RESEARCH HIGHLIGHT

A metabolomics multivariate statistical approach for obtaining data-driven information in neuropharmacological research

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Brain metabolism is exquisitely responsive to activation or inhibition of brain activity, including activation or inhibition of receptors. By multivariate statistical analysis of the metabolic labelling patterns produced following one hour incubation with [3-^{13C}] pyruvate in the presence of receptor ligands of known activity, we have developed a metabolic "footprint" of the GABAergic system. Using this experimental paradigm, any compounds potentially acting on the GABAergic system can then be compared against the footprint and their mode of action as well as the preferred receptor sites identified and characterized. It seems obvious that such an approach would be most valuable for drugs of uncertain pharmacological profiles acting on multiple targets. This approach has already proven useful for γ-hydroxybutyrate (GHB) and was recently applied to ethanol [1]. We showed that the effects of ethanol on reducing glucose metabolism are not via substitution of ethanol for other substrates, or by production of acetate but likely occur via action at GABA receptors, specifically α4β3δ receptors.

Keywords: alcohol; metabonomics; NMR spectroscopy; ^{13}C metabolism; GABA(A)

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Brain metabolism is exquisitely responsive to brain activity with established relationships between glutamatergic activity and metabolic rates in the brain [2,3]. The relationship in the case of GABAergic activity is less clear, with increases or decreases in energy metabolism being possible, depending on the net balance between the increase required by the move away from resting state membrane potential to hyperpolarization, and the resultant overall decrease in activity caused by inhibition of other cells [4].

There are multiple metabolic compartments in the brain; these are classically divided into neuronal and glial compartments but in fact there are multiple subcompartments both in astrocytes and neurons [5-7]. This metabolic compartmentation means that activation of receptors whose localization is restricted in some

