RELATIONSHIP BETWEEN ETHANOL-INDUCED CHANGES IN BRAIN REGIONAL METABOLISM AND ITS MOTOR, BEHAVIOURAL AND COGNITIVE EFFECTS

WEI ZHU*, NORA D. VOLKOW1, YEMING MA2, JOANNA S. FOWLER3 and GENE-JACK WANG4

Department of Applied Mathematics and Statistics, State University of New York, Stony Brook, NY 11794-3600, USA, 1National Institute on Drug Abuse, 6001 Executive Boulevard, Bethesda, MD 20892-9561, USA, 2National Institute on Alcohol Abuse and Alcoholism, 6000 Executive Boulevard, Bethesda, MD 20892-7003, USA and 3Chemistry and 4Medical Departments, Brookhaven National Laboratory, Upton, NY 11973-5000, USA

(Received 3 April 2003; first review notified 9 May 2003; in revised form 20 October 2003; accepted 27 October 2003)

Abstract — Aims and Methods: Acute alcohol administration marked decreases in glucose metabolism throughout the human brain. However, the relationship between alcohol's effects on brain metabolism and the behavioural changes that occur with intoxication are still unclear. Here we assessed this association using principal component analysis for dimension reduction and canonical correlations to gauge inter-class relationships. We also used canonical correlations in the polynomial space to assess for possible nonlinear relationships. Results: After normalizing the regional measures to account for the large whole brain decreases observed with intoxication we show that the largest decreases occurred in occipital cortex and that there were relative increases in basal ganglia. Principal component analysis of the changes in the normalized measures revealed that 60% of the variance was accounted for by two factors; one that contrasted cerebellum versus frontal and anterior cingulate metabolism, and another that contrasted basal ganglia and insula. The square of the first factor was significantly correlated with the deterioration in cognitive performance. The second factor showed a significant linear correlation with self-reports of intoxication and with deterioration in cognitive and motor performance. Conclusions: These findings suggest that the contrasting effects of alcohol in basal ganglia versus the insula are involved in the perception of 'feeling drunk' and that its contrasting effects in cerebellum versus those in frontal and parietal cortices are involved in its motor incoordinating effects. On the other hand alcohol's impact on cognitive performance implicates a more complex pattern of brain effects that includes linear as well as non-linear associations.

INTRODUCTION

Historically, it is well documented that acute alcohol intoxication could result in changes in regional brain function, as assessed by changes in glucose metabolism or cerebral blood flow (Volkow et al., 1988, 1990), cognitive performance (Lau et al., 1995; Curtin et al., 2001), motor function (Lemon et al., 1993; Quillian et al., 1999) and behaviour (Bates, 2000; Giancola, 2000). Back in 1990, Eckardt and colleagues at the National Institute on Alcohol Abuse and Alcoholism (NIAAA) anticipated that future Positron Emission Tomography (PET) studies would include cognitive challenges so that one could ascertain the association between changes in brain regional metabolism and cognitive functions due to acute alcohol intoxication. However, most studies measuring changes in regional brain glucose metabolism have been unable to map the behavioural and motor changes with the metabolic changes during intoxication. Failure to see an association could reflect: (1) the large number of correlations usually performed in these studies, which after correction for multiple tests renders most correlations insignificant; (2) the large decrements in whole brain metabolism induced by alcohol that may hide relatively smaller specific regional effects, which may be the ones responsible for the behavioural effects of alcohol; and (3) that the association between metabolic and behavioural changes may be non-linear (previous studies have only investigated linear association between metabolic changes and behavioural effects).

Indeed, after normalizing for global effects, studies have reported marked change in the patterns of normalized metabolic activity (decreases in posterior areas of the brain and relative increases in striatum during intoxication when compared with sobriety). Here we assess if the changes in the normalized metabolic measures correlate better with the behavioural effects of alcohol than the absolute measures. To address the problem of multiple comparisons when performing correlational analysis between regional measures and behavioural effects, we used principal component analyses for dimension reduction and canonical correlations to summarize the relationship between two sets of measurements (e.g. metabolic measures and cognitive tests). We predicted that while for certain behavioural effects the association with the metabolic changes would be linear, for others that relationship could be non-linear. For this purpose, we assessed regional brain glucose metabolism with PET and FDG in 20 healthy subjects who were tested at baseline and during alcohol intoxication. The results on the metabolic effects of alcohol have been previously published (Wang et al., 2000).

