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Science **328**, 309 (2010);
DOI: 10.1126/science.1177200

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

Comment on "Modafinil Shifts Human Locus Coeruleus to Low-Tonic, High-Phasic Activity During Functional MRI" and "Homeostatic Sleep Pressure and Responses to Sustained Attention in the Suprachiasmatic Area"

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Minzenberg *et al*. (Reports, 12 December 2008, p. 1700) and Schmidt *et al*. (Reports, 24 April 2009, p. 516) reported blood oxygen level–dependent (BOLD) responses in the human locus coeruleus (LC). Here, we show that these LC responses do not correspond to the anatomical location of the LC and present cautionary data concerning the quality of BOLD signals measured from the LC using standard functional magnetic resonance imaging acquisition parameters.

Because the locus coeruleus (LC) plays a major role in modulating brain activity, there is great interest in using neuroimaging techniques to study its function in humans. Two recent studies (*1*, *2*) reported blood oxygen level–dependent (BOLD) responses in the human LC, but neither provided anatomical evidence that activations were located in the LC, and their claim conflicts with the known location of human LC.

The LC is a small, rod-shaped nucleus located within the dorsal wall of the rostral pons in the lateral floor of the fourth ventricle and is the principle site for synthesis of noradrenaline in the brain. The LC in each hemisphere contains 9500 to 23,000 cells and has a rostrocaudal extent of ~16 mm. The cross-sectional area of the LC is 17.2 to 32.8 mm^2 if regions with the lowest cell densities are measured but is only 0.3 to 5.6 mm^2 (*3*) if regions with the mean density (1281 to 1920 cells/mm^2) and above are measured. The LC has been localized in humans based on postmortem examinations (*3*, *4*) and on in vivo magnetic resonance imaging (MRI) scans that visualize neuromelanin pigment within the LC (*5*–*8*).

We adapted the neuromelanin imaging technique to the Siemens Trio 3T scanner (TR = 600 ms, TE = 14 ms, flip angle = 120, voxel dimensions 0.43 × 0.43 × 2.5 mm, 20 axial slices) and scanned 10 subjects. A bite-bar stabilized head position. On axial images, the LC appeared as an area of high signal intensity in the upper pontine tegmentum immediately anterolateral to the floor of the fourth ventricle (Fig. 1A). In all 10 subjects, the LC corresponded to the location reported in previous imaging (*5*, *8*) and histological studies (*3*–*5*) and in human brain atlases (*9*, *10*). To compare the LC location across subjects, individual images were registered to a brainstem atlas template (fig. S1A), and a group-average neuromelanin image was computed (fig. S2). The high accuracy of this procedure was evidenced by the close match of individual brainstem outlines to the template (fig. S1), the presence and high contrast of the LC in the group-average neuromelanin image (fig. S2), and the low variability across individuals of the LC coordinates in the MNI152 (Montreal Neurological Institute) atlas [average (SD): left LC = –3.2(0.39), –38.2(0.63), –24.8(1.01); right LC = 5.4(0.28), –37.9(0.57), –24.7(1.61) (fig. S1B)]. Variability was greatest along the dorsocaudal axis, the longest axis of the LC in previous anatomical studies.

In contrast, the "bilateral pontine clusters" identified as LC in figure 1C in (*1*) were located in a more anterolateral and superior position, which is inconsistent with previous anatomical studies and human brain atlases. The average MNI152 coordinates for Minzenberg *et al*.'s (*1*) task-dependent and task-independent contrasts were 15.2 mm (right LC) and 15.4 mm (left LC) in vector distance from our LC coordinates. Moreover, the variability across subjects was largest along the anteroposterior axis rather than the dorsocaudal axis. Finally, some of the reported LC clusters (*1*) contained more than 300 voxels (2565 mm^3 and 5144 mm^3), greatly

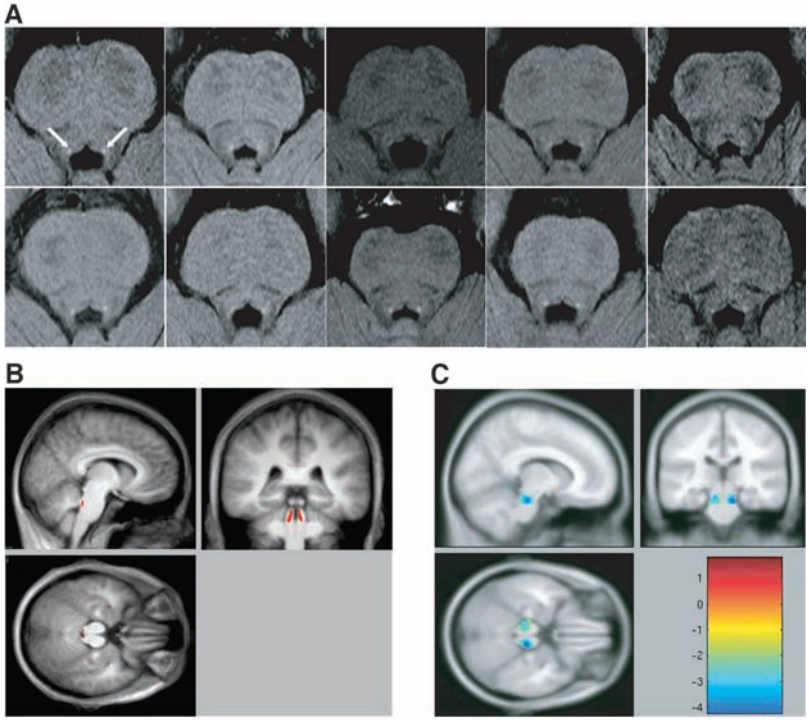

Fig. 1. (**A**) The localization of the human LC in neuromelanin scans of 10 subjects. (**B**) High-contrast LC voxels from the group-averaged neuromelanin image in atlas space (see fig. S2) are colored in red on the group-averaged anatomical image in atlas space. The location of the LC in this atlas-space image can be compared with the atlas-space activations of (*1*), which is shown in (**C**). Adapted version of figure 1 in (*1*). [Reprinted with permission from AAAS]

