Distinguishing GABA from lysine in vitro and in vivo by 2D localized correlated spectroscopy

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Introduction: The coupled protons on GABA carbons 3 and 4 resonate around (F_2 = 2.0 ppm, F_1 = 3.0 ppm) in 2D correlated spectroscopy (COSY) [1]. The unequivocal assignment of this cross-peak to GABA *in vivo*, however, is complicated by the suspicion of macromolecules co-resonating near these frequencies, on the basis of NMR experiments using guinea pig brain extracts containing thymosin β4 [2], a lysine-rich protein [3]. Given the structural similarities between the amine end of GABA, and the amine side chain of lysine, macromolecule co-resonance may be due to lysine side chains in proteins [2]. As such, we tested whether GABA and lysine could be differentiated *in vitro* and *in vivo* by 2D localized COSY at 3T.

Methods: Expected chemical shifts were obtained via simulation (www.nmrdb.org) [4]. Solutions of 10 mM lysine, 10 mM GABA, and a mixture of both were made in phosphate-buffered saline; the pH was adjusted to 7.4. Each phantom also contained 10 mM creatine (Cr) as a chemical shift reference. Water-suppressed localized COSY data from a 3 x 3 x 3 cm^3 voxel were obtained on a Siemens Tim-Trio (VB17A) 3T scanner, using a 12-channel head coil. For *in vivo* data, one of the authors was scanned on a Siemens Tim-Verio (VB17A) 3T magnet with a 32-channel head coil, as previously described [5]. A 3 x 3 x 3 cm^3 voxel was centered on the posterior cingulate cortex. Parameters for phantom and human 2D localized COSY were: TR = 1500 ms, minimal TE = 30 ms, Δt_1 = 0.8 ms, and 8 averages each of 64 increments. Total scan time was approximately 12 minutes. Windowing, zero-filling, Fourier transformation, and peak volume measurements were facilitated with Felix 2007 (Felix NMR, Inc., San Diego, CA).

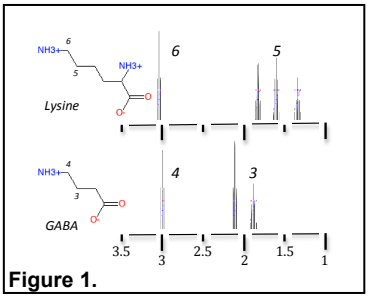

Figure 1.

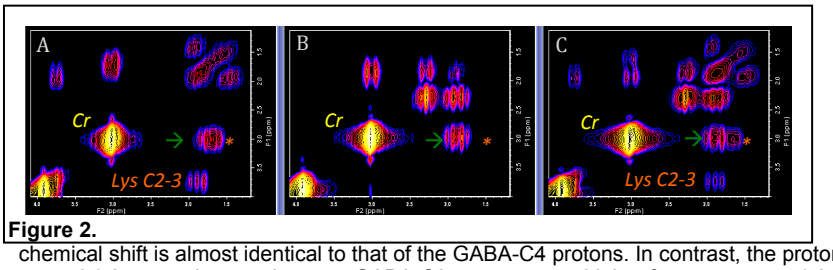

Figure 2.

Results: The structures and simulated 1D spectra for GABA and lysine at physiological pH are shown in Figure 1. The lysine-C6 protons are geminal to the side chain amine; consequently, their chemical shift is almost identical to that of the GABA-C4 protons. In contrast, the protons on lysine-C5 resonate around 1.6 ppm, whereas those on GABA-C3 resonate at a higher frequency near 1.9 ppm due to the deshielding effect of the closer carboxyl group.

In a pure lysine phantom (Figure 2*A*), the lysine-C5-6 cross-peak lies, as expected, adjacent to an orange asterisk set at (F_2 = 1.5, F_1 = 3.0), but far from the green arrow indicating where the GABA-C3-4 cross-peak would be. In a GABA phantom, the more deshielded GABA-C3-4 cross-peak is evident beside this arrow (Figure 2*B*). In a phantom containing both molecules, Figure 2*C* shows the separation of these cross-peaks along the F_2 dimension.

To test this separation *in vivo*, spectra from a healthy human brain was obtained (Figure 3*A*). The region inside the white box was magnified in Figure 3*B*, confirming a separate, putative GABA-C3-4 cross-peak along F_2 (green arrow). Of note, the lysine-specific cross-peak associated with the protons on carbons 2 and 3 (Figure 2*C*) was not detected (Figure 3*B*). The peak volume ratio of this putative GABA cross-peak to that of glutamate plus glutamine (Glx) was 16%.

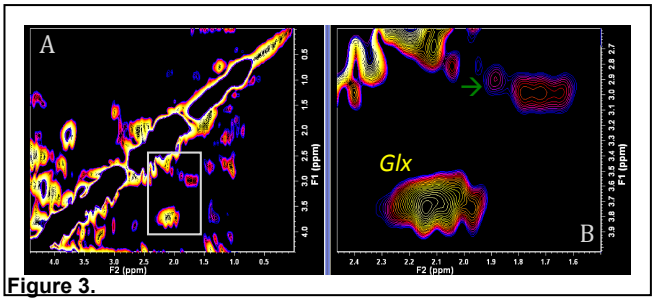

Figure 3.

Discussion: Specific assignment of the GABA-C3-4 cross-peak in 2D COSY has been inhibited by concerns over possible contamination from co-resonating macromolecules. Lysine residues have been reported to represent a possible source of this co-resonance. We have shown here the potential for 2D localized COSY to separate the lysine-C5-6 and GABA-C3-4 cross-peaks, resulting in a GABA:Glx ratio comparable to reported values [reviewed in 6]. Interestingly, we also noted the lack of a lysine-C2-3 cross-peak *in vivo* (Figures 2*C* & 3*B*), and question whether lysine-containing macromolecules are, in fact, MR-visible in living tissue. If not, the cross-peak resonating nearby may represent an entirely different chemical.

A limitation is that molecules formed by bonding to the carboxyl end of GABA, such as homocarnosine, will still possess almost identical proton chemical shifts near the amine end; however, the clinical relevance of such molecules relative to GABA remains uncertain. If this assignment is correct and distinctive, it would represent an important finding; and further studies on its reproducibility and sensitivity to known or experimentally-induced GABA changes *in vivo* would be warranted.

References:

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