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Association between endogenous lactate accumulation and dysregulated activation of the NLRP3 inflammasome pathway in schizophrenia

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In this study, we retrospectively analyzed whether serum lactate levels were elevated in patients with schizophrenia (SCZ) and explored cognitive deficits, abnormalities in lactate metabolism, and neuroinflammation in an dizocilpine (MK-801)-induced N-methyl-D-aspartate (NMDA) receptor (NMDAR) inhibition model using the Morris water maze (MWM) test, biochemical assays, immunofluorescence (IF), Western blot (WB), and enzyme-linked immunosorbent assay (ELISA). We found that serum lactate levels were significantly higher than the normal range in patients with schizophrenia, and they were significantly and positively correlated with both length of hospitalization and serum triglyceride levels. In addition, we found that MK-801 induced cognitive deficits in Sprague-Dawley (SD) rats, accompanied by markedly elevated levels of lactate, pyruvate, glutamate and lactate dehydrogenase (LDH) activity in serum and frontal cortex (FCX). MK-801 caused a significant increase in the expression of NOD-, LRR-, and pyrin-containing protein 3 (NLRP3) and Caspase-1 proteins in FCX of rats; and elevated the levels of interleukin (IL)-1 β and IL-18 in serum and FCX ($P < 0.05$). We also found that serum lactate was significantly and positively correlated with serum pyruvate and glutamate levels, LDH activity, and IL-1 β and IL-18 levels in SD rats. These data suggest that serum lactate is abnormally elevated in both SCZ patients and NMDA receptor inhibition models. Furthermore, there may be a link between MK-801-induced cognitive impairment, elevated serum lactate, and aberrant activation of the NLRP3/Caspase-1/IL-1 β inflammatory pathway in rats. Modulation of serum/brain lactate levels and the NLRP3/Caspase-1/IL-1 β pathway in SCZ patients may serve as potential targets for improving cognitive impairment in SCZ.

Clinical trial registration number: ChiCTR2400091186.

Keywords Schizophrenia, Lactate, MK-801, NLRP3, Caspase-1

Schizophrenia (SCZ) is considered to be one of the most common psychiatric disorders, with mortality rates in patients ranging from two to four times higher than in the healthy population¹. And both environmental and genetic factors can increase the risk of developing schizophrenia. Excessive glutamate during the early stages of life may cause excitotoxicity and structural brain defects². This disorder is clinically characterized by the presence of positive and negative symptoms and cognitive dysfunction. And cognitive deficits mainly include impaired memory, attention deficits and executive dysfunction³. Cognitive dysfunction is considered to be a core feature of SCZ⁴, and one of the main causes of poor functional outcomes in patients. However, the etiology and pathogenesis of SCZ remain complex and not fully understood. With the rise and development of animal models in the field of mental disorders, breakthroughs have been made in the exploration of the pathogenesis

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of schizophrenia and the mechanism of action of antipsychotic drugs. Currently, animal models used to simulate schizophrenia mainly include pharmacological models, neurodevelopmental models and genetic models⁵. A pharmacological model of schizophrenia with N-methyl-D-aspartate (NMDA) receptor (NMDAR) hypofunction can summarize the range of positive, negative, and cognitive symptoms of schizophrenia. The pharmacological approaches to induce NMDAR hypofunction include phencyclidine (PCP), ketamine (KET), and dizocilpine (MK-801)⁶. The NMDAR, an ionotropic glutamate receptor, plays a critical role in glutamatergic neurotransmission, synaptic plasticity, and local rhythmic activity⁷. And, it is also involved in a variety of brain physiological processes, such as learning, memory, and long-term neuronal enhancement. Faure A et al. found that after acute intervention with ketamine and MK-801 in C57BL/6 J mice, these mice generally exhibited communication and socialization deficits⁸.

Studies have shown that the pathological mechanisms of schizophrenia are related to a variety of factors such as impaired energy metabolism, mitochondrial dysfunction, neuroinflammation, redox dysregulation, and NMDAR hypofunction. These pathological processes create a positive feedback loop in the brain centered on oxidative stress. They interact and amplify each other, which is the basis for the dysfunctions that occur in patients' sensory, cognitive, emotional and social function^{9–11}. Glausier JR et al. found significant changes in the expression of genes related to mitochondrial function in the prefrontal gray matter of patients with schizophrenia. A total of 871 mitochondria-related genes were detected, of which 83% (296 genes) were downregulated and 17% (60 genes) were upregulated. The authors concluded that the down-regulated genes, which accounted for 83%, were associated with reduced brain energy demand and decreased neuronal activity¹². Interestingly, lactate is the most important source of energy for the brain, L-lactate is the major form of lactate in living organisms. Brain lactate is mainly produced by astrocyte glycolysis, and it can subsequently be shuttled from astrocytes to neurons via the astrocyte-neuron lactate shuttle (ANLS) to energize neurons^{13,14}. In addition, lactate in peripheral blood can also cross the blood-brain barrier (BBB) to enter the brain. Importantly, under physiological conditions, the BBB exerts a limited regulatory effect on this process¹⁵. Proper levels of L-lactate in the brain are essential for normal learning, memory, and behavior¹⁶. Previous studies have confirmed that abnormal lactate levels are closely linked to various psychiatric disorders. An animal study showed that lactate levels were significantly elevated in the brain homogenates of mouse models of autism, bipolar disorder, and schizophrenia¹⁷, and that there was a significant negative correlation between elevated lactate levels and decreased PH values. In our published article¹⁸, we also extensively discussed lactate metabolism abnormalities in SCZ patients and animal models. In addition, Rahman T et al. also found that increased neuroinflammation in the cerebral cortex of SCZ patients was associated with lower levels of the GluN1 subunit of the NMDA receptor¹⁹. During the neuroinflammatory response, hyperactivation of microglia can lead to the production of large amounts of proinflammatory cytokines, increase astrocyte activity, and promote the release of glutamate, which can have neurotoxic effects on the central nervous system²⁰. Neuroinflammation is closely linked to the activation of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome²¹. The NLRP3 inflammasome is made up of the NLRP3 protein, apoptosis-associated speck-like protein (ASC), and pro-Caspase-1²². When NLRP3 inflammasome is activated, it promotes the release of cytokines such as interleukin (IL) -1 β , IL-18, IL-2, IL-6, tumour necrosis factor α (TNF- α), high mobility group box-1 (HMGB1), inducible nitric oxide synthase, cyclooxygenase-2, and type I interferon - γ ^{23,24}. We have thoroughly discussed the aberrant activation of the NLRP3/Caspase-1/IL-1 β pathway in both SCZ patients and animal models in our published article²⁵. However, it remains uncertain whether there is a link between lactate metabolism dysfunction and the aberrant activation of the NLRP3 inflammasome pathway in schizophrenia (see Fig. 1). An in-depth study of the potential link between the two and their possible mechanisms of action has important clinical implications for the diagnosis and treatment of schizophrenia.

