Neural correlates of vocal learning in songbirds and humans

Kant, A.M. van der

Citation

Kant, A. M. van der. (2015, January 28). *Neural correlates of vocal learning in songbirds and humans*. Retrieved from https://hdl.handle.net/1887/31633

Author: Kant, Anne Marie van der
Title: Neural correlates of vocal learning in songbirds and humans : cross-species fMRI studies into individual differences
Issue Date: 2015-01-28

CHAPTER 4

Neural correlates of zebra finch tutor song perception during development: changes in selectivity and lateralization

---

Birdsong learning and human speech acquisition share interesting similarities. Since both songbirds and humans need to be exposed to the vocalizations of their parents within a limited time window early in life, a sensitive period, in order to develop their own, songbirds present an excellent model for studying the sensitive period for human speech acquisition. During this sensitive period, perceptual sensitivity to adult vocalizations is important for learning in both species. Here we used functional MRI to investigate the development of conspecific song and tutor song selectivity in the auditory midbrain of juvenile zebra finches. Selective responses were previously identified in the auditory midbrain of adult birds (Poirier et al., 2009; Van der Kant et al., 2013). Our data show neural selectivity for conspecific song in both left and right auditory midbrain of female zebra finches early in development, while in male zebra finches tutor song selectivity is mainly present in the left auditory midbrain nucleus towards the end of the sensory phase of song learning. Moreover, the lateralization of tutor song selectivity changes towards adulthood. The strong tutor song selectivity near the end of the sensory period in males might reflect consolidation of a memory trace for the tutor's song. The difference in lateralization between juveniles and adults is reminiscent of the change in lateralization of human language in the course of development. While language in infants is largely bilateral, most human adults show a left lateralization of language.

4.1 Introduction

Both juvenile songbirds and human infants learn their vocalizations by imitation and need to do so within a limited time window, called the sensitive period for vocal learning. Songbirds are therefore the animal model of choice to study vocal learning. Memories of adult vocalizations play an important role in this process, because vocal practice does not always take place in the vicinity of a tutor. Zebra finches memorize the song of their tutor during a sensory phase, which is followed by a sensorimotor phase, during which the birds match their vocalizations to the tutor's song (Eales, 1985). When reared in isolation from adult vocalizations, a memory will not be formed and neither songbirds nor humans will successfully develop their own vocalizations (Doupe & Kuhl, 1999; P. H. Price, 1979).

Several studies have proposed the auditory lobule (London & Clayton, 2008) and the caudomedial nidopallium (NCM) in particular (Bolhuis et al., 2001, 2000; Gobes, Zandbergen, & Bolhuis, 2010; Phan et al., 2006) as candidate regions for the neural substrates for tutor song memory in adult zebra finches. In juvenile zebra finches NCM has been demonstrated to show tutor song induced responses around 56 days of age (Gobes et al., 2010), but not before the start of the sensorimotor phase (Amin et al., 2007). In our previous study (Van der Kant et al., 2013) we showed tutor song selectivity in the right auditory midbrain nucleus (MLd) of adult male zebra finches.

The present study aims to longitudinally map tutor song perception throughout song development within-subject in order to show the dynamics of tutor song memory during song learning. We used in vivo Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) in juvenile male and female zebra finches at different stages of song learning in order to address tutor song selectivity in MLd. We compared the BOLD responses elicited by the birds tutor song and an unfamiliar conspecific song in each bird at 30, 40, 60 and 100 days post hatching (DPH). Additionally, we addressed conspecific song selectivity by comparing BOLD responses to unfamiliar conspecific and heterospecific song. Additional male data were obtained at $\sim$500 DPH for comparison with our previous adult results. Furthermore, we explored lateralization differences in tutor song selectivity over development.

Based on our previous study (Van der Kant et al., 2013) we expect males to show a gradual development of this neural selectivity for the tutor song throughout the sensory phase, exceeding adult levels. The enhanced selectivity is expected to drop towards adult levels at the end of the sensitive period, when it is no longer used for sensorimotor learning. Females do not use tutor song memory for motor learning, but have a preference for the tutor song in adulthood (Riebel, 2000). Female tutor song selectivity is therefore expected to be present at a lower level compared to adult males.

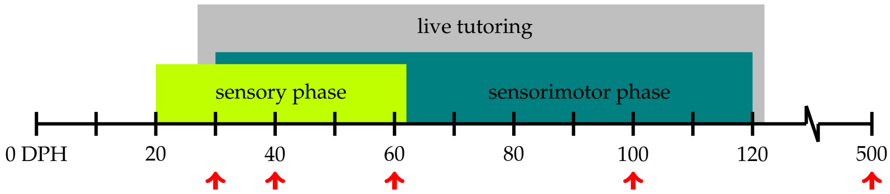

Figure 4.1: Tutoring- and measuring paradigm. fMRI data were acquired at 30, 40, 60 and 100 DPH for both sexes and additionally at 500 DPH for males (red arrows). Lime and teal bars show the sensory and sensorimotor phases, which partly overlap in zebra finches. The gray bar indicates the period during which the birds were individually housed with an adult tutor.