SUBJECTS AND METHODS

Subjects

Twenty healthy subjects, 10 males and 10 females, with a mean age ± SD of 38.4 ± 9.3 years were recruited for the study. Subjects received a complete physical, neurological and psychiatric examination as well as routine laboratory tests including urine toxicology. Subjects with medical, neuro-psychiatric illnesses, treated with any prescription drugs, history of substance misuse including alcohol, or who consumed more than five 12 ounce-cans of beer or more than five drinks of 1 ounce hard liquor per week and/or two packs of cigarettes per day were excluded. Subjects were instructed...
to refrain from alcohol drinking and to discontinue any over-the-counter medication one week prior to the scan. Written informed consent was obtained from each participant after the nature of the experiment was fully explained. Studies were approved by the IRB at Brookhaven National Laboratory.

**PET scan**

Subjects received two PET scans with FDG on two separate days within 1 week of each other. For the females, the studies were done in the mid-luteal phase (16–22 days after the onset of menstruation). On the first day, subjects drank a placebo (100 ml of diet noncaffeinated soda) 40–50 min prior to FDG administration. On the second day, subjects drank a mixture of 95% alcohol (0.75 g/kg) with diet soda added up to 100 ml, 40–50 min prior to FDG. The two scans for a given subject were done at the same time of day (±1 h). The subjects were placed in the scanner with their eyes open and their ears unplugged, in a dimly lit room with minimal noise. PET scans were performed with a Siemens HR+ tomograph (resolution 4.5 × 4.5 × 4.5 mm full width half-maximum, 63 slices). Metabolic images were computed as described previously. Thirteen composite brain regions (frontal, parietal, temporal, occipital cortices, basal ganglia, thalamus, limbic system, midbrain, cerebellum, insular, anterior cingulate gyrus, posterior cingulate gyrus, paracentral) were extracted. Measurement of global brain metabolism was obtained by averaging the values from all of the regions of interest.

**Motor, behavioural and cognitive evaluation**

Motor coordination was evaluated at the beginning and at the end of the study. The following items were assessed: gait (walk heel-to-toe in a straight line for 20 feet); the Romberg test (subject touches the tip of his nose with his fingers while keeping his eyes closed); rhythm (tapping the back and the front of the hand with the other hand) and equilibrium (eyes closed, arms 90 degrees up in front and then stand on one foot for 30 s). For gait and rhythm, the response variable was time required to complete each task and for equilibrium and Romberg the response variable was the number of errors. Subjects’ responses under placebo or alcohol influence were rated with respect to baseline (measures prior to placebo or alcohol) as 0 = no change, 1 = minimal change, and 2 = marked change.

Before placebo or alcohol and at 20, 40, 55, 80 and 140 min after initiation of placebo or alcohol drinking, subjects were asked to evaluate on an analog scale (rated 0–10) their subjective perception of intoxication (feeling drunk), sleepiness, dizziness and high. Cognitive effects of alcohol were evaluated using the Stroop tests [reading colour names (Stroop read)], describing the colour (Stroop colour), and reading colour names coloured with discrepant colours (Stroop interference), the Controlled Oral Word Association test (COWA), the Symbol Digit Modality test (SDMT), and arithmetic calculations (Woods et al., 1992). The behavioural effects of alcohol were greatest at 80 min after alcohol consumption and therefore measurements at this time point after alcohol or placebo consumption were adopted in the ensuing analyses.

**Statistical analysis**

Correlational analyses were performed to evaluate the relationship between alcohol’s effect on behavioural, cognitive and motor functions and alcohol-induced changes in relative regional brain metabolism. These include the usual canonical correlation to evaluate the linear relationship between two sets of variables and a novel measure, the canonical correlation in the polynomial space, to gauge possible nonlinear relationship between two sets of variables. Principal component analysis was performed for dimension reduction prior to the correlational analyses.

**Canonical correlations.** There are four sets of variables: metabolism of 13 brain regions of interest (ROIs), six cognitive tests, four motor functional measurements and four behavioural evaluations. These would result in a total of 182 Pearson product moment correlations between changes in metabolism and other measures. Multiple-test corrections such as the Bonferroni adjustment would wipe out virtually any test significance. Besides, variables within the same class are often highly correlated which renders the correlations redundant. Consequently, we adopted the canonical correlations to gauge the relationship between two sets of variables directly. Canonical correlation is essentially the Pearson correlation between the linear combination of variables in one set and the linear combination of variables from another set. The pair of linear combinations having the largest correlation is determined first. Next, the pair of linear combinations having the largest correlation among all pairs uncorrelated with the initially selected pair is identified, and so on. The pairs of linear combinations are called the canonical variables, and their correlations are called the canonical correlations. The first canonical correlation, which is often the only significant one as in our case, is usually adopted to describe the inter-class correlation. Here we will report the first canonical correlation, its test statistic—Wilks’ Lambda (λ), the equivalent F-statistic and the P-value.

