Statistical shape and position analysis on 3D structural MRI data of a motor region involved in vocal behavior of songbirds

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Introduction

The refinement of motor skills depends on factors such as learning capacity, practice and physiological status. Refinement of a behavior is often paralleled by reshaping an involved brain region or circuit such as the precentral gyrus in musicians. This can occur at the ultrastructural and molecular level, but also at the gross morphological level. In the latter case it is important to determine whether a brain region reshapes in competition with adjacent areas or whether a predetermined (larger) space assigned for a specific task is more or less efficiently occupied.

Assessment of variations in anatomical structures within a population and within the same individual (e.g before and after extensive training) benefits from the 3D-nature and digital format of Magnetic Resonance Images (MRI) and permits analysis of gross morphological developments in the brain circuits. In humans MRI is often used to make comparisons between groups of people with a different behavioral background, e.g. musicians versus non-musicians. This type of analysis, however, never rules out completely a genetic cause of the observed difference. The use of animal models provides a means to perform longitudinal studies on experience dependent mechanisms of brain plasticity on a shorter time scale. The songbird is a model which shows an extreme plasticity in both brain and behavior. Here we analyze plasticity in shape and position of a small vocal motor region in the brain of female starlings during a natural transition from a period of high song output to low song output. We determined whether known changes in volume were accompanied by changes in shape and/or position. Moreover, we evaluated whether these changes could be localized to a specific point of the nucleus.

If the changes are localized to one point they are likely to occur in competition with an adjacent functional area coding for a specific behavior. Non-localized changes could indicate the existence of a predetermined functional space which is completely or only partially occupied, depending on the behavioral requirements.

Methods

Data acquisition: The functional area of the robust nucleus of the arcopallium (RA) within the song control circuit was determined in 10 female starlings by Manganese Enhanced-MRI after a 70 nl, 100 mM manganese injection in HVC (abbreviation used as proper name) in the right hemisphere. Manganese injection and subsequent visualization was performed on a 7T magnet (SMIS, Surrey Medical Imaging Systems, MRRS, Guildford, UK) using a T1-weighted spin echo 3D sequence (TR/TE = 300/18; FOV 25 mm; reconstruction matrix = 256x256x256) in March (period of high song output) and July (period of low song output). Right RA and the ipsilateral telencephalon were segmented (Amira 3.1; Mercury Computers Systems, San Diego, CA, USA) (for full procedure see ref 1).

Anatomical analysis of 3D RA measures: For each individual the telencephalon obtained in both measurements was registered to evaluate the matching of the RA position within each season at the population level (Fig. 1A-1B) and between seasons at the individual level (Fig 1C).

A correspondence was established between the surface of each nucleus and the surface of the sphere using spherical parameterization, maintaining their orientation, but disregarding the position within the telencephalon. The Frobenius norm was used to visualize the variation between individuals of the position of a point at the nucleus' surface for each season (Fig. 1D-E). The average difference in shape deviation from the sphere between March and July was measured by the average Euclidian distance from each point on the nuclei surface to the corresponding points on the sphere surface (Fig 1F).

Anisotropy measures of the RA-shape were determined independent from the orientation or position. Finally we evaluated shifts in the center of gravity of RA at the individual level.

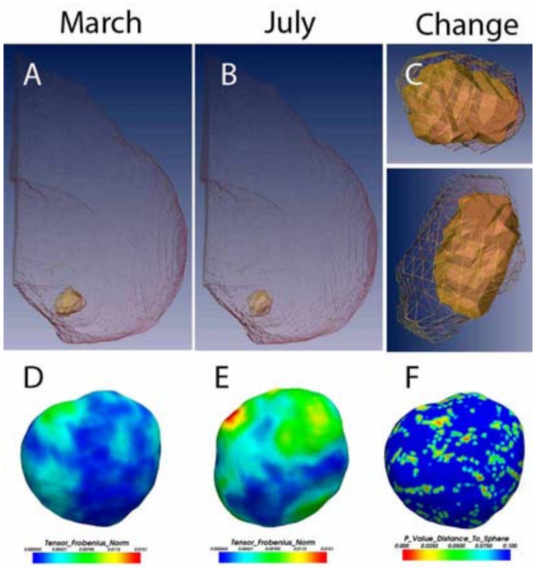

Fig 1. A-B. Position of RA regions (yellow) from all individuals superimposed in the standardized right telencephalon (transparent); C. two individual examples of superimposed RA measures from March (transparent) and July (solid) during a small (top) and a large (bottom) change in volume; D-E Frobenius representation of local variation in RA-shape; F. Statistical representation of local shape changes using Euclidian distance measures.
The warmer the colours, the higher the variance (D-E) or the higher the statistical significance (F)

Results

The mean volume of RA was smaller in July than in March (see ref. 1). Within each individual the area occupied in July either fitted within the March-volume or showed a great extend of overlap (Fig 1C). When the orientation of RA is taken into account, the variance in deviation of the RA-shape from the sphere within each season did not indicate any particular difference between the seasons (Fig 1D-E), which was confirmed by the absence of a localized significant difference in Euclidian distance from the sphere surface between March and July (Fig. 1F).

However, when the orientation of RA was disregarded by comparing the anisotropy measures of the individual RA shapes between seasons we found that RA became more cigar-shaped (linear component; $p=0.0273$) in summer, while planar component ($p=0.131$), spherical component ($p=1.000$) and fractional anisotropy ($p=0.375$) did not change.

Between the two subsequent measures, the displacement of the center of gravity was significantly correlated with the change in volume ($r=0.673$; $p=0.039$). Fig. 1A&B indicates that the overall region in the standardized brain occupied by the RAs of all individuals is comparable between seasons, indicating that the overlap in position of RA between all individuals is larger in March than in July.

Discussion

The results indicate that the sphere-like shape of RA at the time when song output is high (March) regresses to a more cylindrical shape when song output is low (July). Together with the observed shift in position, which is correlated to the volume decrease, these data suggest that the regression occurs to the edge of the original region occupied in March. The absence of a localized deformation of RA on the level of the population when the orientation of the nucleus within the telencephalon is taken into accounts suggests that the regression towards the edge is in a random direction.

The large area of overlap between the individuals in March might suggest the existence of a predetermined (larger) space which is largely occupied when song output is high, maximizing the capacity to produce song. When quality and output of the song behavior deteriorates, occupancy of the RA-area, as determined by its axonal input from the song control region HVC, shifts to a random position, depending on the individual, as indicated by the smaller degree of overlap in July.

The behavior of RA can be explained by two physiological mechanisms. First, the axonal input from HVC to RA is non-topographical and the volume decrease is due to a decrease in cell density and intracellular space while the neuron number is stable (3). Second, recently DTI measures of male starling brains showed that the region around RA is densely packed with fiber structures (4), which are less dense in July as compared to March. Future studies will focus on comparison of all song control regions which occupy different anatomical positions and have different physiological strategies to acquire plasticity (3).

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4. De Groof et al. 2006, Neuroimage. 29:754-963.