4.2 Materials and methods

4.2.1 Subjects

Nine juvenile zebra finches (Taeniopygia guttata) (5 males; 4 females) from the breeding colony of the Bio-Imaging Lab at the University of Antwerp (Belgium) were used for the present study.

The birds were raised by both parents in an individual cage from 0 to 7 DPH, by their mother from 8 DPH until they were fully fledged and able to feed independently (29 DPH) and were housed singly with an adult male tutor in a sound attenuated chamber from 30 to 120 DPH. This protocol is known to optimize song learning (Tchernichovski, Mitra, Lints, & Nottebohm, 2001). Following tutoring and after the last fMRI measurement, the male and female birds were housed in same-sex common aviaries.

All birds were maintained under a 12h light: 12h dark photoperiod throughout the experiment and had access to food, water and baths ad libitum. All experimental procedures were performed according to the Belgian laws on the protection and welfare of animals and were approved by the ethical committee of the University of Antwerp, Belgium (EC no. 2011/08).

4.2.2 fMRI procedure

The birds included in the present study received fMRI measurements at a number of time points during their song development (Figure 4.1). All nine birds were measured at the first four time points: 30, 40, 60 and 100 DPH. In order to match our present results with those from our last study which showed tutor song selectivity in the right MLd of adult males (Van der Kant et al., 2013), the male birds were also measured later in adulthood (around 500 DPH). Because one of the male birds was lost before the last measurement, data could only be obtained from four of the male birds at this time point.

Stimuli

For each experimental bird, three undirected songs were used as stimuli in the fMRI experiment: the tutor song (TUT), the song of an unfamiliar conspecific (CON) and a starling song (HET).

Stimuli were constructed using song material previously recorded from all tutors and colony birds. For each bird, the most representative motif (based on frequency of occurrence) was selected for use as a stimulus (Van der Kant et al., 2013). These motifs were concatenated into strings repeating the same motif, interleaved with silent periods. The duration of the silent periods was selected such that the total amount of song and silence was equal across all stimuli and mean intensity was normalized at 70dB SPL. An equalizer function was applied to the stimuli using WaveLab software (Steinberg, Germany) to correct for enhancement of frequencies between 2500 and 5000 Hz in the magnet bore (Poirier et al., 2010).

Experimental setup

During the experiment birds were continuously anesthetized with isoflurane (anesthesia induction, 3%; maintenance, 1,5%). The anesthetic gas was inhaled with a mixture of oxygen and nitrogen (at flow rates of 100 and 200 cm^3/min, respectively) through a mask over the birds beak. Body temperature was continuously monitored with an internal probe and maintained at 40°C by a feedback-controlled heating system (SA-Instruments, Stony Brook, NY, USA). The respiration rate was continuously monitored with a pneumatic sensor (SA-Instruments) positioned under the chest of the bird.

Auditory stimulation was delivered through dynamic loudspeakers (Visation, Germany) from which the magnets were removed, placed at both sides of the bird's head, close to the ears of the bird (Van Meir et al., 2005). Stimulus delivery was controlled by Presentation 0.76 software (Neurobehavioral Systems Inc., Albany, CA, USA).

Procedures

During fMRI acquisition each bird was presented with one TUT, one CON and one HET stimulus, pseudo-randomly repeated within a single experiment. Each bird received the same auditory stimuli at each time point during development. Stimuli were presented in an ON/OFF blocked design alternating 16s stimulation (ON blocks) and 16s rest periods (OFF blocks). During each block two images were acquired. Each stimulus type was presented 31 times, resulting in the acquisition of 62 images per stimulus and per subject. Three different stimulus orders were used, randomized between birds.

fMRI data acquisition

Blood Oxygen Level Dependent (BOLD) fMRI images were acquired using a 7T Bruker Pharmascan small animal MRI system (Bruker, Erlangen, Germany). The system and the RF coils used for the first four time points of the experiment have been described in a previous publication (Boumans et al., 2007). The last (500 DPH) time point was acquired with a RF transmit volume coil and a RF receive quadrature linear array mouse head surface coil (Bruker, Erlangen, Germany) using the same imaging parameters. For each bird, a time series of 382 T_2-weighted SE RARE images was acquired in the sagittal plane, covering the whole brain (15 slices, 750 μm thick, 50 μm gap, 250 μm^2 in-plane resolution, Effective Echo time (TEeff) / Repetition time (TR): 60/2000 ms, RARE factor: 8, Field of View: 16 x 16 mm), which resulted in a good signal in bilateral MLd. The first 10 images from each time series were considered dummy images to allow the bird to get accustomed to the scanner noise before the presentation of the stimuli was started. Following the fMRI acquisition, a high-resolution structural 3D SE RARE volume (voxel size 125 μm^3; TEeff/TR: 60/2000 ms; RARE factor: 8; Field of View: 16 x 16 mm) was acquired for each bird to allow for spatial registration with the zebra finch atlas (Poirier et al., 2008).