Methods

Clinical research

A retrospective study method was used to find and select all schizophrenia patients hospitalized from April 2022 to December 2022 in the electronic medical record system of Yunnan Provincial Psychiatric Hospital, and 142 patients were finally included according to strict inclusion and exclusion criteria (see Fig. 2.). The diagnosis of the patients strictly followed the diagnostic criteria for schizophrenia in the Diagnostic and statistical manual of mental disorders, Fourth edition (DSM-V) in the U.S. To ensure diagnostic accuracy and consistency, the patient's medical record data was reviewed, double-checked, and entered by another master's student with extensive clinical experience. The information involved in the study included the patient's basic condition, number of hospitalizations, length of stay, severity of clinical symptoms, and biochemical test results. The severity of psychiatric symptoms was assessed using the positive and negative syndrome scale (PANSS), which is commonly used in clinical practice. The biochemical tests included serum lactate, lactic dehydrogenase (LDH), fasting blood glucose, triglyceride (TG), and low-density lipoprotein (LDL), and the results of the included biochemical tests were obtained within one week of the patient's admission to the hospital. The patients were 18–80 years old, of any gender, and of Han Chinese ethnicity (There was no significant correlation between cerebrospinal fluid lactate levels and age, gender, and whether or not they smoked²⁶, but there was a significant correlation with ethnicity²⁷). No history of mental retardation or other psychiatric disorders. No history of infection or alcohol and/or psychoactive substance abuse prior to one week. No viral encephalitis, diabetes mellitus, thyroid dysfunction, hypertension, fatty liver disease, gout, cardiac failure, renal failure, or other medical disorders. Exclude patients who are wheelchair or bedridden or have eating disorders. Exclude female patients who are pregnant or breastfeeding. Exclude patients who have used or are using hypoglycemic, lipid-lowering or antihypertensive drugs.

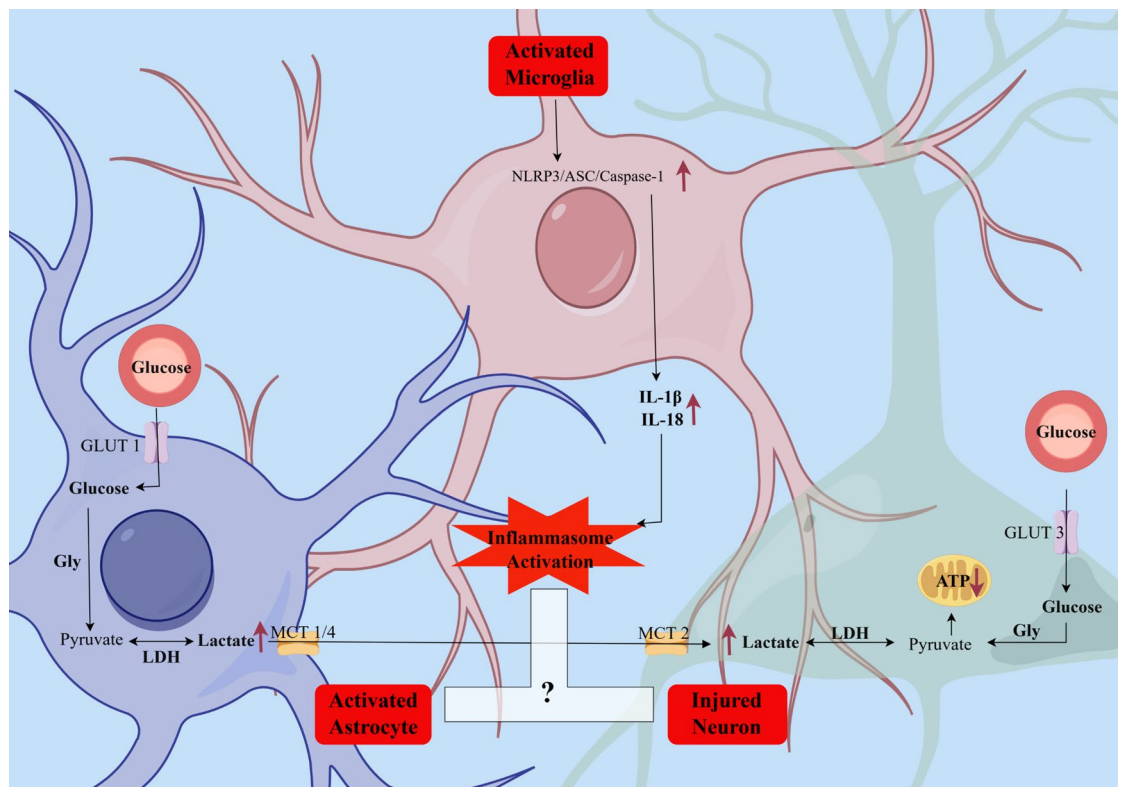


Fig. 1. Astrocyte-neuron lactate shuttle disorders and abnormal activation of microglia in schizophrenia. Astrocyte-neuron lactate shuttle disorders in schizophrenia can lead to abnormal accumulation of lactate in the brain, while abnormal activation of microglia can further lead to abnormal activation of the NLRP3/ASC/Caspase-1/IL-1 β inflammatory pathway^{18,25}. This figure was created by Figdraw2.0, <https://www.figdraw.com>.

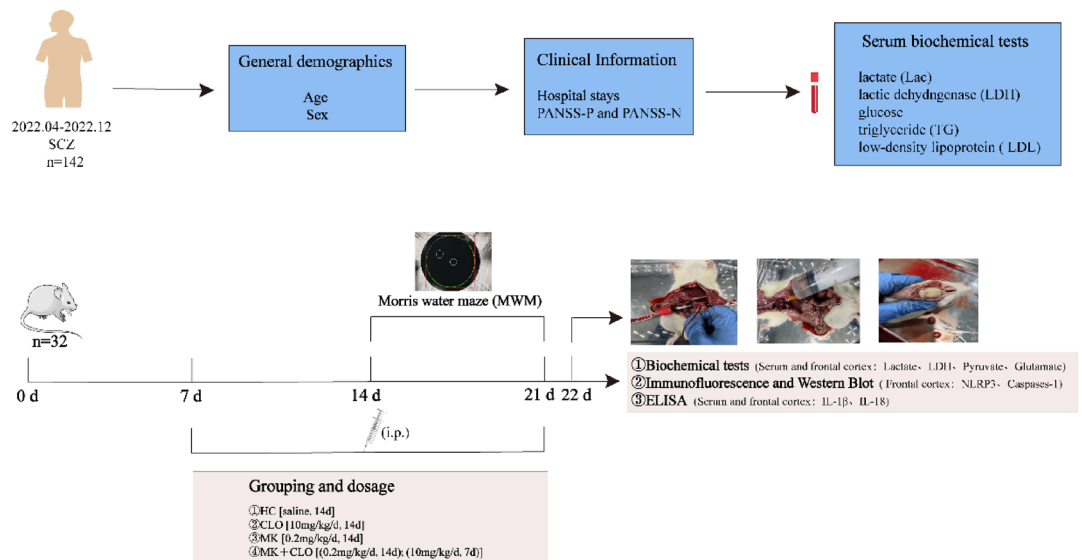


Fig. 2. Flowchart.

Ethics approval and China clinical trial registration

The clinical retrospective study was conducted in compliance with China's Administrative Measures for Clinical Trials. This study protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Medical Ethics Committee of Yunnan Provincial Psychiatric Hospital (Ethics Approval No. YNJS-20241015-001). According to Chinese law and the Medical Ethics Committee of Yunnan Provincial

Psychiatric Hospital, there was no necessity to retroactively obtain informed consent due to the importance of this study subject and the retrospective study design [we only analyzed the available medical record data for correlations and did not directly intervene in the patient's course of treatment. In addition, this researcher can ensure that patients' personal information is not disclosed. Taken together, this study meets the second condition of the waiver of informed consent for Yunnan Provincial Psychiatric Hospital "research that utilizes medical records and biospecimens obtained in previous studies (secondary use of research medical records/biospecimens)"]. In addition, this study was registered with the China Clinical Trial Registry (ChiCTR, <https://www.chictr.org.cn/>, registration number: ChiCTR2400091186, PID: 247916).

