in adjacent individual cages with visual, auditory, and olfactory, but no tactile contact, that result in physical restraint and absence of physical interaction;
- communally-housed birds interacting freely within a social group.

Brain activity was assessed using immunoreactive nuclei density of the IEG Zenk (egr-1). Because brain structures of the SBN are constituted of several neural populations that differ both in the neuro-peptides they express [49] and in the way they respond to social stimuli [30], we conducted double labelling of Zenk and VT (vasotocin) to further identify the active neural populations. Indeed VT cells could be an interesting cell population to follow because, on the one hand, their activity has been associated with the perception of social stimuli eliciting affiliation in the BSTm [30], the display of