fMRI data analysis

Data were preprocessed and analyzed using Statistical Parametric Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). The first 10 (dummy) images from each time series were excluded from the analysis. Each fMRI time series was realigned using a least-squares procedure and a six-parameter rigid-body spatial transformation. Head movement did not exceed 500 μm for any bird. To enable localization of activations in specific brain regions, the high-resolution 3D image of each subject was spatially registered to the MRI atlas of the zebra finch brain (Poirier et al., 2008). The resulting spatial transformation matrix was then applied to the functional time series and re-sampled to obtain a resolution of 125 x 125 x 400 μm. These steps resulted in a good correspondence between anatomical areas in the atlas and the fMRI data. Finally, the fMRI images were smoothed with a Gaussian kernel of 500 μm FWHM in-plane and 800 μm FWHM in the slice direction.

For each bird the BOLD response was modeled as a box-car function convolved with a canonical hemodynamic response function using the General Linear Model and a classical restricted maximum likelihood algorithm. Subject-level fixed-effect analyses were then performed for the comparison of each stimulus with the rest condition. To model the possible effects of head motion (which is not convolved with the hemodynamic response), motion (realignment) parameters were included in the model as regressors for each subject.

The contrasts obtained in the subject-level analysis were entered into a group-level random effects analysis. Based on earlier results (Van der Kant et al., 2013), the group analysis was restricted to right and left MLd (the auditory midbrain nuclei), defined as Regions of Interest (ROI's). For the anatomical location of these ROI's, see Figure 3.3 on page 54. Developmental changes in the BOLD response were expected in MLd based on TUT selectivity previously measured in MLd (Van der Kant et al., 2013). Right and left MLd could be clearly identified and delineated on the zebra finch atlas (Poirier et al., 2008).

Voxel-based statistical differences between stimulus-related BOLD signal changes were assessed using a one-way repeated measures ANOVA followed by post-hoc uni-lateral paired t-tests in the two regions of interest. Differences between the sexes following from interactions were assessed with two-sample t-tests on the contrasts of interest. Only activations with a volume of at least 5 voxels were considered. P-values were adjusted for the number of independent tests performed using Family Wise Error correction for each anatomical Region of Interest (ROI), based on Random Field Theory (Worsley et al., 1996). When an ROI is reported to contain "no clusters", no clusters are found in the uncorrected data with a threshold of $p = 0.05$. Lateralization indices were used to assess laterality of the contrasts of interest. These indexes were computed between left and right MLd in a threshold-free manner using the "AveLI" SPM add-on (Matsuo, Chen, & Tseng, 2012), freely available at http://mrilab.mc.ntu.edu.tw/aveli/). This add-on computes the laterality index based on the sums of positive T-values within a selected ROI (in this case MLd) weighted for the number of voxels with positive T-values within that ROI. The LI represents the portion of the signal that is found either left or right with -1 representing 100% right lateralization and 1 representing 100% left lateralization.

4.3 Results

4.3.1 Tutor song selectivity

Tutor song selectivity independent of sex

Group analyses with males and females pooled showed that bilateral MLd was selectively activated by the tutor song compared to a novel conspecific song only at the age of 60 DPH (60 DPH [TUT-CON] in left MLd: mean sum T (the mean sum of positive t-values within the ROI) $= 147.8$, $sd = 103.3$, $p = 0.005$; [TUT-CON] in right MLd: mean sum T $= 140.5$, $sd = 169.5$, $p = 0.004$). However, no interaction of the TUT-CON contrast with age and sex was found in MLd. NCM did not show tutor song selectivity at any of the time points with males and females pooled and in analyses per sex and was thus excluded from further analysis. Because tutor song selectivity was expected to be more pronounced in male birds, a t-test was used to assess sex differences in the TUT-CON contrast at this age. This analysis revealed that the tutor song selective response in left MLd was significantly stronger in male compared to female birds at 60 DPH (Males-Females 60 DPH: sum T at group level = 185.0, p = 0.019). Based on this result, we further analyzed tutor song selectivity separately in males and females, in order to accurately describe the changes in tutor song selectivity throughout development for males and females.