Animal experiments

Animals and groups

The study protocols were approved by the Ethical Review Committee for Animal Experiments of Kunming Medical University (Approval No.: kmmu20230164). We can confirm that all methods were performed in accordance with the China's Regulations on the Administration of Laboratory Animals and Measures for the Administration of Ethical Review of Laboratory Animals, as well as the International Council for Laboratory Animal Science (ICLAS), and in compliance with the ARRIVE guidelines. Thirty-two male SD rats were purchased from Kunming Medical University (KMMU) and housed in a pathogen-free animal house at KMMU with a 12/12 h light/dark cycle, and medication was administered after 1 week of acclimatization. All rats were randomly and equally divided into four groups (8 rats in each group), i.e., MK (MK-801) group, MK + CLO (MK-801 + clozapine) group, healthy control (HC) group, and CLO (clozapine) group [Chronic intraperitoneal injection (i.p.) of CLO significantly increased the postmortem lactic acid level in adult male SD rats²⁸. The MK group was induced with MK-801 (Sigma, 0.2 mg/kg/d, 14 days, i.p.) in the SCZ animal model²⁹. The MK + CLO group was injected with clozapine (10 mg/kg/d, 7 days, i.p., Shanghai Jingfeng Biotechnology Co., Ltd.) on the 8th day of MK-801 injection (0.2 mg/kg/d, 14 days), and the clozapine was injected 15 min after MK-801 injection. The HC group received an intraperitoneal injection of saline for 14 days and the CLO group received an intraperitoneal injection of clozapine (10 mg/kg/d) for 14 days. Behavioral tests were performed on days 8–14 of drug administration. The Flowchart of the experiment is shown in Fig. 2.

Morris water maze (MWM) test

MWM can be used to assess spatial learning and memory in rats under different experimental conditions³⁰. The water maze was 120 cm in diameter and 45 cm in height, filled with 25 cm of water ($25 \pm 1^\circ\text{C}$) and divided into four quadrants. Finding platform training was performed on the 7th day of drug administration, the platform was fixed in one quadrant (the fourth quadrant), the platform was 1 cm above the water surface, rats were randomly placed from the four quadrants tightly attached to the wall of the water tank (the fourth quadrant), and the time taken by the rats to climb up the platform was recorded for a total of 4 times/day, and the average value was taken. The localization navigation experiment was conducted on days 8–14 of drug administration by placing the platform 1 cm below the water surface in the four quadrants, placing rats randomly from the four quadrants immediately adjacent to the wall of the aquarium (fourth quadrant), and recording the time required for the rats to climb onto the platform for 4 times/day for 7 d, and taking the average value. The spatial exploration experiment was conducted on the 14th day, the platform in the pool was withdrawn 1 h after the end of the localization navigation test, and the SD rats were put into the tank from the original entry point so that the rats were facing the tank wall, and the number of times the rats traversed the platform and the swimming time in the target quadrant (the fourth quadrant) inside the platform were recorded in 60 s. The average time was taken 4 times/day for 7 d consecutively.

Serum and brain tissue collection

After the behavioral test, rats were gas-anesthetized with isoflurane, and blood was collected from the abdominal aorta using blood collection tubes without anticoagulant or procoagulant, centrifuged, and the upper serum and lower precipitate were separately preserved and stored at -80°C for use in serum biochemical assays, including assays for serum lactate, pyruvate, and glutamate levels and lactate dehydrogenase activity. Four of each group were randomly selected for brain tissue perfusion sampling, 4% paraformaldehyde fixation for immunofluorescence detection. The remaining 4 portions of each group were subjected to fresh sampling of brain tissue, quick-frozen in liquid nitrogen and stored at -80°C for biochemical detection (lactate, pyruvate, and glutamate levels and lactate dehydrogenase activity), WB and ELISA. The rats were euthanized immediately after sampling by cervical dislocation to ensure rapid loss of life and minimize pain and discomfort. The kits used for biochemical testing include lactate test kits (Nanjing Jiancheng Bioengineering Institute A019-2-1), glutamate test kits (Nanjing Jiancheng Bioengineering Institute A074-1-1), pyruvate assay kits (Nanjing Jiancheng Bioengineering Institute A081-1-1), and lactate dehydrogenase (LDH) kits (Nanjing Jiancheng Bioengineering Institute 020-1-2).

Immunofluorescence

Brain sections were placed in a constant temperature oven at 60°C for at least 30 min. Sections were routinely dewaxed to water. Rinsing in 0.01 mol/L Tris-Buffer saline (TBS) solution, 5 min \times 3 times; Antigen repair, citrate at pH 6.0, microwave treatment on high for 20 min. Next, 0.01 mol/L PBS solution rinsing, 5 min \times 3 times; After blotting the water around the sections with filter paper, a circle was drawn around the sections with a histochemical pen to prevent liquid leakage during subsequent staining; 5% sheep serum containment, 37°C , 1 h; Shake off the excess sealing liquid, add 2% sheep serum diluted with the appropriate concentration of primary antibody including anti-NLRP3 (1:100; bioss), anti-Caspase-1 (1:100; bioss), and refrigerate at 4°C overnight. Then, rinse with 0.01 mol/L PBST solution for 5 min \times 5 times. Add secondary antibody including CY3-labeled goat anti-rabbit IgG (1:800; Servicebio) and incubate at 37°C for 60 min. Finally, rinse with 0.01 mol/L PBST

for 5 min×3 times. Seal the film with fluorescent sealer containing DAPI (ZSGB-BIO) and scan holographically (nuclei were blue and antibody positive expression was red) and analyzed by Image J software.

Western blot (WB)

The rats were put to death in compliance with animal ethics, and four randomly selected from each group were subjected to fresh sampling of brain tissue, which was preserved in liquid nitrogen until further processing. Protein blotting analysis was performed using 50 mg of prefrontal cortex tissue. GAPDH was chosen as an internal control to ensure equal amounts of protein. Densitometric quantification of the bands was performed using Image J software. The primary antibodies used in the experiment and their dilution ratios: Caspase-1 (proteintech 22915-1-AP, 1:2000), NLRP3 (proteintech, 68102-1-Ig, 1:2000), GAPDH (ZSGB-BIO TA-08, 1:2000). The secondary antibodies used in the experiment and their dilution ratios: generalized secondary antibody (Servicebio GB23303, 1: 4000), generalized secondary antibody (abmart M21001S, 1: 4000).

Enzyme linked immunosorbent assay (ELISA)

The quantitatively analyzed of IL-1β, IL-18 in the serum and prefrontal cortex was carried out using ELISA kit according to the manufacturer's instructions, including Rat Interleukin 1β (IL-1β) ELISA Kit (Keqiao Biotechnology KQ112342) and Rat Interleukin 18 (IL-18) ELISA Kit (Keqiao Biotechnology KQ112340). The concentration was calculated according to the corresponding standard curve. Duplication was set for each sample.

Statistical analysis

All statistical analyses were performed by SPSS 19.0 (IBM, USA). Measures that conformed to normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$), and those that did not conform to normal distribution were expressed as M (P25, P75). For measures that conformed to normal distribution with homogeneous variance, independent samples t-test was used for between-group comparisons; for measures that conformed to normal distribution with heterogeneous variance, independent samples t' test was used for between-group comparisons; and for data that did not conform to normal distribution, Mann-Whitney U-test was used for between-group comparisons in the nonparametric test for independent samples. Count data were expressed as rates or component ratios, and the Pearson chi-square test was used for comparison between groups. For correlation analysis, Pearson correlation analysis was used for information that satisfied normal distribution and met the test of chi-square, and Spearman correlation analysis was used for severely skewed data or data with noncontinuous variables. The level of all tests was set at 0.05, with $P < 0.05$ being considered a statistically significant difference. Graph Pad Prism 5.0 biostatistics software was used for graphing.