**Principal component analysis (PCA).** The sample size in a PET study is intrinsically small (20 here), which would frequently render the degrees of freedom insufficient to detect any significant canonical correlation. We adopted two measures for preliminary dimension reduction. First, only variables that changed significantly (α = 0.05, 2-sided) after alcohol intoxication were included. Second, PCA were performed on the remaining variables in each set for further dimension reduction. The major PCs were selected as follows. For each class, the first few PCs that would account for at least 60% of the variations were selected. Pearson correlations of the selected PCs were obtained and PCs on the behavioural, cognitive or motor functions that were not significantly correlated with PCs of the metabolic measures or vice versa were dropped. Canonical correlations were obtained using the remaining PCs.

**Canonical correlations in the polynomial space.** To detect possible nonlinear relationship we have included powers of variables in each set, and termed the resulting canonical correlations between the extended variable sets as the canonical correlations in the polynomial space. The usual test of significance for a canonical correlation — Wilks’ Lambda (λ), is still valid as long as the set of variables without the polynomial terms is jointly normal (Kshirsagar, 1972). This is a simple and yet effective measure because any function can be written as a polynomial referred to as the Taylor series. Historically, two previous attempts were made to extend the canonical correlations to account for possible nonlinear relationships. The first was by Gregg et al. (1992) who
developed a semi-linear canonical correlation to measure the effect of opioids on the central nervous system. However, it would only allow variables in one set to be non-linear. More recently, Hsieh (2000) has developed non-linear canonical correlations using neural networks. His method is more general but difficult to implement and interpret because the neural net would not reveal the explicit nonlinear relationship under investigation. In contrast, our method is explicit and expedient. It could be further generalized to include interactions among variables in each set. However, since the principal components are orthogonal to each other, the interaction terms are not necessary here.

Multiple-test correction. To correct for multiple tests, we set the family-wise error rate to be 0.05 and adopted the improved Bonferroni procedure based on the ordered \( P \)-values (Simes, 1986). In summary, let \( P_{(1)} \leq P_{(2)} \leq \cdots \leq P_{(k)} \) be the ordered \( P \)-values for testing hypotheses \( H_0 = \{ H_1, H_2, \ldots, H_k \} \). Then \( H_0 \) is rejected if \( P_{(j)} < j \alpha / k \) for any \( j = 1, \ldots, k \). All \( P \)-values reported are 2-sided.

RESULTS

Motor, behavioural and cognitive measurements

Descriptive statistics on the pre-, post-, and changes due to alcohol intoxication, for the behavioural, motor and cognitive measurements are provided in Table 1. At the significance level of 0.05 (2-sided), all variables changed significantly with alcohol intoxication except ‘Stroop colour’. Consequently, ‘Stroop colour’ was dropped from the ensuing correlational analysis.

Absolute metabolism

For the absolute metabolism, every ROI was found to have decreased significantly in metabolic level after alcohol intoxication (Table 2). The first PC alone accounted for 86% of the variations. It was positively and evenly loaded on all 13 ROIs, and therefore is a measure of overall changes in alcohol intoxication except ‘Stroop colour’. Consequently, ‘Stroop colour’ was dropped from the ensuing correlational analysis.

Table 1. Summary statistics of behavioural measures, cognitive function and motor function at baseline, during alcohol intoxication, and the differences (pre–post)

<table>
<thead>
<tr>
<th>Behavioural Measures</th>
<th>Pre-</th>
<th>During</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleepy; mean (±SD)</td>
<td>1.65 (2.83)</td>
<td>4.45 (3.52)</td>
<td>–2.80 (2.71)</td>
</tr>
<tr>
<td>High; mean (±SD)</td>
<td>0.90 (1.83)</td>
<td>5.30 (3.53)</td>
<td>–4.40 (3.10)</td>
</tr>
<tr>
<td>Drunk; mean (±SD)</td>
<td>1.15 (2.39)</td>
<td>5.70 (3.64)</td>
<td>–4.55 (3.58)</td>
</tr>
<tr>
<td>Dizzy; mean (±SD)</td>
<td>0.90 (1.62)</td>
<td>4.80 (3.50)</td>
<td>–3.90 (2.88)</td>
</tr>
</tbody>
</table>