Tutor song selectivity in male MLd

In male zebra finches, a trend towards a [TUT-CON $*$ time point] interaction was found in left MLd (sum F at group level = 321.9, p = 0.081). Further analyses per time point showed that no tutor song selective response could be found in male birds at the early stages of song learning. At 30 and 40 DPH no tutor song selective clusters were present in the left MLd or in the right MLd (Figure 4.2). As also shown by figure 4.2, the perception of the individual stimuli does not show the same pattern at the ages of 30 and 40 DPH. While at 30 DPH the activation in left as well as right MLd is absent or only minor for all three stimuli (30 DPH: no clusters for [TUT-Rest] and [CON-Rest] in left and right MLd; [HET-Rest] in Left MLd: mean sum T = 58.9, sd = 36.1, p = 0.22; [HET-Rest] in Right MLd: mean sum T = 65.6, sd = 67.3, p = 0.09), left MLd does get significantly activated by TUT and CON as opposed to HET at 40 DPH ([TUT-Rest] at 40 DPH: mean sum T = 177.0, sd = 85.2, p = 0.012; [CON-Rest] at 40 DPH: mean sum T = 160.0, sd = 65.2, p = 0.008; [HET-Rest] at 40 DPH: no clusters). There is, however, no tutor song selective activation.

At 60 DPH, when the sensory phase reaches its end, male zebra finches show a strong tutor song selective response in the left auditory midbrain (MLd) ([TUT-CON] in left MLd: mean sum T = 207.3, sd = 101.4, $p < 0.001$). A post-hoc analysis of the individual stimuli showed that at this time point the activation in response to the tutor song remained high ([TUT-Rest] in left MLd: mean sum T = 145.7, sd = 123.6, p = 0.008), while in left MLd no activation was found for conspecific or heterospecific song. A TUT-CON cluster ([TUT-CON]: mean sum T = 207.5, sd = 207.6, p = 0.002) was also found in male right MLd at 60 DPH. However, due to the absence of a significant difference between the tutor song and heterospecific song related activity within

		TUT-CON		CON-HET		TUT-HET	
	DPH	T(sd)	P	T(sd)	P	T(sd)	P
Left MLd	60	207(101)*	0.001				
	100	223(282)	0.11				
Right MLd	500	43(31)	0.081	87(23)*	0.011	119(54)*	0.001

Table 4.1: Sums of T-values and P-values for male birds. Values as reported in section 4.3. For contrasts for which male birds did not show a significant BOLD response ($p < 0.05$), values are not reported. All values are FWE-corrected at the ROI level and only clusters which contain at least 5 voxels are reported

