### Title

Glutamate Changes in Anterior Cingulate Cortex Following CCK-4 Infusion




### Journal

Neuropsychopharmacology, 39(11)

### ISSN

0893-133X



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### Publication Date

2014-10-01

### DOI

10.1038/npp.2014.93

Peer reviewed

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Dear Editor,

Zwanzger and colleagues 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 (Zwanzger et al. 2013). 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: $F(1,17) = 15.94$, $p = .001$. 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. 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 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. 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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