Response to Comment on "Homeostatic Sleep Pressure and Responses to Sustained Attention in the Suprachiasmatic Area"

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Science **328**, 309-c (2010);
DOI: 10.1126/science.1177949

Response to Comment on "Homeostatic Sleep Pressure and Responses to Sustained Attention in the Suprachiasmatic Area"

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Astafiev *et al*. question whether the blood oxygen level–dependent (BOLD) response that we reported in the brainstem was located in the locus coeruleus (LC). Using high-resolution T1-turbo spin echo images (T1-TSE) acquired in an independent group of subjects, we show that the reported task-related BOLD response in the brainstem is actually compatible with the anatomical location of the LC.

The commentary of Astafiev *et al*. (*1*) illustrates the fast pace at which neuroimaging techniques are currently progressing. Long-term studies requiring the recruitment of highly selective populations and complex, time-consuming designs constrained by the physiology of sleep/wake regulation are bound to be outdated by some of their technical aspects when they come to be published. However, this does not necessarily entail that their findings are invalidated.

We used functional magnetic resonance imaging to study the effects of sleep-wake regulation on the cerebral mechanisms supporting cognition (*2*). We originally identified the localization of the blood oxygen level–dependent (BOLD) response using human brainstem atlases (*3*, *4*). Statistical inferences were based on a priori coordinates from previous independent publications reporting responses in the LC [see methods described in the Supporting Online Material for (*2*)]. The potential anatomical inaccuracy of these approaches led us to cautiously label the reported activation as LC-compatible, a localization disputed by Astafiev *et al*.

Here, we confirm the localization of the reported activation in the LC in a follow-up investigation with an independent group of 20 healthy young subjects (mean age, 23.6 ± 2.35), using a magnetic resonance (MR) sequence sensitive to neuromelanin-related contrast. Three data sets were consecutively acquired using a 3 Tesla Allegra MR scanner (Siemens, Erlangen, Germany): (i) an echo planar imaging (EPI) temporal series [voxel size, 3.4 × 3.4 × 3 mm^3; matrix size, 64 × 64 × 32; repetition time (TR), 2130 ms; echo time (TE), 40 ms; flip angle (FA), 90°]; (ii) a T1-weighted structural image [3D-MDEFT (modified driven equilibrium Fourier transform); voxel size, 1 × 1 × 1 mm; matrix size, 256 × 224 × 176; TR, 7.92 ms; TE, 2.4 ms; TI, 910 ms; FA, 15°]; and (iii) a high-resolution T1-turbo spin echo image (T1-TSE; voxel size, 0.43 × 0.43 × 3 mm^3; matrix size, 400 × 512 × 10 voxels; TR, 600 ms; TE, 14 ms; FA, 90°) (Fig. 1A). The LC was manually outlined on the T1-TSE image for each subject (masked-LC image, mLC).

Each individual data set was processed using the same procedure as in (*2*), allowing reliable comparisons with our published data set. The warped T1 and mLC images averaged across subjects reflected group-wise variability in overall brain, brainstem, and LC anatomy, as observed in (*2*). Figure 1B (left panel) shows the location of the peak activation reported as "LC compatible" in Schmidt *et al*. (*1*) on the mean T1 image, overlaid with the mean mLC image. The activation peak was located in the ventral and rostral part of the independently and anatomically identified LC, confirming our original claim that this activation was localized in an area compatible with this nuclear structure.

To make sure that this result was not induced by an inadequate warping of the brainstem, we further applied an optimized brainstem normalization procedure to the data (*5*). Warped T1 images were submitted to a second brainstem-specific normalization step (affine transform, brainstem-masked T1 template), and T1, T1-TSE, and mLC images were warped accordingly. The same procedure was applied to functional