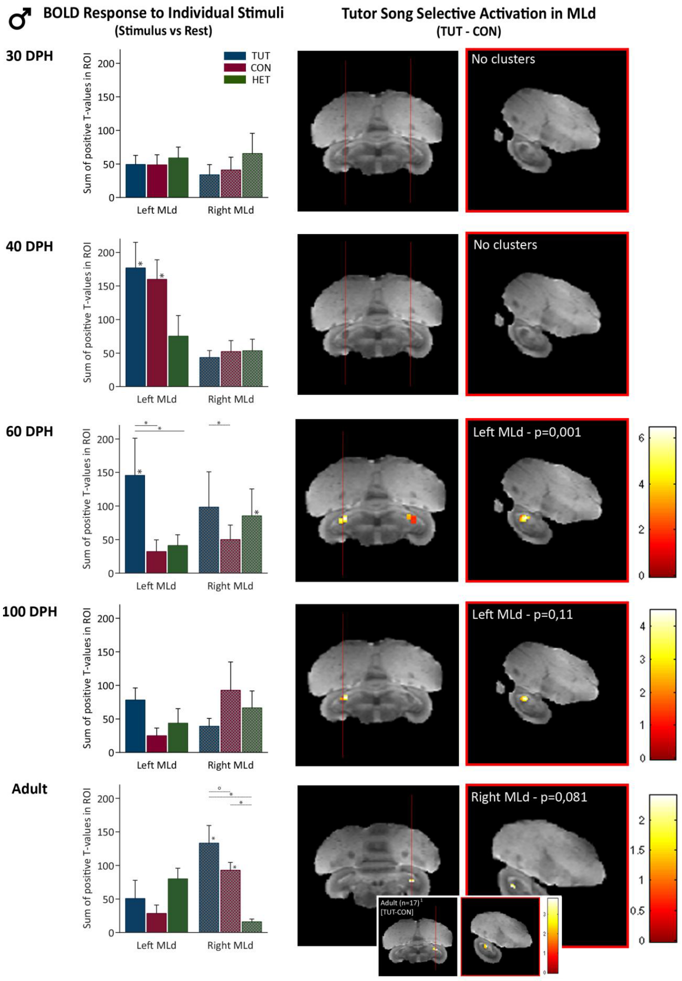

Figure 4.2: LEFT: Mean BOLD response of Males to the individual stimuli (TUT, CON, HET). The sum of positive T-values within each ROI (Left MLd / Right MLd) is used as a measure of BOLD activity. The zero level corresponds to the mean activation level during rest periods (exposure to scanner noise). Stars on top of the bars indicate statistically significant difference between stimuli vs. rest. Horizontal lines above the graphs indicate statistical significant differences between responses to the individual stimuli within the ROI ($^{\circ}p < 0.10$; $*p < 0.05$, corrected for multiple comparisons). **RIGHT: Male tutor song selectivity in MLd.** Only voxels showing a significant difference (threshold: $p < 0.05$) in BOLD response to TUT compared to CON in left and right MLd are displayed. The specified p-values in the sagittal images are FWE-corrected for the anatomical ROI (Left MLd or Right MLd) and only clusters containing at least 5 voxels are included. T-values are color-coded according to the scale displayed on the right side of each panel.

the cluster ([TUT-HET]: no clusters), this activation could not be confirmed as selective for the tutor song. Although right MLd was significantly more activated by tutor song compared to conspecific song, a large portion of the activation in right MLd at this age was induced by the heterospecific song ([TUT-Rest]: mean sum T = 98.4, sd = 117.7, p = 0.188; [CON-Rest]: no clusters; [HET-Rest]: mean sum T = 85.3, sd = 89.7, p = 0.018).

At 100 DPH, around the time of song crystallization, the tutor song selective response no longer reached significance in the males left MLd ([TUT-CON]: mean sum T = 223.0, sd = 281.7, p = 0.11; see figure 4.2). Furthermore, no tutor song selectivity was found in right MLd at this age (no [TUT-CON] clusters). Analysis of the individual stimuli in left MLd showed a trend for tutor song related activation, but left MLd was not activated by the other stimuli (Left MLd: [TUT-Rest]: mean sum T = 78.2, sd = 40.0, p = 0.09; [CON-Rest]: no clusters; [HET-Rest]: no clusters).

Earlier results showed adult male tutor song selectivity in right MLd (Van der Kant et al., 2013). In order to test whether the tutor song selective response in left MLd could be attributed to the age of the birds, male tutor song selectivity was additionally assessed at 500 DPH. At this age, the four remaining males showed a tutor song selective cluster in right MLd, which did not survive correction for multiple comparisons, but showed a trend (500 DPH [TUT-CON] : mean sum T = 42.8, sd = 30.9, p = 0.081; [TUT-Rest]: mean sum T = 133.2, sd = 52.5, p = 0.004; [CON-Rest]: mean sum T = 92.8, sd = 23.5, p = 0.01; [HET-Rest]: mean sum T = 16.1, sd = 7.7, p = 0.004).

Lateralization of male tutor song selectivity

Due to the interference of heterospecific song related activity in right MLd at 60 DPH, when tutor song selectivity is found to be prominent in males, a direct assessment of [TUT-CON] lateralization during song learning would not be informative. Instead, assessment of the activation induced by the individual stimuli does reveal a pattern throughout development.

In left MLd we see an initial increase of activation in response to both TUT and CON at 40 DPH. At the age of 60 DPH the CON related activation shows a sharp drop, leaving left MLd purely tutor song selective at this age. Later in development, the activation in response to TUT also drops, leaving left MLd selective for neither TUT nor CON. Activation in response to HET shows relatively minor changes throughout development in left MLd. In contrast, no large peaks in TUT or CON related activation are found in right MLd before adulthood.

At 500 DPH, TUT related activation shows a peak in right MLd, which is not found in left MLd. However, lateralization of the tutor song selective signal was highly variable at this age (mean LI [TUT-CON] = -0.13, sd = 0.8, p = 0.36). Individual analysis of the subjects showed that tutor song selectivity was highest in right MLd in three out of four subjects (LI's [TUT-CON]: -0.02, -0.99 and -0.45), while in the fourth subject, tutor song selectivity was highly left lateralized (LI [TUT-CON] = 0.92).

No significant tutor song selectivity in female MLd

In females, no interaction between tutor song selectivity and time point was found in either right or left MLd. Like the males, the females did not show TUT selectivity at early age in left Mld ([TUT-CON] at 30 DPH: mean sum T = 47.3, sd = 42.6, p = 0.28; [TUT-CON] at 40 DPH: no clusters) nor in right Mld ([TUT-CON] at 30 DPH: no clusters; [TUT-CON] at 40 DPH: no clusters). In contrast to the males, however, tutor song did not evoke significant differential activation in left or right MLd of female zebra finches at 60 DPH, when compared to conspecific song ([TUT-CON] at 60 DPH in left MLd: mean sum T = 73.5, sd = 38.8, p = 0.19; in right MLd: no clusters). Analysis of the individual stimuli at this age confirms that none of the stimuli significantly activates left or right MLd in females ([TUT-Rest]; [CON-Rest]; [HET-Rest]: no clusters in left or right MLd). At 100 DPH no tutor song selectivity was found in MLd of female zebra finches ([TUT-CON]: no clusters in right or left MLd).

