Archival Report

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Large-Scale Persistent Network Reconfiguration Induced by Ketamine in Anesthetized Monkeys: Relevance to Mood Disorders

Qian Lv, Liqin Yang, Guoliang Li, Zhiwei Wang, Zhuangming Shen, Wenwen Yu, Qinying Jiang, Baoyu Hou, Jian Pu, Hailan Hu, and Zheng Wang

ABSTRACT

BACKGROUND: Ketamine is a highly attractive candidate for developing fast-onset antidepressant agents; however, the relevant brain circuits that underlie sustained, efficacious antidepressant effects remain largely unknown.

METHODS: We used a holistic scheme combining whole-brain resting-state fMRI and graph theoretical analysis to examine the sustained effects on brain networks after administration of a single dose of ketamine and to identify the brain regions and circuits preferentially targeted by ketamine. Topological differences in functional networks of anesthetized macaque monkeys were compared between ketamine (.5 mg/kg) and saline treatment after 18 hours.

RESULTS: We observed persistent global reconfiguration of small-world properties in response to ketamine intake, accompanied by large-scale downregulation of functional connectivity, most prominently in the orbital prefrontal cortex, the subgenual and posterior cingulate cortices, and the nucleus accumbens. Intriguingly, intrinsic connectivity with the medial prefrontal areas in the reward circuits were selectively downregulated. Global and regional regulations of the brain networks precisely opposed the maladaptive alterations in the depressed brain.

CONCLUSIONS: Our results demonstrated that local synaptic plasticity triggered by blockade of *N*-methyl-D-aspartic acid receptors was capable of translating into prolonged network reconfiguration in the distributed cortico-limbic-striatal circuit, providing mechanistic insight into developing specific loci or circuit-targeted, long-term therapeutics.

Keywords: Functional connectivity, Graph theory, Ketamine, Macaque monkey, Mood disorders, Neural network plasticity

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Mood disorders, such as major depressive disorder (MDD), are likely accompanied by distributed system-level disturbances in brain circuitry (1–6). There is increasing recognition that therapeutic interventions could generate structural and functional network reorganization through the possible neural plasticity (4,7) and regulate system-level abnormalities in the brain, eventually leading to complete rehabilitation. Converging evidence has demonstrated that a single subanesthetic dose of ketamine, a noncompetitive *N*-methyl-D-aspartic acid (NMDA) receptor antagonist, produces a rapid-acting and long-lasting antidepressant response (8–13) attributable to synaptic plasticity rather than simple antagonism of NMDA receptors (7,11). These features are extremely attractive for novel pharmacological strategies (14) and have inspired a flurry of preclinical and clinical studies of the neurobiological mechanisms of ketamine action (15,16). To date, however, several key questions regarding the antidepressant action of ketamine remain largely unsolved. First, what are the relevant brain circuits that underlie the long-term, efficacious action of ketamine? Second, why do many patients show a response 24 hours after ketamine administration? (17). Accordingly, is there sustained neural network reconfiguration after acute ketamine treatment, considering the metabolic half-life of ketamine is shorter than 3 to 4 hours? (18). Finally, although synaptic plasticity triggered by the transient blockade of NMDA receptors could help restore disrupted homeostatic regulation and lost synaptic connections (4,7,11), the involved brain regions where synaptic plasticity occurs in complex mood and emotion circuitry, thereby contributing to long-lasting antidepressant effects, remains unknown.

To address these questions, we tested the therapeutic effects of a single dose of ketamine on healthy adult macaque monkeys. Growing evidence has validated the use of resting-state functional magnetic resonance imaging (rsfMRI) in anesthetized macaques (19–27) and has underscored its potential to bridge information between human and animal models (28–31). Although the effect of anesthetic agents on functional brain networks remains poorly understood, recent study has demonstrated that stable functional connectivity patterns under a narrow range of medium level isoflurane

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(e.g., ±.25%) are suitable for anesthetized nonhuman primate investigations (26). Furthermore, whole-brain rsfMRI allows unbiased, simultaneous identification of all pharmacologically affected regions in the brain. Through transiently and reversibly controlled neurochemical perturbations, nonhuman primate models may serve as ideal testing benchmarks to clarify the underlying biomechanisms of drug action in the absence of any pre-existing homeostatic dysfunction as part of the complex disease process (e.g., multidimensional symptomatology in psychiatric disorders), comorbidity, or medication status.

