

Among the absolute changes between baseline and follow-up, we found only a significant difference between the groups for creatinine clearance ($P < 0.01$), whereas u-NGAL, u-NGAL_{CR} (urinary neutrophil gelatinase-associated lipocalin adjusted for creatinine), and urine output did not deviate significantly. Among the relative changes, calculated as (follow-up – baseline)/follow-up \times 100, only creatinine clearance deviated significantly ($P = 0.02$), whereas no significant differences were found in u-NGAL, u-NGAL_{CR}, and urine output. Thus, creatinine clearance increased slightly in the HES group and was unchanged in the saline group.

Competing Interests

The authors declare no competing interests.

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Being Conscious of Methodological Pitfalls in Functional Brain Network Analysis

To the Editor:

We have read with interest the work by Khodayari-Rostamabad *et al.*,¹ but we would like to point out that volume conduction and uncorrected network measures may have influenced the findings described in this study.

In this double-blinded, placebo-controlled, crossover study, Khodayari-Rostamabad *et al.* performed coherence and graph theoretical (“small world”) analyses of electroencephalogram recordings to characterize the effects of remifentanyl on the functional brain network in healthy subjects. A reduction of mean coherence was found in the α (8 to 12 Hz) and β 1 (12.5 to 18 Hz) frequency bands after infusion of remifentanyl, whereas no differences were found after placebo infusion. Network analysis revealed an increase in path length (*i.e.*, decreased integration) and decrease in clustering coefficient (*i.e.*, decreased segregation) in the same frequency bands. A negative correlation was found between the path length in the α frequency band and the continuous reaction time index, which was used as a measure of sustained attention and vigilance. On the basis of these findings, they stated that infusion of remifentanyl disrupts the complex cortical network, which was associated with reduced attention.

The field of functional connectivity studies is growing exponentially and has increased our understanding of cognition, neuropsychiatric diseases, and physiological effects of pharmacological agents. Simultaneously, methodological limitations of initial approaches of functional connectivity and network analysis of electroencephalogram recordings have been described, which may potentially bias results.² Some concerns may apply to the study by Khodayari-Rostamabad *et al.*

First, coherence was used to calculate functional connectivity, which is likely to be affected by volume conduction, and therefore, coherence might be influenced by alterations in the power spectrum. Multiple electroencephalogram channels will pick up activity of a single source because of transmission of the signal through tissue between the cortex and the electrodes (*i.e.*, volume conduction) and spreading of the electrical field. As a consequence, coherence and correlation can give erroneous estimates of functional connectivity.³ Spectral power changes because of remifentanyl have been described previously, including an increase in α frequency band and a decrease in β frequency bands.⁴ Therefore, the reduction of coherence found by Khodayari-Rostamabad *et al.* might be explained by power changes in different frequency bands, which could also lead to spurious alterations of network characteristics.

Several measures of connectivity have been developed to overcome this problem,² including the imaginary coherence and the phase lag index. These measures are relatively insensitive to volume conduction and field spread, because they discard zero phase lags.³ The phase lag index has already been

used in previous studies during loss and recovery of consciousness because of anesthetics^{5,6} and in vegetative state.⁷ Therefore, these findings may be considered as more reliable, especially where the study of Khodayari-Rostamabad *et al.* reports conflicting results.

Calculating the characteristic path length and clustering coefficient provides information on the topology of the underlying functional network. Khodayari-Rostamabad *et al.* used weighted graph analysis to compare differences in path length and clustering coefficient between remifentanyl and placebo. In a weighted graph, the functional connectivity strength is used to weigh the connection between any two nodes in the network. These weights of the connections have a direct influence on the network characteristics, *i.e.*, clustering coefficient and path length.⁸ When the functional connectivity strength is increased, the path length will reduce and clustering coefficient will increase automatically. As the authors found a lower functional connectivity strength during remifentanyl infusion *versus* placebo, this may at least partly explain the higher path length and lower clustering coefficient during remifentanyl infusion.

A commonly used correction procedure for network analysis based on a weighted graph is normalization of network measures. Normalized measures can be obtained by dividing the path length and clustering coefficient by the characteristics of simulated random networks with the same connection density and strength. However, this normalization does not solve the bias completely.⁸ A recently introduced method to evaluate the underlying functional network is the minimum spanning tree, which may solve the need for normalization.⁹ The minimum spanning tree is an acyclic subgraph in which the most important connections are included. This results in a mathematically defined subnetwork with a fixed density and degree, which may be more appropriate for group comparisons of network topology, and increase comparability between different studies.¹⁰

Studies exploring the effects of anesthesia using functional connectivity and network analysis are important to increase our understanding of the complex phenomena such as consciousness. Recent work provided an interesting view of the underlying mechanism of anesthetics. Unconsciousness induced by propofol results in reconfiguration of the network by a shift of the primary hub (*e.g.*, the highest connected node) from the parietal to the frontal lobe.⁵ Furthermore, in healthy subjects, a change of directionality of the functional connectivity was found during anesthesia-induced loss of consciousness, which normalized after consciousness was regained.^{11–13}

In conclusion, the work by Khodayari-Rostamabad *et al.* should be interpreted with caution because of the methodological limitations described earlier. As long as the field connectivity analysis is evolving rapidly and analysis techniques are constantly being optimized, it is important to use state-of-the-art methodology when studying functional brain networks in a clinical setting.

Competing Interests

The authors declare no competing interests.

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