4.3.2 Conspecific song selectivity

Neural selectivity for conspecific song was assessed by comparing the BOLD activity evoked by a novel conspecific to that evoked by a novel heterospecific (European starling) song. The familiarity of both songs might increase with each measurement due to hearing the song under anesthesia, but the amount of cumulative exposure is the same for both songs throughout the experiment.

Selectivity for conspecific song as shown by the [CON-HET] contrast was found to be present in left MLd with sexes and time points pooled. Conspecific song selectivity showed a significant interaction between sex and time point in left MLd (sum F on group level = 355.8, p = 0.018). Therefore, conspecific song selectivity was further investigated in males and females separately.

Conspecific song selectivity in males

Although analysis of the individual stimuli shows a large peak of activation for TUT and CON in left MLd at 40 DPH in males (Left MLd at 40 DPH: [TUT-Rest]: mean sum T = 177.0, sd = 85.2, p = 0.012; [CON-Rest]: mean sum T = 160.0, sd = 65.2, p = 0.008; [HET-Rest]:no clusters), there is no significant conspecific song selectivity at this age (Left MLd: [CON-HET]: mean sum T = 99.3, sd = 51.2, p = 0.13; [TUT-HET]: mean sum T = 115.9, sd = 58.3, p = 0.15).

In adulthood, at 500 DPH, the right auditory midbrain is selectively activated by conspecific over heterospecific song (Right MLd [CON-HET]: mean sum T = 86.8, sd = 23.0, p = 0.011). This is confirmed by the tutor song ([Right MLd [TUT-HET]: mean sum T = 119.3, sd = 54.2, $p < 0.001$). There were no significant differences between conspecific and heterospecific song activations in males at 30, 60, 100 and 500 DPH in left MLd and at any juvenile time points in right MLd.

Conspecific song selectivity in females

		TUT-CON		CON-HET		TUT-HET	
	DPH	T(sd)	P	T(sd)	P	T(sd)	P
Left MLd	30			178(91)*	0.001	178(67)*	0.001
	60	74(39)	0.19				

Table 4.2: Sums of T-values and P-values for female birds. Values as reported in section 4.3. For contrasts for which female birds did not show a significant BOLD response ($p < 0.05$), values are not reported. All values are FWE-corrected at the ROI level and only clusters which contain at least 5 voxels are reported

Analyses per time point demonstrated that MLd is only selective for conspecific song in females at 30 DPH, when they have spent only limited time with their tutor ([CON-HET] in left MLd at 30 DPH: mean sum T = 178.3, sd = 90.8, p < 0.001) (Figure 4.3). This selectivity was shown only in the left MLd. In right MLd none of the stimuli induce a significant activation at this age. After 30 days of age, no significant selectivity for conspecific song was found in left MLd ([CON-HET] at 40 DPH: mean sum T = 66.9, sd = 71.5, p = 0.248; [CON-HET] at 60 DPH: no clusters; [CON-HET] at 100 DPH: mean sum T = 71.1, sd = 27.2, p = 0.238).

In order to further investigate whether the selectivity at 30 DPH originated from hearing a novel conspecific rather than selectivity for conspecific song in general, we also assessed the differences in BOLD response elicited by the tutor song, which is familiar to the bird, compared to a heterospecific song. This analysis also showed significant differential activation in the left MLd (Left MLd [TUT-HET] at 30 DPH: mean sum T = 178.2, sd = 66.7, p < 0.001) (Figure 4.3). At 30 DPH left MLd is indeed significantly activated by tutor song as well as conspecific song (Left MLd [TUT-Rest]: mean sum T = 108.5, sd = 60.0, p = 0.01; [CON-Rest]: mean sum T = 107.6, sd = 93.9, p = 0.049), while showing no activation in response to heterospecific song (Left MLd [HET-Rest]: no clusters).