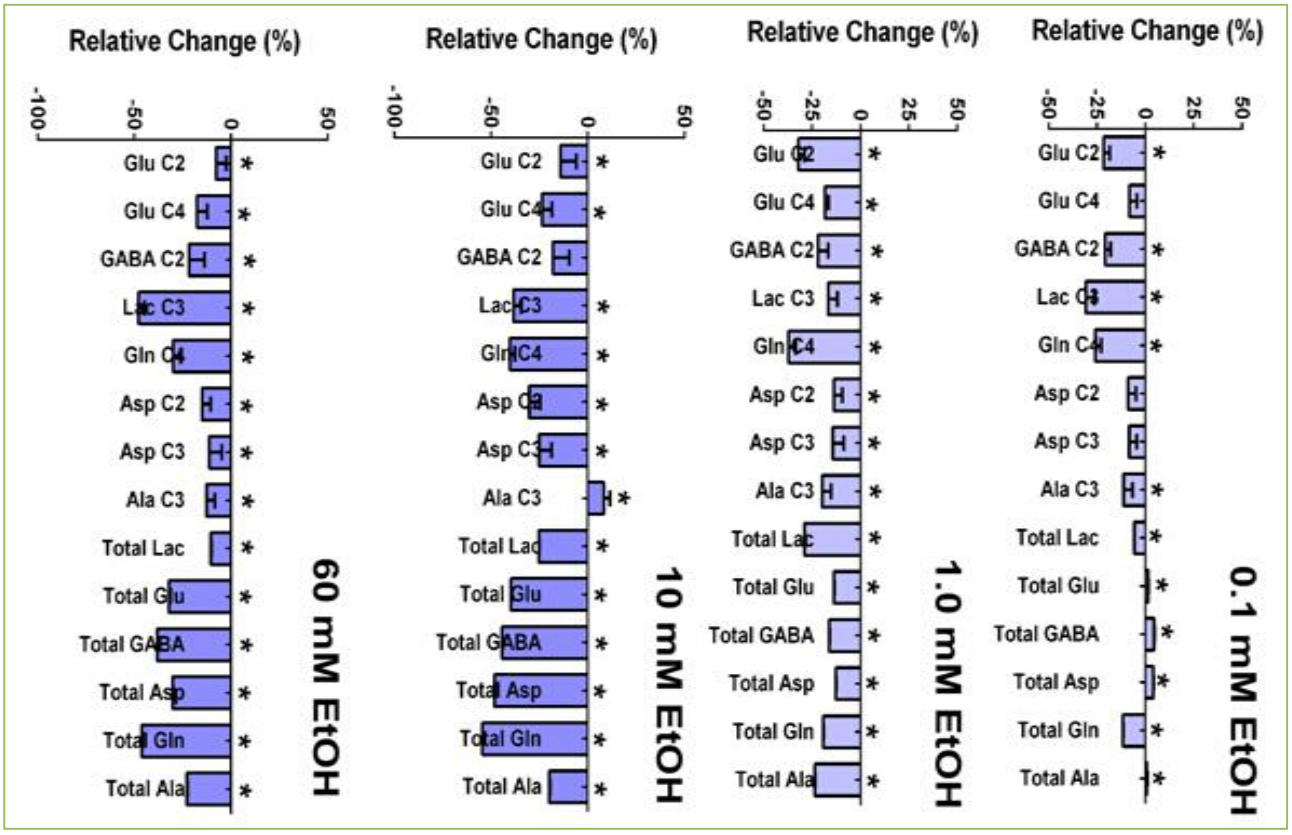

Figure 1. Relative effect of different concentrations of exogenous ethanol on ^{13}C incorporation and on total metabolite pool sizes in brain cortical tissue slices incubated 1 h with sodium [3-^{13}C]pyruvate. Data are shown as relative to the control mean, with control metabolism centered at zero. Error bars represent standard deviations. Statistically significant changes (calculated on the raw data not the relative change in flux or pool size, see Methods) are indicated by * ($P < 0.05$, different to control). Figure adapted from [1].

manner, such as to a particular population of neurons or to a particular extrasynaptic location, can invoke a unique metabolic response derived from the preferential perturbation of the metabolic (sub) compartments most influenced by (a) particular receptor(s).

Using the stable isotope ^{13}C, supplied as [3-^{13}C]pyruvate, it is possible to sample these unique metabolic responses. By allowing brain cortical tissue slices to metabolise [3-^{13}C]pyruvate for a set period of time under exposure to ligands active at particular receptors or transporters one can obtain a metabolic "fingerprint" of activity evoked by that ligand, relative to metabolic activity in the absence of ligand. If one obtains enough of these fingerprints, a comprehensive picture of types of metabolic activity can be constructed.

For example, we have been constructing a metabolic picture of the GABAergic system. Our GABAergic "footprint" is a multivariate statistical analysis of the fingerprints generated by 43 different ligands known to be active in the GABAergic system, including agonists and antagonists at GABA(A), GABA(B), $GABA(A)_{rho}$ receptors, and the GABA transporters GATs1-3 and the betaine/GABA transporter BGT.

We used this footprint to identify candidate receptors at which the party drug γ-hydroxybutyrate (GHB) might be active [8]. One of these candidate receptors was subsequently independently verified [9]. These findings are consistent with very high degree of reliability and veracity of the metabolomics approach that we have developed for identifying unknown drug targets [10]. Moreover, the metabolomic approach - examining drug actions on brain tissue with much of its structural and functional integrity intact and a spectrum of potential pharmacological targets still *in situ* - could provide a vital link between the understandings of what the drugs does at a single type of receptor studied *in vitro* and its actual effects on brain function *in vivo*. This was our

