Supplementary material

Material and methods

Mean concentration maps for patients (with and without medication overuse) and healthy controls were calculated using the ImCalc function in the Statistical Parametric Mapping 8 package (Wellcome Trust Center for Neuroimaging, Institute of Neurology, University College London, London, UK) and overlaid on the study-specific anatomical template in MRIcono (http://cnl.web.arizona.edu/mricro.htm).

The regions of interest were generated by first binarizing the individual warped NAA images, using the ImCalc function and a threshold of 1 mM, and then adding them up for all participants, separately for each hemisphere. Parts of the images with a magnitude smaller than the total number of participants used in the analyses were discarded. The resulting images were then binarized and cut using MRIcono to yield the final regions of interest. For the other metabolites, to exclude discarded voxels (zeroed voxels with CRLB > 30%) from the averaging over voxels within each region, the respective concentration maps were also binarized, added up and thresholded before being masked with each of the regions of interest. It is noteworthy that only few voxels were discarded and that most of these were located along the outer rim of the images.

To extract the anatomical locations covered by the regions of interests, the 2D region of interest images were resliced according to the 3D high-resolution anatomical image of the chosen template subject. The 3D anatomical image was then normalized into MNI space using the Statistical Parametrical Mapping 8 package and the warp parameters were applied to the resliced region of interest images. These images were then imported
into xjView 8 (http://www.alivelearn.net/xjview/) where anatomical labeling was performed. The major regions covered by the thalamic regions of interest were the ventral lateral nucleus, ventral posterior lateral and medial nuclei, medial dorsal nucleus, mammillary body, and subthalamic nucleus. For the anterior cingulate region of interest, the major regions covered were the medial frontal gyrus and anterior cingulate cortex, including Broadmann areas 10, 24 and 32. For the mid cingulate region, the major regions covered were the anterior cingulate cortex, cingulate gyrus and the medial frontal gyrus, including Broadmann areas 6, 24 and 32. Finally, for the posterior cingulate region of interest, the major regions covered were the cingulate gyrus, posterior cingulate cortex and precuneus, including Broadmann areas 7, 23 and 31.