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BRAIN RESEARCH

Research Report

Sexually dimorphic expression of the genes encoding ribosomal proteins L17 and L37 in the song control nuclei of juvenile zebra finches

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ARTICLE INFO

Article history:
Accepted 3 August 2006
Available online 30 August 2006

Keywords:
Sexual differentiation
Masculinization
Ventricular zone
In situ hybridization
Songbird

ABSTRACT

Studies evaluating the role of steroid hormones in sexual differentiation of the zebra finch song system have produced complicated and at times paradoxical results, and indicate that additional factors may be critical. Therefore, in a previous study we initiated a screen for differential gene expression in the telencephalon of developing male and female zebra finches. The use of cDNA microarrays and real-time quantitative PCR revealed increased expression of the genes encoding ribosomal proteins L17 and L37 (RPL17 and RPL37) in the male forebrain as a whole. Preliminary *in situ* hybridization data then indicated enhanced expression of both these genes in song control regions. Two experiments in the present study quantified the mRNA expression. The first utilized 25-day-old male and female zebra finches. The second compared a separate set of juveniles to adults of both sexes to both re-confirm enhanced expression in juvenile males and to determine whether it is limited to developing animals. In Experiment 1, males exhibited increased expression of both RPL17 and RPL37 compared to females in Area X, the robust nucleus of the arcopallium (RA), and the ventral ventricular zone (VVZ), which may provide neurons to Area X. Experiment 2 replicated the sexually dimorphic expression of these genes at post-hatching day 25, and documented that the sex differences are eliminated or greatly reduced in adults. The results are consistent with the idea that these ribosomal proteins may influence sexual differentiation of Area X and RA, potentially regulating the genesis and/or survival of neurons.

1. Introduction

Sex differences in neural structure and function have been described in diverse vertebrate models. In many cases, they are permanently organized by exposure to gonadal hormones early in development (reviewed in Cooke et al., 1998; De Vries and Simerly, 2002). However, increasing evidence suggests roles for non-steroidal, genetic, factors (e.g., Arnold, 2002). This idea has become particularly relevant to the song system of zebra finches. In these birds, adult male sing, but females do not, and the forebrain regions that control song learning and production are far larger in males than in females, due in part to increased neuron number and soma size (reviewed in Wade, 2001). Exogenous estradiol can substantially, although not completely, masculinize the brain and behavior of females, and estrogen produced in the brain masculinizes at least one

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doi:10.1016/j.brainres.2006.08.002

projection within the song circuit (Holloway and Clayton, 2001). However, the natural mechanisms controlling the increase in the volume of these structures, the size and number of neurons within them, and the capacity to display song are largely unknown (see Wade and Arnold, 2004).

To facilitate our understanding of these processes, we used cDNA microarrays to screen for differential gene expression in the forebrains of developing male and female zebra finches. The results were validated with real-time quantitative (q) PCR, and then *in situ* hybridization was used in a few animals to determine the brain regions in which they were expressed. Among others, genes encoding two ribosomal proteins (RP), RPL17 and RPL37, were clearly detected in the song control nuclei of juveniles, and their expression appeared in some cases to be increased in males compared to females (Wade et al., 2005).

The present studies were undertaken to characterize the expression of these genes more completely. In addition to completing the protein coding sequence, in Experiment 1 we used *in situ* hybridization on a larger sample of 25-day-old individuals to determine whether the expression of these genes was indeed increased in song control nuclei of males compared to females. This age is during the juvenile period when males are memorizing the songs of their fathers and when morphological differentiation of the song circuit is occurring (Nordeen and Nordeen, 1997; Doupe et al., 2004; Wade and Arnold, 2004). Experiment 2 was then designed to both provide an additional replication of male-biased mRNA expression at this developmental stage and to determine whether the sex difference is maintained into adulthood. If RPL17 or RPL37 is specific to the process of sexual differentiation, then one would predict diminished and/or sexually monomorphic expression in adulthood.