Given the ubiquitous distribution of NMDA receptors throughout the brain (14), a randomized, placebo-controlled functional connectome study was conducted in anesthetized monkeys 18 hours after ketamine and saline administration. The dose used (.5 mg per kg) has achieved clinically meaningful improvements in MDD patients (8–10,17), and the time point was chosen for the most prominent antidepressant response (9), without additional schizophrenia-like symptoms (32). Although recent rsfMRI studies have evaluated the antidepressant effects of ketamine at a priori selection of candidate brain regions or pathways (33–35), a global view of the action of ketamine on the whole-brain scale is still lacking. Therefore, we applied graph theoretical analysis to quantitatively examine the overall topological organization of brain networks (36) and used network-based statistics (NBS) (37) to characterize specific features within a network. This study represents the first fMRI investigation of the antidepressant effects of ketamine in nonhuman primates, using a holistic scheme that could possibly identify hitherto unidentified systems relevant to novel therapeutic targets in the brain reward circuitry. Our primary hypotheses were that a single subanesthetic dose of ketamine would produce sustained network reconfiguration in healthy subjects after it was metabolically eliminated and that this prolonged action, which counteracts abnormalities of functional networks in MDD patients, would provide a plausible mechanistic underpinning for the long-term antidepressant effects of ketamine.

METHODS AND MATERIALS

Animal Preparation

All experimental procedures in nonhuman primate research in this study were approved by the Institute of Neuroscience Animal Care and Use Committee and by the Shanghai Institute for Biological Sciences Biomedical Research Ethics Committee and conformed to the National Institutes of Health guidelines for the humane care and use of laboratory animals.

Nine adult macaque monkeys (3 male and 1 female *Macaca fascicularis* and 5 male *Macaca mulatta*) weighing 5.0 to 10.0 kg (7.7 ± 1.8 kg) were prepared and maintained for fMRI scans. Animals were intramuscularly injected with either a single subanesthetic dose of ketamine (.5 mg per kg) or placebo saline (.90% w/v of NaCl) in random order (Figure 1A). In light of previous nonhuman primate (38) and human clinical studies (17), the ketamine dose was determined to produce sufficient regulatory effects in subjects. Follow-up fMRI scans were scheduled 18 hours after ketamine or saline administration. The imaging interval between saline and ketamine administration was spaced at least 1 month apart to avoid possible carry-over effects. Before each scanning session, an anesthesia cocktail of dexmedetomidine (18–30 μg per kg) and midazolam (.2–.3 mg per kg), supplemented with atropine sulfate (.05 mg per kg), was introduced to the animals. After monkeys were intubated, under an anesthesiologist's guidance (GL), the anesthesia was maintained using the lowest possible concentration of isoflurane gas. For all animal scans, the mean (±SD) inhaled concentration was .92% (±.26%). Subjects received intermittent positive pressure ventilation by an MRI-compatible ventilator (CWE Inc., Weston, Wisconsin) to ensure a constant respiration rate (31.8 ± 2.3 breaths/min). Vital signs, including blood oxygenation, rectal temperature, heart rate (Small Animal Instruments Inc., Stonybrook, New York), respiration rate, and end-tidal CO_2 (Smiths Medical ASD Inc., Dublin, Ohio), were continuously monitored during the experiment and maintained tightly within normal limits. Oxygen saturation was maintained over 95%, and body temperature was kept constant using a hot water blanket (Gaymar Industries Inc., Orchard Park, New York). Lactated Ringer's solution was given at 10 mL per kg per hour during the anesthesia procedure.

Image Acquisition

A cohort of image data was acquired from the Institute of Neuroscience on a 3-T whole-body scanner (Siemens Healthcare, Erlangen, Germany) running with or without an enhanced gradient coil insert (AC88; 80 mT/m maximum gradient strength, 800 $mT \cdot m^{-1} \cdot s^{-1}$ maximum slew rate). A total of 57 of 96 imaging datasets were obtained from the standard Trio system, using a protocol similar to that used with AC88. Two sets of custom-made 8-channel phased-array transceiver coils were used for the imaging sessions on the AC88 and Trio systems (provided by Dr. Lawrence Wald's laboratory at Massachusetts General Hospital, Boston, Massachusetts, and developed in-house), respectively.

Whole-brain resting-state fMRI data were collected using a gradient-echo echo-planar imaging (EPI) sequence (TR = 2000 ms; TE = 29 ms; flip angle = 77°; slices = 32; field of view = 96 × 96 mm; 1.5 × 1.5 mm^2 in plane resolution; slice thickness = 2.5 mm; GRAPPA factor = 2). For each experiment, 4 to 6 EPI runs were acquired, and each run contained 200 functional volumes. A pair of gradient echo images (echo time: 4.22 ms and 6.68 ms, respectively) with the same orientation and resolution as the EPI images were acquired to generate a field map for distortion correction of the EPI images. High-resolution T1-weighted anatomical images were acquired using an MPRAGE sequence (TR = 2500 ms; TE = 3.12 ms; TI = 1100 ms; flip angle = 9°; acquisition voxel size = .5 × .5 × .5 mm^3; 144 sagittal slices). Six whole-brain anatomical runs were acquired and averaged for better brain segmentation and three-dimensional cortical reconstruction. Each experiment lasted for approximately 3 to 4 hours, and a total of 96 imaging datasets (47 for ketamine condition and 49 for saline condition) were collected from 9 monkeys.

Network Construction

Node and Edge Definition. A network is composed of nodes and edges between nodes. Herein, nodes represent