Results

Analysis of serum lactate test results in patients with schizophrenia

142 patients with schizophrenia were included in the study and their mean age was 46.42 ± 16.36 years. The mean serum lactate value of the patients when they were first admitted to the hospital was 2.71 ± 0.65 mmol/L, and the normal value range of lactate from the lactate testing instrument was 1.33–1.78 mmol/L. The serum lactate level of the patients when they were first admitted to the hospital was analyzed by using a one-sample t-test, and the result showed that serum lactate level of the patients with schizophrenia was significantly higher than the normal range ($T = 17.043, P < 0.000$). In addition, we found significant positive correlations between serum lactate levels and patients' age ($r = 0.211, P < 0.05$), LDL ($r = 0.196, P < 0.05$), glucose ($r = 0.157, P > 0.05$), triglycerides ($r = 0.352, P < 0.001$), and hospital stays ($r = 0.243, P < 0.01$) (Tables 1 and 2).

In all patients with schizophrenia included in this study, there was a significant positive correlation between the age of the patients and the hospital stays ($r = 0.496, P < 0.01$), triglycerides and low-density lipoprotein ($r = 0.426, P < 0.01$) were also significantly positively correlated (Table 1). Partial correlation analysis revealed that after controlling for the covariate of age, there was a significant positive correlation between patients' serum lactate concentration and hospital stays ($pr = 0.180, P < 0.033$) (Table 3; Fig. 3A); and after controlling for serum

	Age	Hospital stays	Lactate	LDH	Glucose	TG	LDL	PANSS-P	PANSS-N
Age	1								
Hospital stays	0.496**	1							
Lactate	0.223**	0.243**	1						
LDH	0.141	−0.035	0.030	1					
Glucose	0.261**	0.100	0.157	0.205*	1				
TG	0.067	−0.040	0.352**	0.127	0.234**	1			
LDL	0.106	0.024	0.214*	0.144	0.114	0.426**	1		
PANSS-P	−0.257**	0.092	−0.141	0.068	0.007	−0.022	0.087	1	
PANSS-N	0.166*	0.307**	0.110	0.047	0.099	0.071	0.122	0.348**	1

Table 1. Spearman's correlation analysis between general demographic data, clinical variables and biochemical indicators. Abbreviations: lactate dehydrogenase, LDH; triglyceride, TG; low-density lipoprotein, LDL; positive and negative syndrome scale-Positive Symptom, PANSS-P; positive and negative syndrome scale-Negative Symptom, PANSS-N. Note: * $P < 0.05$, ** $P < 0.01$.

	Lactate	
	<i>r</i>	<i>P</i>
Age (Pearson)	0.211	0.012
LDL (Pearson)	0.196	0.019
Hospital stays (Spearman)	0.243	0.004
PANSS-P (Spearman)	−0.141	0.093
PANSS-N (Spearman)	0.110	0.193
LDH (Spearman)	0.030	0.721
Glucose (Spearman)	0.157	0.062
TG (Spearman)	0.352	0.000

Table 2. Correlation analysis of serum lactate at admission with clinical variables and biochemical tests. Abbreviations: low-density lipoprotein, LDL; positive and negative syndrome scale-Positive Symptom, PANSS-P; positive and negative syndrome scale-Negative Symptom, PANSS-N; lactate dehydrogenase, LDH; triglyceride, TG.

	Lactate	
	<i>pr</i>	<i>P</i>
Hospital stays (Age)	0.180	0.033
LDL (TG)	0.079	0.351
TG (LDL)	0.313	0.000
Age (Hospital stays)	0.094	0.268

Table 3. Partial correlation analysis between serum lactate at admission and length of hospitalization, glucose, low-density lipoprotein, triglycerides, and age in patients. Abbreviations: low-density lipoprotein, LDL; triglyceride, TG. Note: (), control variable; *pr*, partial correlation coefficient.

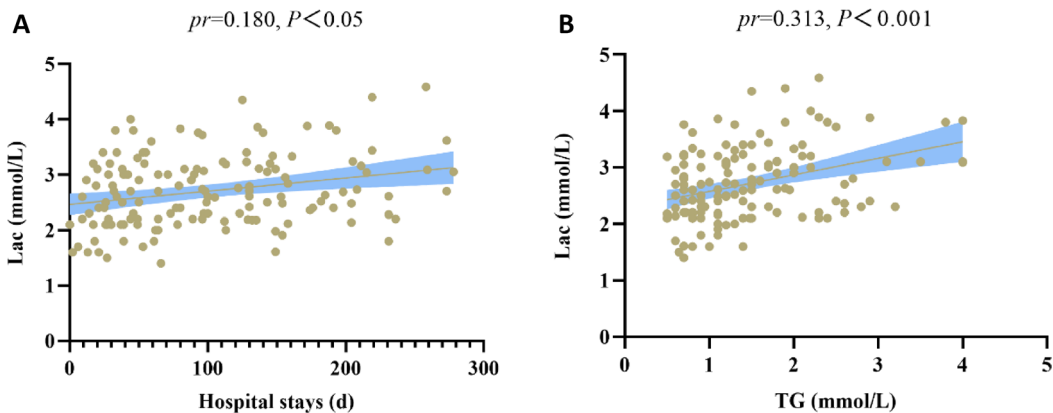


Fig. 3. (A) Correlation between serum lactate at admission and hospital stays in SCZ patients. (B) Correlation between serum lactate and serum triglycerides at admission in patients with SCZ. Note: *pr*, the partial correlation coefficient. Abbreviations: lactate, Lac; triglyceride, TG.

LDL, there was a significant positive correlation between patients' serum lactate concentration and serum triglyceride level ($pr = 0.313, P < 0.001$) (Table 3; Fig. 3B).

Analysis of the detection results of MWM localization and navigation stages in each group of rats
Escape latency was tested in four groups of rats during the 7-day orientation navigation phase using the Morris water maze. The results showed that the main effect of testing time was significant ($\text{Wald}\chi^2 = 58.801, P_{\text{time}} < 0.001$), i.e., the difference of the time factor (day) was statistically significant; the main effect between the treatment groups was significant ($\text{Wald}\chi^2 = 15.127, P_{\text{group}} < 0.01$), i.e., the factor of the subgroup (group) was statistically significant, and there was an interaction between group and time ($\text{Wald}\chi^2 = 159.781, P_{\text{interaction}} < 0.001$). There was a significant difference in avoidance latency between groups on day two ($P < 0.01$) and day five ($P < 0.05$) (Fig. 4A). However, the escape latency was significantly longer in the MK group than in the HC group only on the fifth day ($P < 0.05$). The difference in the latency to escape on the fifth day between the MK