2. Results

2.1. *Experiment 1*

For both RPL17 and RPL37, main effects of sex (both $F>12.44$; $p<0.006$) and brain region ($F>8.07$, $p<0.0001$), as well as significant interactions between the two variables ($F>3.53$, $p<0.015$; Fig. 1) were detected. Specifically, expression of RPL17 was increased in males compared to females in Area X ($p<0.001$), the ventral portion of the ventricular zone (VVZ; $p=0.001$) which may contribute cells to Area X (see DeWulf and Bottjer, 2002; DeWulf and Bottjer, 2005), and probably the robust nucleus of the arcopallium (RA; $p=0.012$, $\alpha=0.010$ for comparisons in 5 brain regions). In parallel, expression of RPL37 was greater in males than females in Area X ($p=0.001$, $\alpha=0.012$ for comparisons within 4 brain regions), the VVZ ($p<0.001$), and RA ($p=0.002$).

When the sexes were analyzed separately, both genes exhibited differences across brain regions within males (both $F>4.78$, $p<0.008$). For RPL17, mRNA in RA was increased compared to Area X, the VVZ, and the control region, A, (all $p<0.05$) in males. In contrast, females ($F=13.09$, $p<0.001$) showed a *decrease* in RPL17 expression in Area X and the VVZ compared to A ($p<0.05$). For RPL37, Area X, RA and the VVZ all showed increases compared to the control region, A, in males (all $p<0.05$). However, no differences existed among the brain regions in females for this gene ($F=0.58$, $p=0.635$).

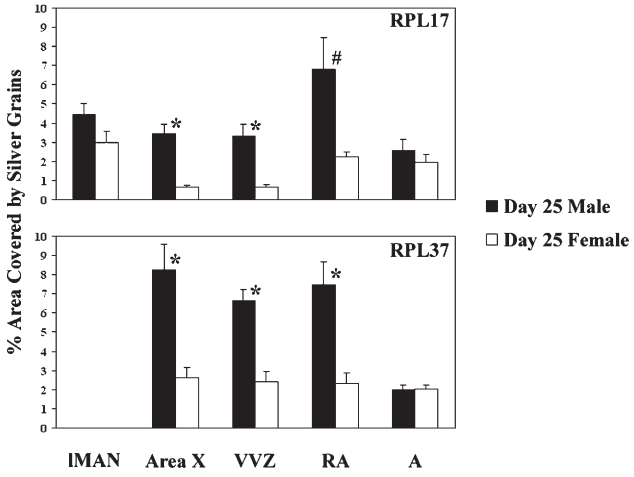

Fig. 1 – Experiment 1: Sex differences in the expression of genes encoding ribosomal protein L17 (RPL17; top) and RPL37 (bottom) in the song systems of 25-day-old zebra finches. In both cases, significant main effects of sex and brain region, as well as interactions between the two variables, were detected. Song control regions showing distinct labeling in a preliminary study (Wade et al., 2005) were evaluated, as was a control region outside of RA in the arcopallium (A). These areas were the same for the two genes, except for lMAN, in which we had detected RPL17 but not RPL37. * = significant effect of sex in pairwise planned comparisons (Bonferroni-corrected); # = one-tailed *p* value of 0.012 ($\alpha=0.01$ for 5 brain regions).

2.2. *Experiment 2*

For RPL17 (Figs. 2 and 3), significant main effects of age ($F=88.23$, $p<0.0001$) and sex ($F=78.65$, $p<0.0001$) were detected, as was a significant age × sex interaction ($F=18.53$, $p=0.0003$). An effect of brain region was also detected ($F=70.88$, $p<0.0001$), and all two- and three-way interactions involving this variable were statistically significant (all $F>5.63$, $p<0.002$). Within each of the regions of primary interest (RA, Area X and the VVZ), two-way ANOVAs revealed significant effects of age and sex, as well as interactions between them (all $F>6.00$, $p<0.024$). In the control region, A, a significant effect of only age was detected ($F=8.44$, $p=0.009$), and the magnitude of the difference between juveniles and adults was far smaller than in the other areas (Fig. 2). Planned, pairwise comparisons between sexes at 25 days of age ($\alpha=0.0125$ for 4 comparisons) revealed enhanced expression in males compared to females in Area X, RA and the VVZ (all $t>5.65$, $p\leq0.0002$), but not A ($t=0.79$, $p=0.939$). In adults, the only significant sex difference was in RA ($t=3.17$, $p=0.010$), and the magnitude of the dimorphism was far smaller than in juveniles (Fig. 2). Similarly, in males, all regions except A ($t=1.85$, $p=0.095$) showed increased expression in juveniles compared to adults (all $t\geq6.05$, $p\leq0.0001$), whereas in females RPL17 expression was statistically equivalent between the ages, although slightly higher in 25-day-old birds, in each of the four regions