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Glutamate Changes in Anterior Cingulate Cortex Following CCK-4 Infusion

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Zwanzger *et al* (2013) recent article entitled 'Acute shift in glutamate concentrations following experimentally induced panic with cholecystokinin tetrapeptide—a 3T-MRS study in healthy subjects' demonstrates a creative experimental design for studying the neurochemical processes underlying panic attacks. In 18 healthy volunteers, they acquired a series of 1H-MRS measurements in the anterior cingulate cortex to measure the Glx signal (arising primarily from glutamate) before and after an intravenous injection of a panic-inducing dose of CCK-4. They concluded that 'CCK-4-induced panic was accompanied by a rapid and significant increase of Glx concentrations in the ACC.' Unfortunately, they used a statistically invalid approach to test the likelihood that their observations could have occurred by chance.

Their experimental design included six 5-minute MRS acquisitions, each yielding a measurement of Glx. The first measurement was made before the CCK-4 injection and was considered the baseline measurement. The five subsequent Glx measurements were considered post CCK-4 measurements. To test their hypothesis that CCK-4 causes an increase in ACC Glx, the authors used a repeated measures analysis of variance to compare the baseline Glx value to the maximum Glx value observed in any of the five post CCK-4 measurements. They reported a highly significant result: $F(1,17) = 15.94$, $P = 0.001$. However, this statistical approach implicitly assumes that the null hypothesis is zero, or 'no difference' between the baseline and the maximum subsequent Glx value. This is clearly incorrect. In a series of six random numbers, the first number will be lower than the maximum of the subsequent five numbers 83% of the time. To illustrate the seriousness of this statistical illusion, I used a random number generator to create three data sets similar to that reported by Zwanzger *et al* (2013). Each data set included 18 'subjects' and each subject was represented by a series of six random numbers. The random numbers were generated to have a mean and standard deviation identical to

those inferred from Figure 2 in the Zwanzger *et al* (2013) article. Running a repeated measures ANOVA comparing 'baseline' to 'Maximum post baseline' gave the following results for the three randomly generated data sets: $F(1,17) = 34.67$, $F(1,17) = 21.85$, and $F(1,17) = 17.55$ —all 'highly significant' if one assumes the null hypothesis is zero difference. Yet, the F ratio reported by Zwanzger *et al* (2013) was lower than any of the F ratios I obtained using random numbers as 'data.' The same statistical illusion invalidates their analysis of the heart rate change following CCK-4.

The article by Zwanzger *et al* tests an interesting hypothesis and includes a scholarly and valuable discussion of the potential role of glutamatergic mechanisms in panic disorder. Unfortunately, the empirical data presented do not support the conclusion that CCK-4 induces a rise in ACC Glx. In fact, the pattern of changes in Glx they observed following CCK-4 resembles what one would expect to see by chance.

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REFERENCE

Zwanzger P, Zavorotnyy M, Gencheva E, Diemer J, Kugel H, Heindel W *et al* (2013). Acute shift in glutamate concentrations following experimentally induced panic with cholecystokinin tetrapeptide—a 3T-MRS study in healthy subjects. *Neuropsychopharmacology* 38: 1648–1654.

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