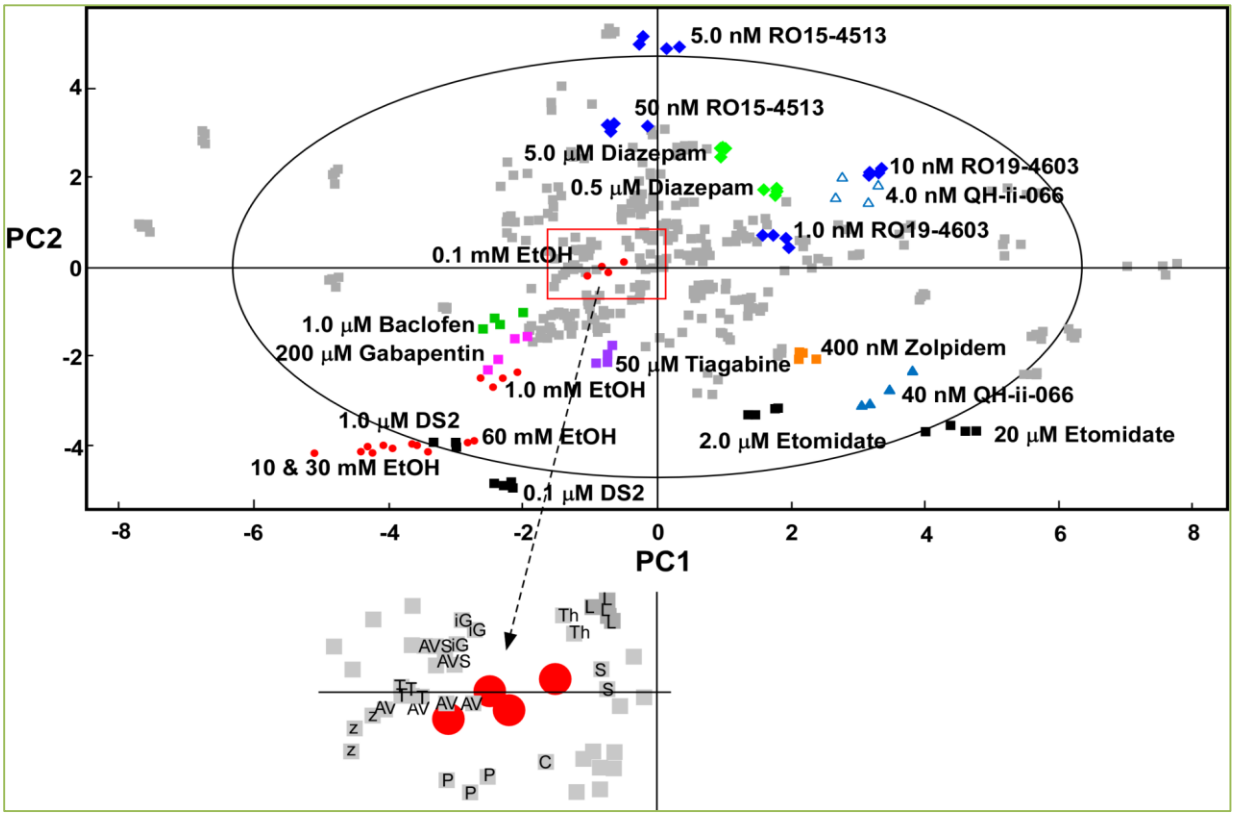

Figure 2. Principal components analysis of ethanol and ligand data shown against metabolic fingerprint data from selected ligands active at GABA(A), GABA(B) and GABA(C) receptors, GAT inhibitors and exogenous GABA. These data generated a three component model accounting for 82% of the variance in the data (47, 26 and 9%, respectively), the major two components of which are shown in this diagram. Ethanol data are shown as red circles, DS2 and etomidate as black squares, RO15-4513 and RO19-4603 as blue diamonds, Gabapentin as pink squares, tiagabine as purple squares, diazepam as green diamonds, QH-ii-066 as blue triangles, Zolpidem as orange squares and baclofen as green squares. All other data are represented as grey squares. The large outer ellipse represents the 95% confidence interval (Hotelling score). The inset to the figure shows an enlargement of the area of the PCA plot immediately surrounding the low (0.1 mM) concentration of ethanol. Key T, 10 μM tiagabine; Th, 10 μM THIP; iG, 10 μM *iso*guvacine; S, 10 μM SGS-742; AV, 100 μM vigabatrin with 5 μM AMPA; z, 40 nM zolpidem; C, 2 μM CI966; L, 0.1 nM L655-708; AVS 100 μM vigabatrin + 5 μM AMPA + 10 μM SKF-89976A; P, picrotoxin. Reprinted with permission [1].

guiding principle when we recently used the footprint to examine the possible effects of ethanol in the GABAergic system [1].

Ethanol is known to be a neuromodulatory agent with actions at a range of membrane proteins including GABA receptors. The most abundant synaptic GABA(A) receptors (mainly comprising α1, β2 and γ2 subunits) are, for practical purposes, non-responsive to ethanol [11] while those containing α4β3δ (and α6 in cerebellum) are about as ethanol-sensitive as *N*-methyl-D-aspartate receptors (except for being activated rather than inhibited by ethanol[12]; see also [13,14]). Ethanol has biphasic outcomes [15] both with stimulatory and "relaxing" effects reported at low concentrations.

In addition, it has also been speculated that ethanol acts as a substrate in the brain, due to the significant reduction in glucose metabolism that is consequent on ethanol consumption. While ethanol itself may not be metabolized in brain to any degree, it has been speculated that acetate, produced following metabolism of ethanol in liver, may substitute as a brain substrate [16]. This question is a problematic one to answer *in vivo*, due to the effects of peripheral metabolism and the subsequent difficulty in determining the source of metabolites found in the brain. In contrast, the cortical tissue slice offers a useful alternative system in which to study effects of ethanol without peripheral interference, and without production of acetate from added ethanol. Acetate is known to be a poor substrate for metabolism in brain, due in part to its low ΔG [17], and the distinct possibility of metabolism of acetate being blocked by acetylation of acetylCoA synthetase [18].