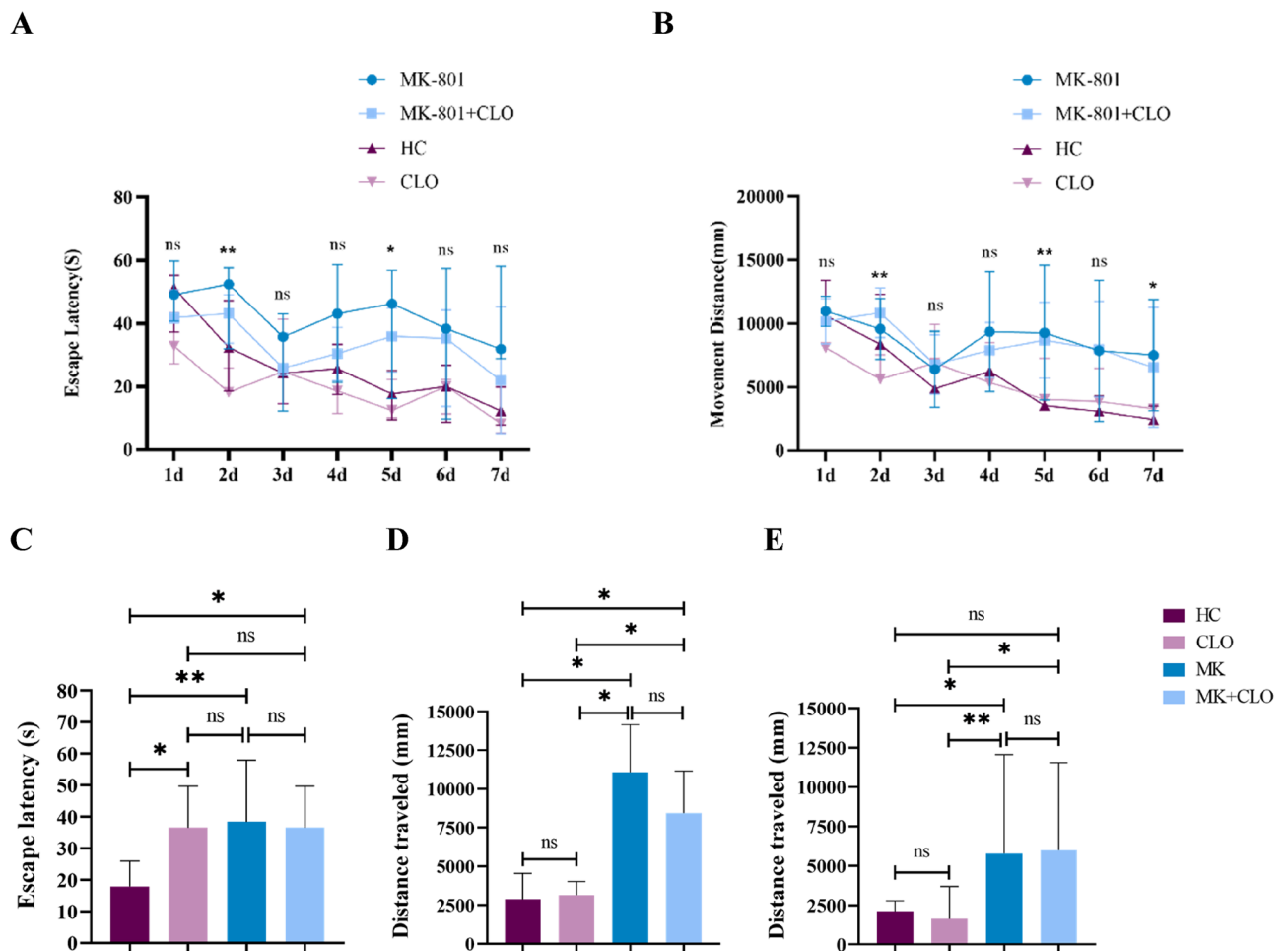


Fig. 4. Morris water maze (MWM) test. **(A)** The escape latency during the 7 days of training period. **(B)** The distance traveled during the 7 days of training period. **(C)** The escape latency on the day 5 of training period. **(D)** The distance traveled on the day 5 of training period. **(E)** The distance traveled on the day 7 of training period. Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P > 0.05$. Abbreviations: healthy control group, HC; clozapine group, CLO; MK-801 group, MK; MK-801 + clozapine group, MK + CLO.

and CLO groups was not statistically significant ($P > 0.05$). Day 5 escape latency was significantly longer in the MK + CLO group than in the HC group ($P < 0.05$). However, we did not find statistical differences ($P > 0.05$) between the MK + CLO and CLO groups and between the MK + CLO and MK groups during the Day 5 escape latency (Fig. 4C).

Four groups of rats were tested for distance traveled during the 7-day orientation voyage phase. The results showed that the main effect of testing time was significant ($\text{Wald}\chi^2 = 73.481$, $P_{\text{time}} < 0.001$), i.e., the difference of the time factor (day) was statistically significant; the main effect between treatment groups was significant ($\text{Wald}\chi^2 = 20.029$, $P_{\text{group}} < 0.001$), i.e., the factor of subgroup (group) was statistically significant; and there was an interaction between group and time ($\text{Wald}\chi^2 = 112.876$, $P_{\text{interaction}} < 0.001$). There was a significant difference in the distance traveled between the groups on the second, fifth and seventh days at $P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively (Fig. 4B). On days 5 and 7, the distance traveled was significantly longer in the MK group than in the HC and CLO groups ($P < 0.05$). The difference in distance traveled between the MK + CLO group and the MK group on days 5 and 7 was not statistically significant ($P > 0.05$). The distance traveled on day 5 was significantly longer in the MK + CLO group than in the HC group ($P < 0.05$); however, we did not find a significant difference in the distance traveled on day 7 between these two groups ($P > 0.05$). The distance traveled on days 5 and 7 was significantly longer in the MK + CLO group than in the CLO group ($P < 0.05$) (Fig. 4D and E). In addition, the four groups of rats were tested for locomotor speed during the 7-day localization voyage phase. The results showed that the grouping factor (group) was not statistically significant ($\text{Wald}\chi^2 = 1.685$, $P_{\text{group}} > 0.05$), so no further analysis was done.

Analysis of the results of serum and frontal cortex (FCX) biochemical tests in each group of rats

We found that lactate levels in serum and FCX were significantly higher in the MK group than in the HC group ($P < 0.001$) (Fig. 5A and E). Lactate levels were significantly higher in serum ($P < 0.01$) and FCX ($P < 0.001$) in the MK group compared with the CLO group (Fig. 5A and E). Lactate levels in FCX were significantly lower

