Noradrenergic modulation of auditory processing in the songbird brain.

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Introduction: Song learning in oscines (birds in the order Passeriformes) shares a large number of features with human speech acquisition. Both songbirds and humans learn their complex vocalizations early in life by forming memories of the adult song they hear. They subsequently match these memories through a sensori-motor process characterized by separate stages of behavioural development that will progressively improve the quality of their imitation as they practice. Both songbirds and humans have evolved a complex network of specialized forebrain areas in which motor and auditory centres closely interact. For these reasons, the songbird brain has developed as an excellent model to study the neural bases of vocal learning and sound processing. Several studies suggest that noradrenaline (NA) plays a role in song perception and song-specific activation. However, little is known about this potential role. The aim of this study was to investigate the role of NA in conspecific songs and bird's own song perception.

Material and Methods: Sixteen adult males zebra finches, anesthetized with isoflurane, were used for this BOLD fMRI experiment. Birds received an i.p. injection of DSP-4 (N-(2-chloroethyl)-N-2-bromobenzyl-amine hydrochloride), a noradrenergic- specific neurotoxin (n = 8) or saline solution (0.9 %) (n = 8). One week later, neural activity in response to auditory stimuli was measured on a 7T Pharmascan scanner (Bruker) with a spin-echo T2W sequence (TE = 55 ms; TR = 2000 ms). Stimuli consisted of familiar heterospecific songs (HET, songs from canaries and starlings), familiar conspecific songs (CON, songs from zebra finches housed in the same cage) and the bird's own song (BOS). Stimuli were presented 25 times each to the anesthetized subject in a block design. Data were realigned, co-registered to an MRI atlas of the zebra finch brain, and smoothed. Statistical results were then analyzed with the General Linear Model implemented in SPM software. BOS-specific activation was investigated by comparing the BOLD response elicited by BOS with the response elicited by CON; CON-specific activation was investigated by comparing the BOLD response elicited by CON vs. HET. Voxels that displayed a differential response to the stimuli as a function of experimental treatments were first identified by the presence of a significant interaction "stimulus x group" in the general 3 X 2 Anova (within factors: three stimuli; between factors: 2 groups). We then further investigated this interaction by testing more specifically for potential interactions between groups considering only two stimuli at one time (BOS-specific activation: BOS and CON stimuli vs. groups; CON-specific activation: CON and HET stimuli vs. groups). Birds were sacrificed one day after the fMRI experiment and stained by immunohistochemistry for dopamine beta-hydroxylase to verify the DSP-4-induced depletion of noradrenergic cells and fibres.

Results: The immunohistochemical staining revealed a decreased number of noradrenergic cells in the Locus coeruleus and in the ventral subcoeruleus and a decreased density of noradrenergic fibres in the cerebellum in the DPS-4 group as compared to the saline group. Functional MRI data revealed a significant difference in BOS-specific activation between groups in a cluster located in the left NCM, a secondary auditory region (F = 13.02, p = 0.008) (fig. 1). Post-hoc t-tests revealed a significant BOS-specific activation in the DSP-4 group (t = 3.52; p = 0.003) that was absent in the saline group (t = 0.03; p = 0.71). The BOS-specific activation present only in the DSP-4 group resulted from a significantly greater neural activity induced by BOS in the DPS-4 group as compared to the saline group (t = 2.64; p = 0.028). The 2 X 2 ANOVA testing for differences in CON-specific activation between groups revealed a significant interaction in a cluster located in the right CM, another secondary auditory region (F = 14.40, p= 0.005) (fig. 2). Post-hoc t-tests indicated a significant CON-specific activation in the DSP-4 group (t = 3.44; p = 0.002) that was again absent in the saline group (t = -1.54; p = 0.62). The CON-specific activation present only in the DSP-4 group resulted from a significantly greater neural activity induced by CON in the DPS-4 group as compared to the saline group (t = 3.31; p = 0.002) and a weaker neural activity induced by HET in the DPS-4 group as compared to the saline group (t = 2.81; p = 0.009).

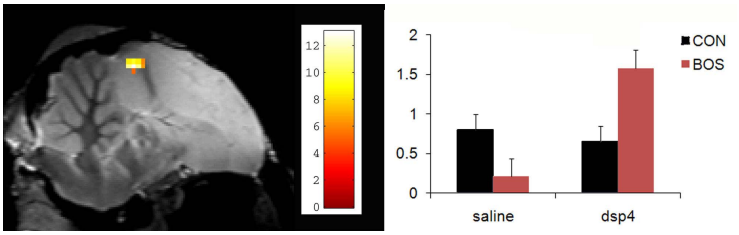

Figure 1: Differential BOS-specific activation between experimental groups (saline and DSP4) Left: statistical parametric F-map of the region where a significant interaction was found at the group level ($p < 0.05$) Middle: Colour scale of F values. Right: Mean relative amplitude (in %, + SE) of neural activations elicited by BOS (in red) and CON (in black) in the voxel presenting the maximal F value among all significant voxels of the cluster illustrated on the left panel.

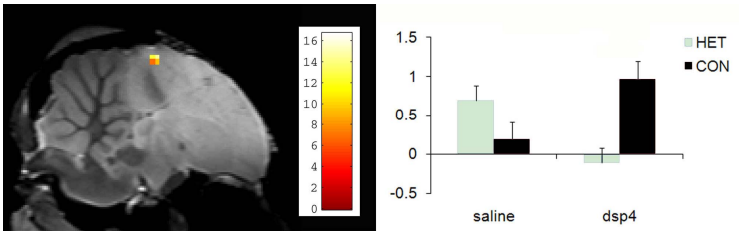

Figure 2: Differential CON-specific activation between experimental groups (saline and DSP4) Left: statistical parametric F-map of the region where a significant interaction was found at the group level ($p < 0.05$) Middle: Colour scale of F values. Right: Mean relative amplitude (in %, + SE) of neural activations elicited by CON (in black) and HET (in light green) in the voxel presenting the maximal F value among all significant voxels of the cluster illustrated on the left panel.

Discussion and Conclusion: Our results suggest the existence of anatomically discrete complex effects of NA on song processing in secondary auditory regions of songbirds. The auditory forebrain of songbirds is densely innervated by noradrenergic inputs, much of which probably arise from the locus coeruleus. Depletion of these noradrenergic inputs results in an enhanced differential activation by socially relevant auditory stimuli suggesting than NA might play an inhibitory role in these discriminations. These results have, however, been observed in anesthetized birds. Future work will need to elucidate the behavioural relevance of such modulatory effects of NA on conspecific song and bird's own song perception in behaving birds.