Using the cortical tissue slice model it has been shown that ethanol has distinct and significant effects on incorporation of ^{13}C-label into metabolites of the Krebs cycle and related amino acids, as well as on total metabolite pool sizes [1]. The high concentrations of ethanol used (10, 30 and 60 mM) were found to induce similar metabolic patterns and these patterns were shown to cluster on the GABAergic footprint with those from DS2(4-Chloro-*N*-[2-(2-thienyl)imidazo[1,2*a*]-pyridin-3-yl]benzamide), a positive allosteric modulator of α4β3δ-containing GABA(A) receptors, suggesting that these concentrations of ethanol act to reduce glucose metabolism directly via action as these receptors. Interestingly, the very low concentration of ethanolthat was used (0.1 mM) produced a quite different metabolic profile which clustered with those from inhibitors of GABA uptake, and ligands showing affinity for α5, and to a lesser extent, α1-containing GABA(A)R. Ethanol is known to have a biphasic effect and we suggest that the stimulatory effect of ethanol is modulated by these low concentrations of ethanol, equating to a single sip of wine.

Conflicting interests

The authors have declared that no competing interests exist.

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References

1. Rae CD, Davidson JE, Maher AD, Rowlands BD, Kashem MA, Nasrallah FA, *et al*. Ethanol, not detectably metabolized in brain, significantly reduces brain metabolism, probably via action at specific GABA(A) receptors and has measureable metabolic effects at very low concentrations. J Neurochem 2014; 129: 304-314.
2. Stagg CJ, Bestmann S, Constantinescu AO, Moreno LM, Allman C, Mekle R, *et al*. Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. Journal of Physiology-London 2011; 589:5845-5855.
3. Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG. Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. Proc Natl Acad Sci USA 1998; 95:316-321.
4. Nasrallah F, Griffin JL, Balcar VJ, Rae C. Understanding your inhibitions. Modulation of brain cortical metabolism by GABA-B receptors. J Cereb Blood Flow Metab 2007; 27:1510-1520.
5. Kreft M, Bak LK, Waagepetersen HS, Schousboe A. Aspects of astrocyte energy metabolism, amino acid neurotransmitter homoeostasis and metabolic compartmentation. Asn Neuro 2012; 4.
6. Waagepetersen H, Sonnewald U, Larsson O, Schousboe A. Compartmentation of TCA cycle metabolism in cultured neocortical neurons revealed by 13C MR spectroscopy. Neurochem Int 2000; 36:349-358.
7. Waagepetersen HS, Sonnewald U, Larsson OM, Schousboe A. Multiple compartments with different metabolic characteristics are involved in biosynthesis of intracellular and released glutamine and citrate in astrocytes. Glia 2001; 35:246-252.
8. Nasrallah FA, Maher AD, Hanrahan JR, Balcar VJ, Rae CD. γ-Hydroxybutyrate and the GABAergic footprint. A metabolomic approach to unpicking the actions of GHB. The Journal of Neurochemistry 2010; 115:58-67.
9. Absalom N, Eghorn LF, Villumsen IS, Karim N, Bay T, Olsen JV, *et al.* alpha 4 beta delta GABA(A) receptors are high-affinity targets for gamma-hydroxybutyric acid (GHB). Proc Natl Acad Sci USA 2012; 109:13404-13409.
10. Enna SJ. Extrasynaptic site of action for gamma-hydroxybutyrate. Proc Natl Acad Sci USA 2012; 109:13142-13143.
11. Mori T, Aistrup GL, Nishikawa K, Marszalec W, Yeh JZ, Narahashi T. Basis of variable sensitivities of GABA(A) receptors to ethanol. Alcoholism-Clinical and Experimental Research 2000; 24:965-971.
12. Wallner M, Hanchar HJ, Olsen RW. Low dose acute alcohol effects on GABA(A) receptor subtypes. Pharmacol Ther 2006; 112:513-528.
13. Kaur KH, Baur R, Sigel E. Unanticipated Structural and Functional Properties of delta-Subunit-containing GABA(A) Receptors. J Biol Chem 2009; 284:7889-7896.
14. Lovinger DM, Homanics GE. Tonic for what ails us? high-affinity GABA(A) receptors and alcohol. Alcohol 2007; 41:139-143.
15. Pohorecky LA. Biphasic action of ethanol Biobehavioral Reviews 1977; 1:231-240.
16. Volkow ND, Hitzemann R, Wolf AP, Logan J, Fowler JS, Christman D, *et al*. Acute effects of ethanol on regional brain glucose metabolism and transport Psychiatry Res Neuroimaging 1990; 35:39-48.
17. Veech RL, Gitomer WL, King MT, Balaban RS, Costa JL, Eanes ED, editors. The effect of short chain fatty acid adminstration on hepatic glucose, phosphate, magnesium and calcium metabolism.: Plenum Publishing Corporation; 1986. 617-646.
18. Hallows WC, Lee S, Denu JM. Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proceedings of the National Academy of Science USA 2006; 103:10230-10235.