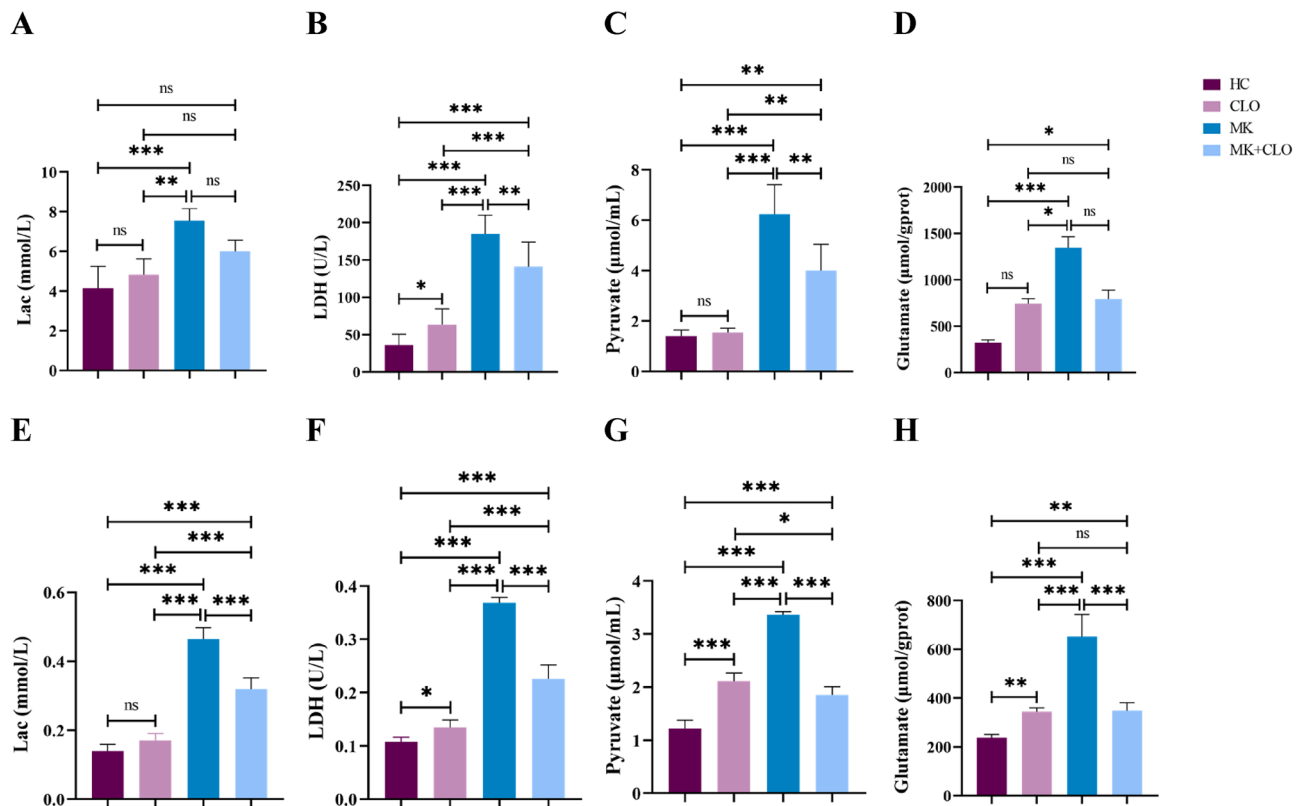


Fig. 5. Analysis of biochemical indices. (A) Statistical diagram of Lac in the serum. (B) Statistical diagram of LDH in the serum. (C) Statistical diagram of Pyruvate in the serum. (D) Statistical diagram of Glutamate in the serum. (E) Statistical diagram of Lac in the FCX. (F) Statistical diagram of LDH in the FCX. (G) Statistical diagram of Pyruvate in the FCX. (H) Statistical diagram of Glutamate in the FCX. Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P > 0.05$. Abbreviations: healthy control group, HC; clozapine group, CLO; MK-801 group, MK; MK-801 + clozapine group, MK + CLO. lactate, Lac; lactate dehydrogenase, LDH.

in the MK + CLO group than in the MK group ($P < 0.001$); however, we did not find a significant difference in serum lactate levels between these two groups ($P > 0.05$) (Fig. 5A and E). In addition, lactate levels in FCX were significantly higher in the MK + CLO group than in the CLO group ($P < 0.001$); however, there was no significant difference in serum lactate between these two groups ($P > 0.05$) (Fig. 5A and E). LDH activity was significantly higher in serum and FCX in the MK group compared with the HC and CLO groups ($P < 0.001$) (Fig. 5B and F). LDH activity in serum ($P < 0.01$) and FCX ($P < 0.001$) was significantly lower in the MK + CLO group than in the MK group (Fig. 5B and F). In addition, LDH activity in serum and FCX was significantly higher in the MK + CLO group than in the CLO group ($P < 0.001$) (Fig. 5B and F). Pyruvate levels in serum and FCX were significantly higher in the MK group compared with the HC and CLO groups ($P < 0.001$) (Fig. 5C and G). Pyruvate levels in serum ($P < 0.01$) and FCX ($P < 0.001$) were significantly lower in the MK + CLO group than in the MK group (Fig. 5C and G). In addition, the level of pyruvate in serum was significantly higher in the MK + CLO group than in the CLO group ($P < 0.01$) (Fig. 5C and G). However, the level of pyruvate in FCX of the MK + CLO group was significantly lower than that of the CLO group ($P < 0.05$) (Fig. 5C and G). Serum and FCX glutamate levels were significantly higher in the MK group than in the HC group ($P < 0.001$) (Fig. 5D and H). Serum ($P < 0.05$) and FCX ($P < 0.001$) glutamate levels were significantly higher in the MK group compared to the CLO group (Fig. 5D and H). FCX glutamate levels were significantly lower in the MK + CLO group compared to the MK group ($P < 0.001$); however, we did not find a significant difference in serum glutamate levels between these two groups ($P > 0.05$) (Fig. 5D and H). However, we did not find significant differences in glutamate levels in serum and FCX between the MK + CLO and CLO groups ($P > 0.05$) (Fig. 5D and H). These results indicate that MK-801-induced schizophrenia model has abnormally elevated levels of lactate, pyruvate, glutamate and LDH activity in serum/FCX. Clozapine ameliorated the abnormally elevated levels of lactate and glutamate in FCX, as well as the abnormally elevated pyruvate levels and LDH activity in serum and FCX.

Analysis of NLRP3 and Caspase-1 protein levels in the frontal cortex of rats in each group

Immunofluorescence results showed that the relative fluorescence intensities of NLRP3 and Caspase-1 were significantly higher ($P < 0.001$) in FCX of the MK group compared with those of the HC group and CLO group (Fig. 6A, B, C and D). The relative fluorescence intensities of NLRP3 and Caspase-1 in FCX of the MK + CLO group were significantly lower than those of the MK group ($P < 0.001$) (Fig. 6A, B, C and D). The relative