4.4 Discussion

The present study for the first time uses a longitudinal fMRI approach to assess song selectivity in juvenile zebra finches. This approach enabled us to follow the same group of birds throughout song learning and address changes in neural selectivity during development. In the present study we showed that the left MLd of juvenile male zebra finches shows strong selectivity for their

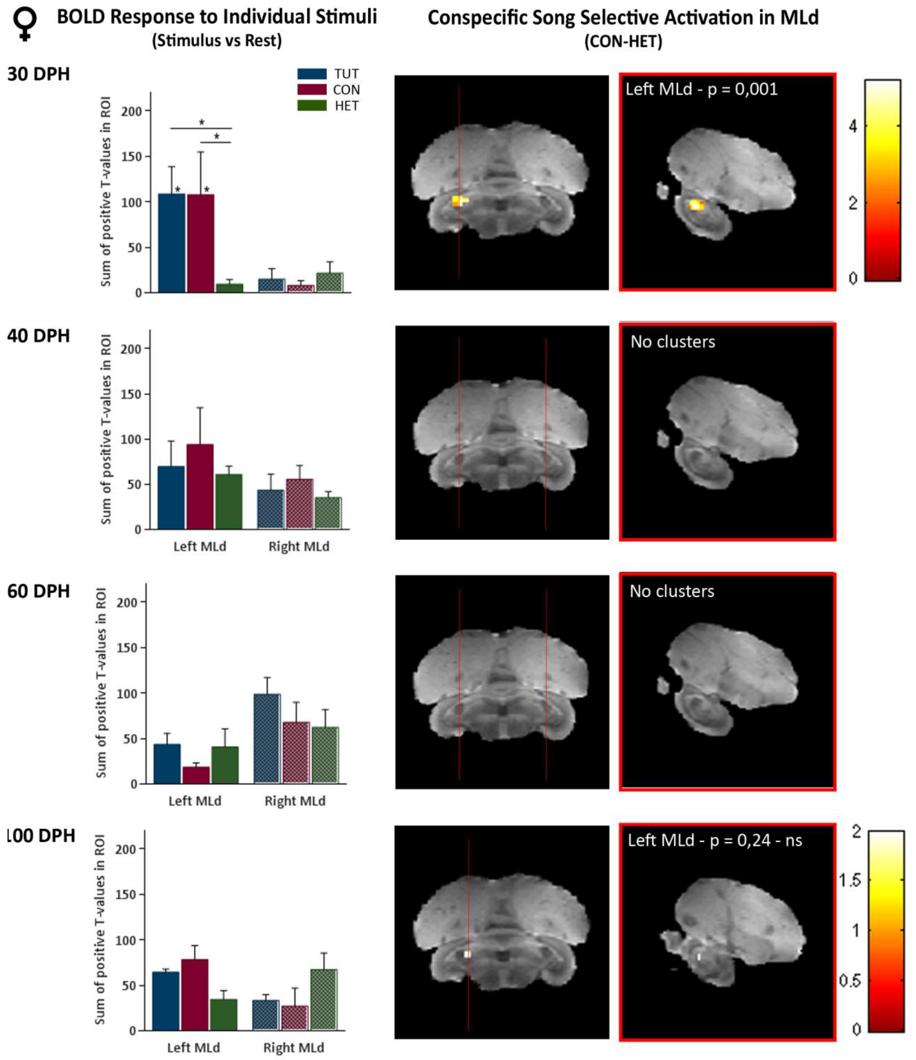

Figure 4.3: LEFT: Mean BOLD response of Females to the individual stimuli (TUT, CON, HET). The sum of positive T-values within each ROI (Left MLd / Right MLd) is used as a measure of BOLD activity. The zero level corresponds to the mean activation level during rest periods (exposure to scanner noise). Stars on top of the bars indicate statistically significant difference between stimuli vs. rest. Horizontal lines above the graphs indicate statistical significant differences between responses to the individual stimuli within the ROI ($p < 0.05$, corrected for multiple comparisons). **RIGHT: Female conspecific song selectivity in MLd.** Only voxels showing a significant difference (threshold: $p < 0.05$) in BOLD response to TUT compared to CON in left and right MLd are displayed. The specified p-values in the sagittal images are FWE-corrected for the anatomical ROI (Left MLd or Right MLd) and only clusters containing at least 5 voxels are included. T-values are color coded according to the scale displayed on the right side of each panel.

tutor song at 60 DPH. Moreover, the left MLd of female zebra finches shows conspecific song selectivity over heterospecific song as early as 30 DPH. At this time, when they have only been exposed to their tutor for a limited time span, the female birds do not show selectivity for the tutor song over other conspecific song.