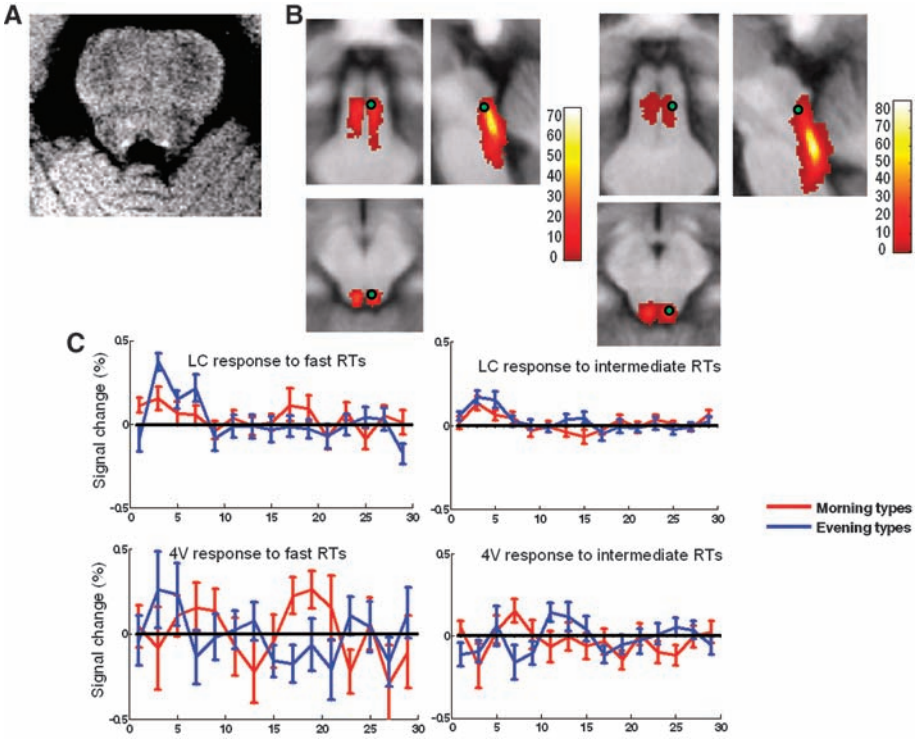

Fig. 1. (**A**) The human LC as seen on a high-resolution T1-TSE scan (transverse slice). (**B**) Location of the peak activation reported as "LC compatible" on the mean T1 image, overlaid with the mean mLC image. (Left panel) Peak activation overlaid on spatial transformations as in (*2*) (green dot; $x = 4$; $y = -32$; $z = -18$), displayed in coronal (top left image), sagittal (top right image), and transverse (bottom image) orientations. (Right panel) After optimized brainstem normalization (green dot; $x = 6$; $y = -34$; $z = -14$). (**C**) Average time course (PSTH) of task-related BOLD response associated with fast RTs (left panels) and intermediate RTs (right panels) during the evening session in the peak voxel located in the LC ($x = 4$; $y = -32$; $z = -18$, upper panels) and in the fourth ventricle (bottom panels), in morning (red) and evening (blue) types.

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data (*2*), leading to a new activation map. Figure 1B (right panel) shows the projection of the new activation peak on the brainstem-warped mean T1 and mLC images. Again, the reported activation peak falls within the estimated anatomical position of the LC. However, the peak now projects to a more lateral area of the LC. These two normalization approaches and ensuing group-wise LC volume distribution highlight the fact that an exact LC localization is made difficult not only because of the small size of the LC and brainstem pulsatility but also because it depends on the specific procedure used to coregister functional and structural data. Thus, one cannot entirely rely on standard template or atlases (*6*) to decide whether an activation map is compatible with LC location. We agree with Astafiev *et al.* that future research should refine the anatomical localization of brainstem responses.

Astafiev *et al.* (*1*) additionally question the biological validity of BOLD responses in the LC area. We further extracted the average time course of task-related BOLD responses associated to the fastest reaction times (RTs) (optimal vigilance regulation) and intermediate RTs (global vigilance regulation) during the evening session, both in the peak voxel located in the LC ($x = 4$, $y = –32$, $z = –18$) (*2*) and in the fourth ventricle, in morning and evening type subjects. As illustrated in Fig. 1C, the LC response to the fast RTs during the evening hours presented higher activity levels in evening as compared to morning types, and its time course followed the expected shape of the hemodynamic response function. In contrast, activity extracted in the fourth ventricle presented much larger inter-individual variability and did not follow any reproducible time course suggestive of consistent event-related hemodynamic response. Contrasting with the results of Astafiev *et al.* (*1*), these findings indicate the possibility to record consistent hemodynamic responses in the brainstem. Nevertheless, this discrepancy itself calls for caution when reporting BOLD responses in brainstem areas where a precise investigation of the BOLD time course requires the use of comprehensive models of hemodynamic response, using a complete set of basis functions, a finite impulse response model (*7*, *8*), or a Bayesian estimation of the hemodynamic response (*9*, *10*).

At the cellular level, the LC and its dendritic fields, which extend in the peri-LC area (*11*), receive a number of various afferents, not all of which have been chemically identified. Some of them originating from the frontal cortex and bulbar reticular formation are thought to be glutamatergic (*11*). Glutamate-immunoreactivity is present in a substantial proportion of synapses terminating on LC adrenergic cells (*12*). However, to add to the complexity of the system, glutamergic afferents to the LC can sometimes result in decreased LC firing (*13*) through mGluR-related activity-dependent depression (*14*). Whether these effects concern the phasic or tonic mode of firing in the LC, and to what extent it influences the BOLD signal, remains also to be assessed.

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10 July 2009; accepted 25 March 2010
10.1126/science.1177949