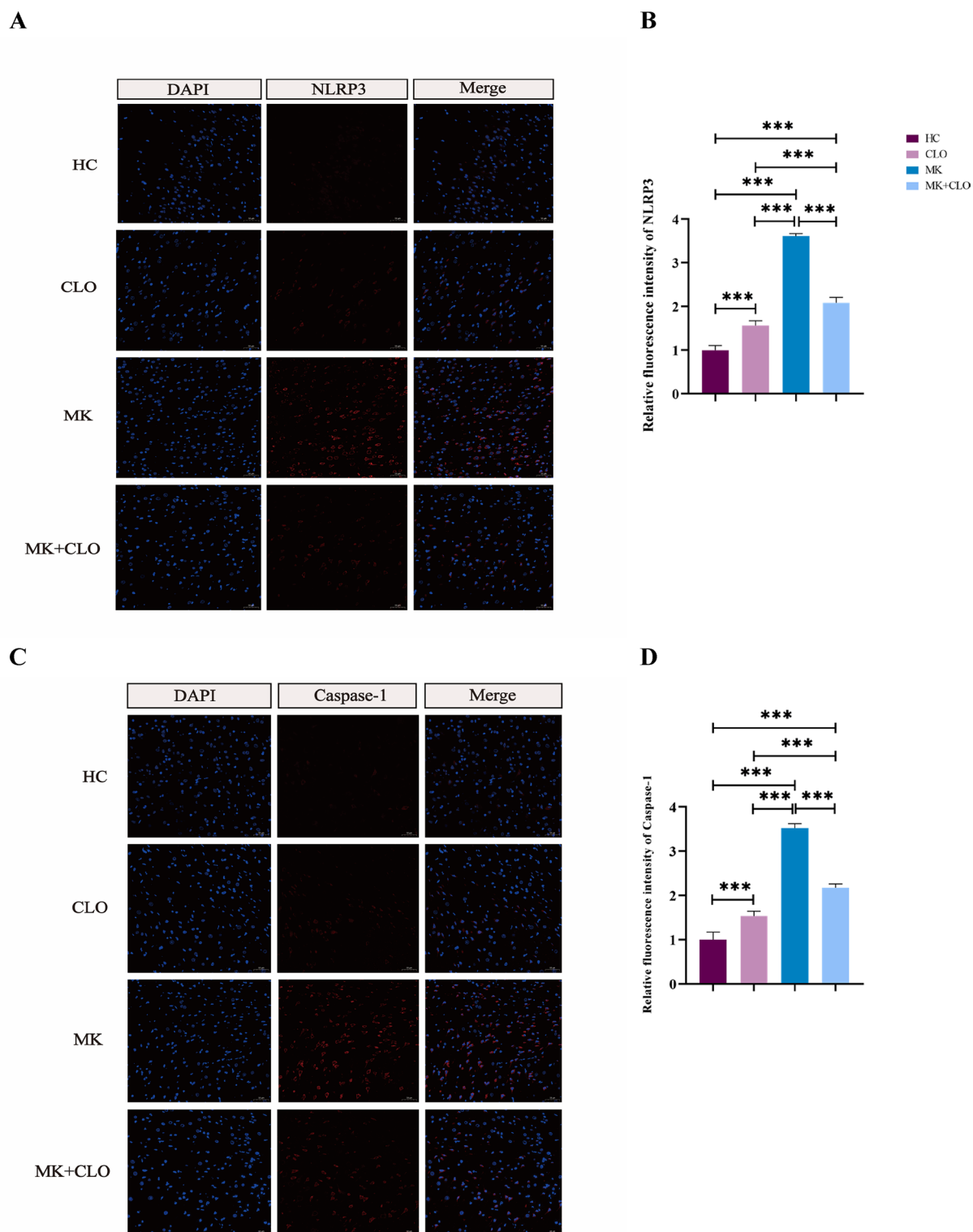


Fig. 6. (A) Representative immunofluorescent (IF) staining of NLRP3 (red) with DAPI (blue) in the FCX. Scale bar = 50 μ m. (B) IF statistical diagram of NLRP3 in the FCX. (C) Representative immunofluorescent staining of Caspase-1 (red) with DAPI (blue) in the FCX. Scale bar = 50 μ m. (D) IF statistical diagram of Caspase-1 in the FCX. (E) Representative western blot images of NLRP3 and Caspase-1 in the FCX, GAPDH was used as a loading control. WB quantitative analysis of NLRP3 (F) and Caspase-1 (G) in the FCX. Quantitative statistics of IL-1 β levels in the serum (H) and FCX (I) detected by ELISA. Quantitative statistics of IL-18 levels in the serum (J) and prefrontal cortex (K) detected by ELISA. Note: * P < 0.05, ** P < 0.01, *** P < 0.001, ns P > 0.05. Abbreviations: healthy control group, HC; clozapine group, CLO; MK-801 group, MK; MK-801 + clozapine group, MK + CLO.

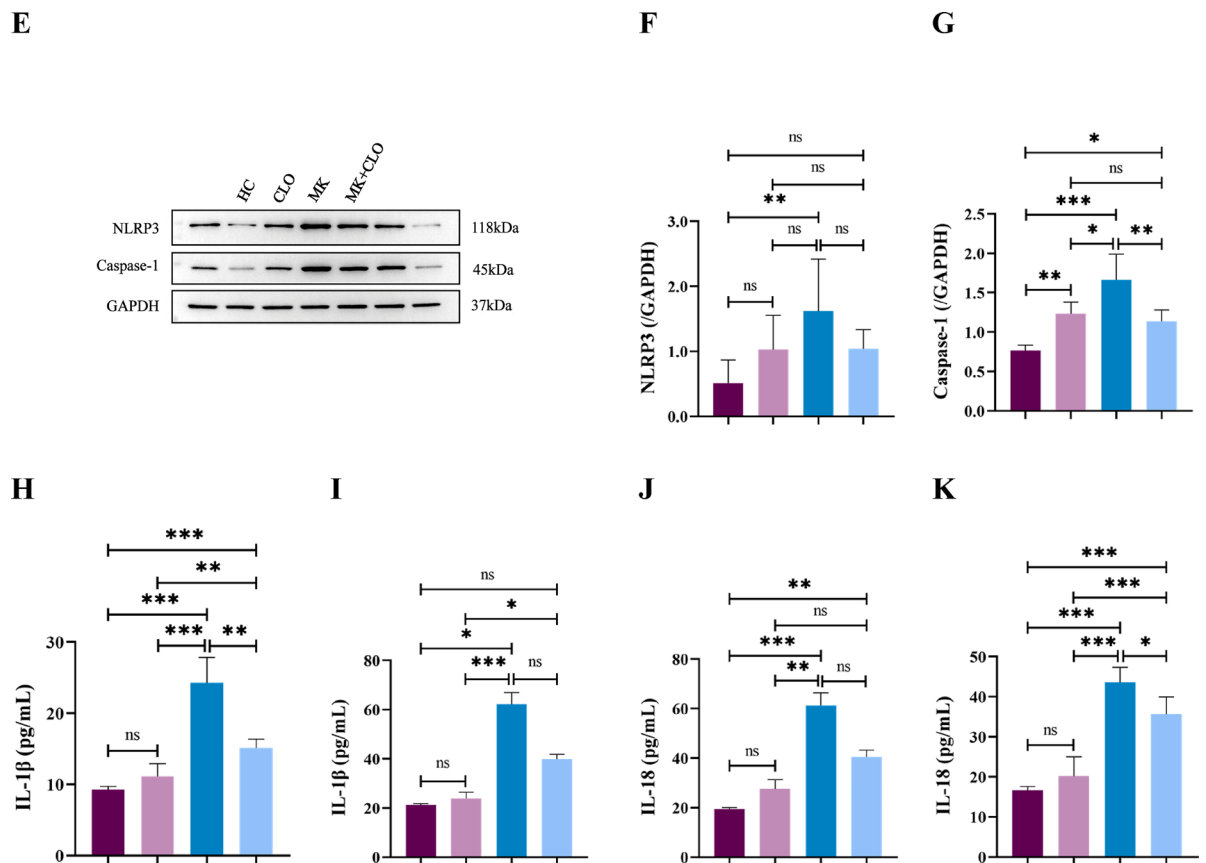


Fig. 6. (continued)

fluorescence intensities of NLRP3 and Caspase-1 in FCX were significantly higher ($P < 0.001$) in the MK + CLO group compared to the HC and CLO groups (Fig. 6A, B, C and D).

In addition, the protein expression levels of NLRP3 ($P < 0.01$) and Caspase-1 ($P < 0.001$) were significantly elevated in FCX from the MK group compared with the HC group (Fig. 6E, F and G). The protein expression level of Caspase-1 in FCX of the MK group was significantly higher than that of the CLO group ($P < 0.05$); however, we did not find a significant difference in the protein expression level of NLRP3 in FCX of these two groups ($P > 0.05$) (Fig. 6E, F and G). The protein expression level of Caspase-1 in FCX of the MK + CLO group was significantly lower than that of the MK group ($P < 0.01$); however, we did not find a significant difference in the protein expression level of NLRP3 in FCX of these two groups ($P > 0.05$) (Fig. 6E, F and G). The protein expression level of Caspase-1 in FCX of the MK + CLO group was significantly higher than that of the HC group ($P < 0.05$); however, we did not find a significant difference in the protein expression level of NLRP3 in FCX of these two groups ($P > 0.05$) (Fig. 6E, F and G). We did not find significant differences in protein expression of NLRP3 and Caspase-1 in FCX between the MK + CLO and CLO groups ($P > 0.05$) (Fig. 6E, F and G). These results support that the protein levels of NLRP3 and Caspase-1 were abnormally elevated in the FCX of the SCZ model induced by MK-801, and that clozapine could ameliorate the abnormally elevated protein levels of Caspase-1.