Tutor song selectivity was found in the male left MLd at 60 DPH. At this age, the sensory period of song learning approaches its end in zebra finches (Slater, Eales, & Clayton, 1988). Analysis of the activation in terms of the individual stimuli showed that this strong selectivity is caused by a combination of factors. Tutor song related activation in left MLd stays high compared to the 40 DPH time point, while conspecific song related activity diminishes. This might relate to the possibility of juveniles including elements from a second tutor in their song, which does still exist at 40 DPH, but is decreased when the birds reaches the age of 60-65 days, indicating that the sensory phase is ending (Eales, 1985). Therefore, when hearing a novel conspecific bird, the auditory system will not be highly activated anymore at this age. However, tutor song is still very important at the age of 60 DPH, because the bird is now in a stage of singing plastic song and needs to compare this song to the tutor song continuously. Therefore the memory of the tutor song needs to be a strong one. Moreover, sensory learning has reached its peak at this age and the memory of the tutor song formed during the sensory phase will be at its strongest. The finding that tutor song selectivity in left MLd cannot be found at 100 DPH indicates that this nucleus is mainly selective for tutor song during learning and is partly lost after song crystallization. Indeed, in an earlier fMRI study in adult male zebra finches (Van der Kant et al., 2013) no tutor song selectivity was found in left MLd.

In the abovementioned adult study, male zebra finches showed tutor song selectivity in the right auditory midbrain nucleus of adult male zebra finches. Although the last time point (500 DPH) of our longitudinal study contains only four males, it does show a trend towards tutor song selectivity in right MLd, which is consistent with our previous study (Van der Kant et al., 2013), showing significant tutor song selectivity only in right MLd, but no significant lateralization. Individual analyses of the four male birds also measured in later adulthood confirmed that three of these birds showed a left-to-right shift of tutor song selectivity between the ages of 60 DPH and 500 DPH. Although these data are too sparse to draw any conclusions towards a left-to-right shift during song development in male zebra finches, they seem to be in accordance our previous data, which showed tutor song selectivity in the right MLd of adult male zebra finches. Moreover, a similar change in lateralization of song perception over development was recently found in the zebra finch NCM using ZENK expression (Moorman et al., 2012). Together, these data might suggest that there is a broad change in lateralization in the brain regions which play a role in song memorization. This is the case in humans, where adult left lateralization for language is not yet present in infants, but emerges during development (for a review, see Minagawa-Kawai, Cristià, & Dupoux, 2011).

If there is indeed a left-right lateralization shift, it might suggest that left MLd tutor song selectivity found during development and right MLd tutor song selectivity in adulthood represent two different processes, possibly related to song learning and song maintenance respectively. In order to investigate this issue and uncover the mechanisms behind the developmental changes, a longitudinal study with a larger group of males is ongoing in the Bio-Imaging Lab.

Although the effect was only significant in males, at 60 DPH the left MLd of females was also activated by the tutor song to a larger extent than the neighboring time points (40 and 100 DPH). This might indicate that the preference for tutor song found earlier in females (Clayton, 1988; Riebel, 2000) also has a neural correlate during development and that the process of sensory learning, although perhaps stronger in males, might be similar in males and females. The timing of the peak of tutor song related activity, which is similar in males and females, suggests that, although females do not learn to sing, they do show signs of a sensory period at the neural level. Female zebra finches showed highly significant selectivity for conspecific songs in the left auditory midbrain nucleus at the age of 30 DPH. At this time, left MLd is significantly activated by the tutor song and an unfamiliar conspecific song, while showing no activation in response to heterospecific song. This early neural sensitivity to conspecific song in female birds might contribute to the selection of quality mates in adulthood (Lauay, Gerlach, Adkins-Regan, & DeVoogd, 2004).

The CON selective activation in left MLd of juvenile female zebra finches is also in line with selectivity for conspecific song in left MLd found earlier in an auditory fMRI study in adult males (Poirier et al., 2009). However, in the present study this early selectivity for conspecific songs was not found in male birds. This is surprising, because male birds would need to distinguish a possible model for song learning from other sounds. Moreover, it has been shown that zebra finches can behaviorally distinguish conspecific from heterospecific songs very early in life and this has even been proposed to be an innate ability (Braaten & Reynolds, 1999). We cannot exclude the possibility that a neural selectivity for heterospecific song is present in the midbrain at an early age the developmental fMRI study, which is ongoing and includes a larger group of birds might be able to address this issue.

A selective response for conspecific songs could be detected in the male right MLd in adulthood, at 500 DPH. However, at this age the birds were no longer kept in isolation and had been exposed to other conspecifics. The familiarity of the conspecific song played during the experiment had not changed at this time, because the conspecifics to which the birds were exposed during scanning were either no longer present in the laboratory or housed in a different aviary. The selectivity at 500 DPH could therefore not be due to recognition of the song from the bird’s daily environment. A more likely explanation is that social interaction in the aviary renders the male birds more sensitive to territorial behavior and thus to relatively novel conspecific song.

Together with our earlier data (Van der Kant et al., 2013), the present data show that the neural substrates of tutor song memory in male zebra finches during song learning and in adulthood show a different lateralization. These findings are in accordance with both songbird and human data on laterality changes during vocal development, suggesting that lateralization changes during birdsong learning are not restricted to MLd, but might occur throughout the auditory system.