In-vivo online monitoring of testosterone-induced neuroplasticity in a seasonal songbird

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TARGET AUDIENCE: Persons interested in longitudinal DTI, neuroplasticity and hormones.

INTRODUCTION: Longitudinal MRI allows us to study the interaction between brain neuroplasticity and behaviour. Using testosterone (T)-implants we can induce in seasonal songbirds a drastic behavioural change in song output. T has also been shown to increase the volume of steroid-sensitive brain nuclei in adulthood in several vertebrate species. The song-control system (SCS) is a network of discrete nuclei in the songbird brain that controls the production and learning of birdsong and exhibits some of the best-studied neuroplasticity found in the adult brain. Photoperiodic growth during the breeding season of the SCS nuclei including HVC (used as a proper name), the robust nucleus of the arcopallium (RA), and Area X is driven, at least in part, by the gonadal steroid T[1]. Previous studies assessed the effect of T after a period of 8–14 days but the exact time course of these effects is unknown [2]. We asked here whether testosterone-dependent SCS plasticity could be observed at shorter latencies. With this study we also wanted to resolve the issue of causality between T, song behaviour and neuroplasticity. Is the increase in singing behaviour induced by T itself or does T induce the neuroanatomical changes in the SCS, which in turn induces the increase in singing behaviour. We used an almost 'real-time' monitoring of T-effects on the brain and on song behaviour by measuring song output and brain plasticity (using DTI) in the same animal during a short period of time.

MATERIALS & METHODS: Nine female starlings (*Sturnus vulgaris*) were kept in a short day photoperiod (8L/16D) in order to remain photosensitive. All starlings underwent a baseline scan without testosterone (T) implant (Day 0 or D0). Then they were implanted with silastic tube implants filled with crystalized T and repeatedly measured on D1, D2, D4, D7, D14 and D21 after implantation. Afterwards the T-implant was removed and starlings were measured again one (D-1) and 2 days (D-2) after implant removal. The starlings were anaesthetized with isoflurane (induction: 2%; maintenance: 1.5%). *In vivo* Diffusion Tensor Imaging (DTI) on starling was performed on a 7T MR system (Bruker Pharmascan). 27 sagittal slices (thickness 0.23mm) were obtained covering the whole starling brain. Diffusion weighted SE-EPI images were obtained with diffusion applied in 60 directions. The image parameters were: FOV 23 mm, TE 23 ms, TR 7000 ms, acquisition matrix (128x128), δ 4ms, Δ 12ms, b-value 670 s/mm^2, 2 repetitions, in-plane resolution of 0.18 mm² and a total scan time of 72 min. During experiments respiration rate and temperature were continuously monitored and kept constant. Data analysis was done with SPM8. Diffusion-weighted images were first realigned using the procedure of the 'Diffusion II Toolbox' for SPM8. The B_0-images of the different time points of the same starling were then co-registered to each other. The diffusion tensor was estimated from the DW-data, and mean diffusivity (MD), eigenvalues (λ_1, λ_2, λ_3) and fractional anisotropy (FA) maps were derived. Mean DTI-values (± SD) and volumes were calculated for relevant regions of interest (ROI) delineated on FA-maps. Linear mixed models (1st order autoregressive model) statistics were performed on the DTI-values and volumes of the same ROI obtained at different time points. Song was also recorded 12 hours before and 12 hours after each DTI measurement using sound boxes and a PC equipped with Sound analysis Pro 2.0 software. Blood samples were also taken at specific time points to assay the blood plasma level of T.

RESULTS: Testosterone concentrations were significantly higher when implanted with T after two days already [P = 0.007; mean ± SEM: baseline D0, 0.018 ± 0.014 ng/ml; D2, 4.22 ± 2.02 ng/ml] and remained high until the T-implant was removed (P = 0.02; mean ± SEM: D7, 4.25 ± 2.38 ng/ml; D-2, 0.16 ± 0.12 ng/ml). Analysis of the data of the FA maps showed that there was significant difference between time points for the HVC to RA motor pathway (F = 2.372; p = 0.049). Paired post-hoc T-tests showed that FA was significantly higher in HVC-RA at D21 compared to D0, D1 and D2 (p = 0.005; p = 0.003; p = 0.033 respectively) and at D-2 compared to D0, D1 and D2 (p = 0.012; p = 0.006 en p = 0.048). Analysis of the RA volume data showed a significant difference between time points (F = 4.028; p = 0.004). Paired post-hoc T-tests that the volume of RA was significantly larger at D14 compared to D0, D1, D2, D7 and D-1 (respectively p = 0.004; p = 0;007; p = 0.041; p = 0.004; p = 0.002). RA volume was positively correlated with DTI parameters MD (p = 0.002), λ_1 (p = 0.008), λ_2 (p < 0.001), λ_3 (p = 0.010) but not with FA in RA (p = 0.960). Song analysis showed that there was a clear increase in 'warbling' song (typical song for starlings) from D4 onwards (D4 vs D0: p = 0.004; D4 vs D1: p = 0.001; D4 vs D2: p = 0.005).

DISCUSSION & CONCLUSION: In this study we wanted to examine how fast neuroplasticity of the songbird brain is triggered by steroid hormones, and more importantly examine the causality of this neuroplasticity. We wanted to see if the steroids would induce singing behaviour which in turn induces the observed neuroplasticity (activity-induced neuroplasticity) or if it is the neuroanatomical changes in the brain (induced by the steroids) that lead to a change in singing behaviour (hormone-induced neuroplasticity). Our results indicate that it is the former kind of plasticity. It would seem that the steroids somehow are capable of inducing singing (plasma T is higher at D2 and singing behaviour is higher from D4), while neuroanatomical changes (in volume or DTI parameters of SCS) are only apparent from day D14 or D21. However another explanation could be that T influences other brain regions (related to the motivation of singing) like e.g. POM which has been found to change in volume after two days of T-treatment in quails[3]. Another interesting finding is the neuroanatomical changes of SCS are relatively slow to accomplish (it takes about two to three weeks before changes are visible) when T is introduced to the system. However if the T-implant is removed from the bird we see a rapid decline in (microstructural) neuroanatomical changes (in a matter of days).

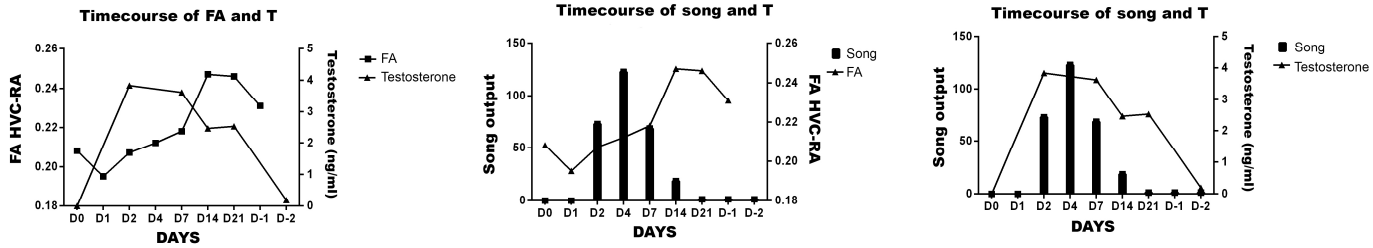

Figure: Example of timecourses of connectivity of HVC-RA (measured using FA), blood plasma testsosterone levels (in ng/ml) and song output (number of songs sung) of one female starling. One can see that the testosterone levels coincide with song output (both higher at day 2), while the connectivity of HVC-RA takes longer before it is visible (14 days).

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