Cognitive Function

Stroop Read; mean (±SD)      | 100.05 (14.66) | 85.10 (17.89)  | 14.95 (15.15) |
Stroop colour; mean (±SD)     | 45.90 (9.31)   | 44.80 (8.31)   | 1.10 (8.56)   |
Stroop Interference; mean (±SD)| 73.50 (10.99)  | 62.30 (13.10)  | 11.20 (15.28) |
SDMT; mean (±SD)              | 46.20 (10.44)  | 41.21 (10.49)  | 5.79 (10.63)  |
COWA; mean (±SD)             | 16.33 (14.70)  | 9.32 (4.56)    | 7.45 (15.50)  |
Arithmetic calculation; mean (±SD)| 11.70 (1.98)  | 10.68 (2.47)   | 1.21 (1.69)   |

Motor Function

Gait; mean (±SD)              | 15.69 (4.55)   | 24.72 (22.47)  | –9.13 (18.80) |
Equilibrium; mean (±SD)       | 0.03 (0.11)    | 0.74 (0.87)    | 0.71 (0.89)   |
Romberg test; mean (±SD)      | 43.01 (27.90)  | 52.60 (35.31)  | –8.48 (20.93) |
Rhythm; mean (±SD)            | 19.09 (5.44)   | 21.27 (6.59)   | –2.18 (3.59)  |

All measures changed significantly at \( \alpha = 0.05 \) (2-sided) except ‘Stroop colour’.

Unlike absolute metabolism where every ROI was found to have decreased significantly with alcohol, only 7 of the 13 ROIs changed significantly (Table 2). Decreases were significant in occipital > posterior cingulate/precuneus > cerebellum and relative increases were significant in basal ganglia > anterior cingulate > insula > frontal cortex. These regions were adopted for the ensuing correlational analysis. The first two PCs of the seven ROIs accounted for 60% of the variations. The first two PCs for motor function and cognitive tests and the first PC for behavioural measurements accounted for 80%, 73% and 75% of the variations, respectively (Table 3). For each class, these were the first few PCs which accounted for at least 60% of the variations and therefore were selected for further correlational analysis. We have thus reduced the 20 original variables to seven PCs — a 65% reduction in dimension.

For the ROIs, the first PC is a contrast of frontal and anterior cingulate versus cerebellum. The second PC is a contrast of basal ganglia versus the temporal insula. For the motor functions, the first PC is positively and evenly loaded on all four measurements, and is therefore an overall indicator of motor function. The second PC is a contrast of gait and equilibrium versus Romberg test and rhythm. For the cognitive functions, the first PC is heavily loaded on Stroop read and Stroop interference whereas the second PC is most heavily loaded on COWA, calculations and SDMT. For the behavioural measurements, the first PC is positively and evenly loaded on all four measurements and reflects a measure of perception of intoxication (Table 3).

Correlational analysis using PCs

With the seven remaining PCs, the first canonical correlations between relative metabolic measures and the other three measures were: (1) metabolism and motor; 0.76 (\( \lambda = 0.43, \))
DISCUSSION

Many correlations, few subjects

In this study we tried to unravel the relationships of alcohol-induced changes in brain regional metabolism, motor function, cognitive function and behavioural measurements through correlational analysis. The large number of correlations (182) under considerations and the multiple tests concern would render most correlations insignificant. For example, the behavioural measure of ‘sleepiness’ appeared to be marginally correlated with four out of seven composite brain regions (relative metabolism) with the 2-sided \(P\)-values being 0.05 (frontal), 0.06 (temporal), 0.06 (cerebellar) and 0.02 (insular). Using the conventional Bonferroni correction for multiple tests, at the family-wise error rate of 0.05, the significance level for each test was 0.05/4 = 0.0125. Therefore, one would have concluded that changes in ‘sleepiness’ due to alcohol intoxication are not correlated with the corresponding changes in relative regional metabolism.

In summary, the three ordered \(P\)-values are 0.0107, 0.0269 and 0.0357. They are smaller than the corresponding threshold values 0.0167, 0.0333, and 0.05 in the ordered \(P\)-value test \((k = 3, \alpha = 0.05)\). Therefore we conclude that changes due to alcohol influence in brain regional metabolism are significantly correlated with the corresponding changes in motor function, cognitive function and behavioural measurements.
which is counter-intuitive. One solution is to adopt the canonical correlations to gauge inter-class relationships. In this case, the canonical correlation between ‘sleepiness’ and the seven ROIs is reasonably high (0.81). But so is the $P$-value (0.616)! This is because the small sample size (20 here) intrinsic for a PET study has rendered the degrees of freedom insufficient to detect any significant canonical correlations. The solution to this second dilemma is to apply the principal component analysis first to reduce the dimension. Here, the canonical correlation between ‘sleepiness’ and the first two principal components of the brain regional metabolism is 0.62 with the $P$-value being 0.015. Thus, one could finally conclude that changes in ‘sleepiness’ are indeed associated with changes in brain metabolism as implicated by the individual Pearson correlations.