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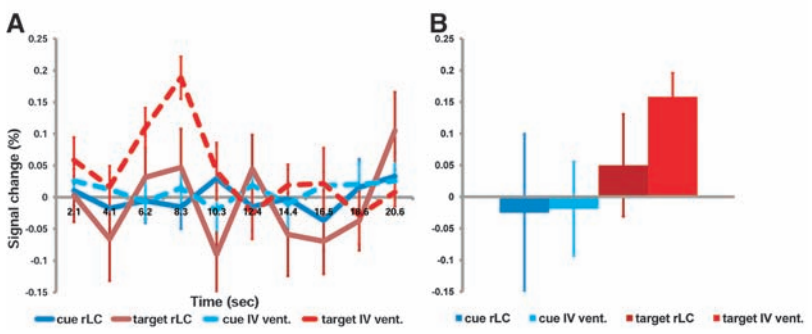

Fig. 2. (**A**) Average time course from 10 subjects extracted from individually defined ROIs in the right LC and the IV ventricle. (**B**) Average magnitudes from the same subjects extracted from the same regions. Cue: response to an informative peripheral cue that either shifted attention or maintained attention at the current location; target: response to correct detection of a target, which required a key press (*13*). rLC: right LC, IV vent.: IV ventricle. Error bars represent SEM.

exceeding the estimated volume of the LC (275.2 to 524.8 mm^3) (*3*).

Minzenberg *et al*. (*1*) acknowledge that the observed "activation clusters extend outside the LC proper," but argue that (i) "LC-NE dendrites, which are the likely site of membrane potential changes associated with BOLD signal change… extend in the pons considerably beyond the LC proper in mammals…including humans" and (ii) "the activation clusters observed here compare favorably, both qualitatively (3D extent) and quantitatively (location of maxima, cluster size), with those reported in several other studies." However, the dendrites of individual LC neurons typically extend for less than 1 mm from the nuclear core of LC [up to 500 μm in rats (*11*) and similarly in humans (*12*)], distances far too small to account for the extended pontine clusters. In addition, the cited studies that reported LC activity did not conduct an independent localizer (i.e., neuromelanin scans) but relied on previously reported atlas coordinates. Accurate LC localization is also unclear in the recent report of Schmidt *et al*. (*2*), who claimed that the dorsal pontine tegmentum area encompasses the locus coeruleus but did not independently localize the LC. Their reported LC MNI coordinates are 12 mm and 9 mm in vector distance from our coordinates.

Even if the LC is properly localized, the measured BOLD response may not accurately reflect the activity of LC neurons because of a mismatch between the size of the LC and the typical size of a functional MRI (fMRI) voxel (3 to 4 mm isotropic), as well as pulsation artifacts from the fourth ventricle. The 10 subjects whose LC was localized using the neuromelanin sequence had previously participated in a fMRI study (*13*) involving standard acquisition parameters ($4 \times 4 \times 4$ mm^3 voxels, whole-brain coverage, no cardiac gating), which separately measured the BOLD activations to peripheral cues that directed attention to a task-relevant location and the activations to targets at that location. Figure 2 shows group-mean BOLD time courses and magnitudes (*13*) from individually defined LC regions of interest (ROIs) ($3 \times 3 \times 3$ mm^3). Neurophysiological studies indicate that LC neurons respond to behaviorally relevant targets (*14*). No response was observed to cues, and a low-amplitude noisy response to targets was observed. A task-specific response was observed from the identically sized ROI in the fourth ventricle, raising concerns about the biological validity of any observed LC response. The use of small voxel sizes to reduce partial volume averaging and cardiac gating to minimize ventricular pulsation artifacts may improve signal quality (*15*).

Finally, a common practice in fMRI research is the reporting of response magnitudes rather than time courses, as in Minzenberg *et al*. (*1*). This is particularly problematic when imaging brainstem nuclei, because a significant positive magnitude can be observed while nonphysiological features of the time course are hidden. Our results, however, do not imply that the BOLD signals of Minzenberg *et al*. are artifactual, only that those signals do not reflect LC activity.

In conclusion, our analysis raises doubts concerning the identification of the LC in several previous BOLD studies. Moreover, the interpretation of BOLD signals from the LC using standard fMRI acquisition parameters can be problematic and requires time-course information. These cautions apply to other brainstem nuclei.

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16. This work was supported by the National Institute of Neurological Disorders and Stroke grant RO1 NS048013, National Institute of Mental Health Grant RO1 MH 71920-06, and the J. S. McDonnell Foundation. We thank D. L. Pope for help with data collection and subject recruitment, S. Adams for help with subject recruitment, M. P. McAvoy for statistical advice, G. Foster and R. Nagel for the adaptation of neuromelanin-sensitive sequence, and D. A. Yablonskiy and X. He for testing alternative sequences for LC localization and for physics advice.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5976/309-a/DC1
Figs. S1 and S2

2 June 2009; accepted 22 March 2010
10.1126/science.1177200