Neuropsychopharmacology (2014) 39, 2707–2708

www.neuropsychopharmacology.org

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'Acute Shift in Glutamate-Concentrations Following Experimentally Induced Panic with Cholecystokinin-Tetrapeptide—A 3T-MRS Study in Healthy Subjects'—A Reply to the Letter to the Editor

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Neuropsychopharmacology (2014) **39,** 2707–2708; doi:10.1038/npp.2014.104; published online 18 June 2014

In his letter and subsequent correspondence, Maddock (2014) comments on our recent manuscript 'Acute Shift in Glutamate-Concentrations Following Experimentally Induced Panic with Cholecystokinin-Tetrapeptide—A 3T-MRS Study in Healthy Subjects' (Zwanzger *et al*, 2013) and the statistical approach.

Since to our knowledge this was the first study investigating temporally dynamic changes in brain glutamate concentrations following a pharmacologically induced panic attack, no *a priori* hypothesis could be proposed with regard to the exact time point of the change. The results of the study showed that brain neurochemical alterations following CCK-4-induced panic do not necessarily occur at a specific time point. Therefore, a comparison of baseline *vs* individual peak concentrations was carried out in order to evaluate potential changes in Glx/Cr concentrations (Zwanzger *et al*, 2013).

Referring to the Reader's concerns in general, we would like to emphasize the specific character of a challenge study, which was designed to investigate predominantly post-interventional effects of Glx/Cr and not changes at predefined time points. In these studies, parameters such as maximum levels or maximum change from baseline have been frequently used before. In particular, other neurobiological outcome parameters such as heart rate or blood pressure (eg, Depot *et al*, 1998; Le Melledo *et al*, 1998; Flint *et al*, 2002; Koszycki *et al*, 2012) and neuroendocrine data (eg, Koszycki *et al*, 2012) have been analyzed using peak values or maximum change from baseline.

Specifically, in his first letter Maddock (2014) suggests the statistical approach to be incorrect, implying an almost impossible null hypothesis (no difference between the baseline and the maximum subsequent value). To our opinion, this objection is unfounded. Naturally, in a series of six random numbers, the first number will be always lower than the maximum of the subsequent five numbers, with a probability of at least 83%. However, this is only applicable if the consecutive measurements are mutually independent. If the consecutive measurements are associated, the aforementioned probability depends on the correlations between the measurements and can take small values too.

Therefore, the Reader's 'random number generator' used to create six random numbers for each of the 18 hypothetical 'subjects' of the considered three groups adduces no evidence in his objection and argumentation.

Since for each of the 18 subjects the subsequently measured Glx/Cr values following CCK-4 injection are interdependent, in order to conduct a valid simulation via a hypothetical model, correlations between randomly generated numbers similar to that of the Glx/Cr values would have been required.

In his further correspondence, the reader has asserted that a null hypothesis based on the maximum increase is not the same as the null hypothesis based on the maximum change. Also, this objection is unfounded to our opinion, since the fact that the observed maximum over time is applied to dependent and not to independent measurements was entirely neglected. Even if one considers the 'maximum increase' instead of the 'maximum change' of Glx/Cr over consecutive measurements the formulation of a null hypothesis such as 'the maximum increase does not differ from the mean baseline value of Glx/Cr' is correct, as long as dependences between the subsequent measurements are expected.

The used statistical approach employing a repeated-measures ANOVA is correct for testing the null hypothesis 'no difference between baseline and the maximum increase

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over the five subsequent glx/cr values'. Contrary to the Reader's random model, repeated-measures ANOVA design does not require independence between baseline and subsequent values or between baseline and the maximum of subsequent values. The same argumentation applies to the analysis of heart rate following CCK-4 injection.

Certainly, since it cannot be ruled out that the results might have been influenced by other factors, we fully agree that the present data have to be interpreted with caution as explicitly discussed in the manuscript.

FUNDING AND DISCLOSURE

This work was supported by 'Innovative Medizinische Forschung' (IMF) of the Medical Faculty, University of Muenster (IMF ZW 210703). All affiliations mentioned below have no relevance to the work covered in the manuscript: PZ has received speaker fees from Pfizer, Servier, Lilly, Astra Zeneca, and Bristol-Myers Squibb; he is on the advisory board of Pfizer, is a consultant for Ironwood Pharmaceuticals, and has received funding from AstraZeneca. All other authors declare no conflict of interest.

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