Cognitive and metabolic measures — the nonlinear brain

The brain is said to be nonlinear (Frackowiak et al., 1997). Thus, the conventional measures for linear relationships including the usual canonical correlations would be insufficient to detect all possible relationships. We have developed a simple new measure, the canonical correlations in the polynomial space, to detect possible nonlinear relationships. The fact that the first PC of the relative metabolism has a nonlinear relationship with the second PC of the cognitive functions confirmed our suspicion. By adding a quadratic term of the first PC of metabolism, the resulting first canonical correlation in the polynomial space between metabolism and cognition increased from 0.50 ($P = 0.3348$) to 0.76 ($P = 0.0357$); a big leap from an insignificant linear relationship to a significant nonlinear relationship. This is the first ever documentation of a non-linear relationship between metabolism and cognition according to our knowledge. This non-linear association suggests that the effects of alcohol on cognitive function may not be evident until after large changes in metabolic activity (shifts in activity in frontal and anterior cingulate versus activity in cerebellum) have occurred. The association between alcohol-induced changes in activity in frontal cortex and anterior cingulate was expected since these brain regions are involved in executive functions, working memory, decision making and attention, all of which were required to perform the cognitive tasks (Duncan and Owen, 2000; Krawczyk, 2002). Though the cerebellum has been traditionally considered to be involved with motor coordination increasing, evidence from imaging studies points to its involvement in cognitive operations (Habas, 2001). It is suggested that this is in part achieved via its neuroanatomical connections with the frontal cortex (Andreasen et al., 1998). Indeed, in this study it was the contrast in the activity between the frontal and anterior cingulate and that in cerebellum that accounted for the association with cognitive performance.

Behavioural and metabolic measures

The significant canonical correlation was mainly due to the association between the second PC (contrast between metabolism in basal ganglia and that in temporal insula) and the behavioural measures that reflected the subjective perception of intoxication (drunk, dizzy, sleep and high). The striatum, which includes the nucleus accumbens, is one of the brain regions directly implicated in the reinforcing effects of drugs of abuse. Specifically it is believed that the ability of drugs of abuse, including alcohol, to increase DA in nucleus accumbens underlies their reinforcing effects (Di Chiara and Imperato, 1988; Koob and Bloom, 1988). Indeed, imaging studies have reported striatal activation during drug intoxication (Breiter et al., 1997; Stein et al., 1998). The insula is also a brain region that had previously been shown to be activated during alcohol intoxication (Sano et al., 1993). In fact, alcohol-induced increases in CBF in temporal cortex have been linked to its reinforcing effects (Ingvar et al., 1998). Moreover, animal studies (Collins et al., 1996; Lyons et al., 1998; Crews et al., 2001) consistently documented the temporal insula as one of the major brain regions affected by acute alcohol intoxication.

Motor and metabolic measures

The significant canonical correlation was largely due to the association between motor functions and the second PC of relative metabolism (contrast between metabolism in basal ganglia and that in the temporal insula). This suggests an involvement of these brain regions in the motor-incoordinating effects of alcohol. Indeed the effects of alcohol on basal ganglia have been associated with its motor effects (Williams-Hemby and Porrino, 1994; Dar, 1998). Though to our knowledge the effects of alcohol on the temporal insula have not been linked with the motor impairing effects of alcohol, imaging studies have documented the role that the temporal insula has on learning of new motor sequences (Ghaem et al., 1997).

In summary, the results from this study suggest that alcohol-induced deterioration in cognitive and motor function as well as its behavioural effects are linked with changes in patterns of brain activity rather than changes in specific brain regions. Specifically, the contrasting effects of alcohol in basal ganglia versus the insula appear to be involved in the perception of ‘feeling drunk’, and the contrasting effects in cerebellum versus those in frontal and parietal cortices are involved in its motor-incoordinating effects. The cognitive effects were also linked with a contrast function between frontal and anterior cingulate versus cerebellum though this relationship was nonlinear, which suggests that a threshold effect may be necessary in order to observe impairment during intoxication.

Acknowledgements — This research was supported in part by the DOE (OBER) (DE-AC02-98CH10886) and NIAAA (AA 09481).

REFERENCES