Levels of IL-1β and IL-18 in serum and frontal cortex of rats in each group

IL-1β levels in serum ($P < 0.001$) and FCX ($P < 0.05$) were significantly higher in the MK group compared with the HC group (Fig. 6H and I). IL-1β levels in serum and FCX were significantly higher in the MK group than in the CLO group ($P < 0.001$) (Fig. 6H and I). Serum IL-1β levels in the MK + CLO group were significantly lower than those in the MK group ($P < 0.001$); however, we did not find a significant difference in IL-1β levels in FCX between these two groups ($P > 0.05$) (Fig. 6H and I). In addition, we found significantly higher IL-1β levels in serum ($P < 0.01$) and FCX ($P < 0.05$) in the MK + CLO group than in the CLO group (Fig. 6H and I). IL-18 levels in serum and FCX were significantly higher in the MK group compared to the HC group ($P < 0.001$) (Fig. 6J and K). IL-18 levels in serum ($P < 0.01$) and FCX ($P < 0.001$) were significantly higher in the MK group than in the CLO group (Fig. 6J and K). The level of IL-18 in FCX of the MK + CLO group was significantly lower than that of the MK group ($P < 0.05$); however, we did not find a significant difference in the serum levels of IL-18 between these two groups ($P > 0.05$) (Fig. 6J and K). In addition, we found that IL-18 levels in FCX of the MK + CLO group were significantly higher than those of the CLO group ($P < 0.001$); however, we did not find a significant difference in IL-18 levels in serum of these two groups ($P > 0.05$) (Fig. 6J and K). These results indicate that IL-1β and IL-18 levels were abnormally elevated in serum/FCX in the MK-801-induced schizophrenia model.

Clozapine treatment improved the abnormally elevated IL-1 β levels in serum and abnormally elevated IL-18 levels in FCX.

Analysis of the correlation between serum lactic acid and other assays in various groups of rats

For the correlation analysis between serum lactate and MWM assays, we did not find significant correlations between serum lactate and escape latency on day five ($r = 0.118$, $P = 0.519$) and distance traveled ($r = 0.307$, $P = 0.087$) (Fig. 7B and D); however, serum lactate was not significantly correlated with escape latency on day two ($r = 0.447$, $P = 0.010$), distance traveled on the second day ($r = 0.497$, $P = 0.004$), and distance traveled on the seventh day ($r = 0.382$, $P = 0.031$) were significantly and positively correlated with each other (Fig. 7A, C and E). Correlation analysis of serum lactate with other serum biochemical indices revealed significant positive correlation between serum lactate and serum levels of LDH activity ($r = 0.744$, $P = 0.000$), pyruvate ($r = 0.765$, $P = 0.000$) and glutamate ($r = 0.694$, $P = 0.000$) (Fig. 7F, G and H). In addition, correlation analysis between serum lactate and serum levels of IL-1 β and IL-18 revealed a significant positive correlation between serum lactate levels and serum IL-1 β ($r = 0.760$, $P = 0.000$) and IL-18 ($r = 0.781$, $P = 0.000$) levels (Fig. 7I and J).

Discussion

Disorders of serum lactate metabolism and abnormalities of glucolipid metabolism in SCZ patients

Our findings showed that serum lactate levels were significantly elevated in patients with schizophrenia ($T = 13.287$, $P < 0.001$). Kim S et al. found higher levels of lactate, glutamate, alanine, serine, and ornithine in patients with chronic schizophrenia compared to the healthy control group³¹. Ravanfar P et al. observed increased lactate and phosphocreatine concentrations in the caudal anterior cingulate cortex of 12 chronic SCZ patients³². Interestingly, lactate levels were also significantly higher in the dorsolateral prefrontal cortex (DLPFC) of postmortem schizophrenic patients ($P < 0.05$)³³. In addition, Halim ND et al. found that lactate levels in the postmortem cerebellum of schizophrenic patients increased by 28% ($P < 0.01$) compared to healthy controls²⁸. They suggested that this disturbance in lactate metabolism may be related to antipsychotic medication rather than primary metabolic disturbances in the prefrontal cortex of the patients. A study by Elmorsy E et al. found no significant difference in arterial blood lactate levels between the schizophrenia patient group and the healthy control group ($P > 0.05$). However, after three months of treatment with antipsychotics (APs), all patients with schizophrenia showed significantly higher arterial blood lactate levels compared to their baseline levels at the time of the first visit³⁴. Furthermore, patients who developed extrapyramidal reactions had significantly higher lactate levels than those treated with the same antipsychotics. Elmorsy E et al. concluded that abnormally elevated blood lactate levels could serve as biomarkers for antipsychotic side effects. In contrast to Halim ND and Elmorsy E et al., Regenold WT et al. found that the mean lactate concentration in the cerebrospinal fluid of patients with schizophrenia (1.61 ± 0.31 mmol/L) was significantly higher by 23% compared to the healthy control group (1.31 ± 0.21 mmol/L). Interestingly, the use of typical/atypical antipsychotic drugs was significantly associated with lower lactate concentrations in the cerebrospinal fluid²⁷. Among the 30 patients (15 with schizophrenia and 15 with bipolar disorder), 5 had a family history of psychiatric disorders. Their mean cerebrospinal fluid lactate was 2.10 ± 0.34 mmol/L. In contrast, patients without a family history had lactate concentrations of 1.59 ± 0.28 mmol/L. This suggests that elevated lactate levels in patients with schizophrenia may be linked to a genetic predisposition rather than being caused by antipsychotic drugs. It has also been shown that patients with schizophrenia exhibit significant changes in mitochondrial number, morphology, and function. These changes promote glycolysis and lead to abnormally high lactate levels³⁵. Furthermore, the theory of tissue hypoxia and acid retention also explains the decreased pH and increased lactate in the brain tissue of schizophrenic patients³⁶.

In contrast to the above findings, Huang JT et al. observed a decrease in lactate levels in the cerebrospinal fluid of patients with first-episode schizophrenia. They attributed this decrease to the depletion of lactate produced by astrocytes in the brain³⁷. Beasley CL et al. found low levels of both lactate and alanine in the ventral internal capsule forelimb of schizophrenia patients, and importantly, only two samples contained detectable APs³⁸. They suggested that this may indicate altered ANLS energy metabolism coupling in the brain of schizophrenia patients. Additionally, the high metabolic activity in the cerebral white matter of these patients may lead to the depletion of lactate produced by astrocytes. However, there are differences in energy metabolism in different brain regions of schizophrenic patients. Importantly, the energy metabolism of patient's brain changes dynamically as the disease progresses. Wijtenburg SA et al. compared schizophrenia patients of shorter illness duration (< 5 years) and longer illness duration (> 5 years). And they found significant differences in lactate, glutamatergic and GABAergic in the DLPFC, hippocampus (HPC), anterior cingulate, and centrum semiovale between the two groups³⁹. They also found significant differences in glutamatergic and GABAergic metabolites between SCZ patients, first-degree relatives, and the HC group in different brain regions. Interestingly, our study also found a significant positive correlation between patients' serum lactate levels and the length of hospitalization ($pr = 0.180$, $P < 0.033$).

The current trend of abnormal lactate metabolism in schizophrenia remains controversial^{26,27,31–33,36,38–40}. Abnormally elevated blood/brain lactate in schizophrenia are recognized by most investigators^{26,27,31–33,36,39,40}, and our study also revealed that serum lactate levels were significantly higher than normal in patients with schizophrenia. Physiologically, blood lactate can cross the BBB to enter and energize the brain, and the BBB has a rate-limiting effect on this process^{15,41}. However, studies have confirmed that damage as well as dysfunction can occur in the BBB in schizophrenia^{42,43}. In addition, disturbances in brain lactate metabolism in schizophrenia may be closely associated with ANLS dysfunction. Raquel Pinacho et al. proposed that abnormal NMDA-dependent glycogenolysis in schizophrenia can lead to a temporary localized energy deficit at the ANLS junction⁴⁴. Sullivan CR et al. suggested that neuronal developmental disorders, hippocampal neuronal apoptosis,

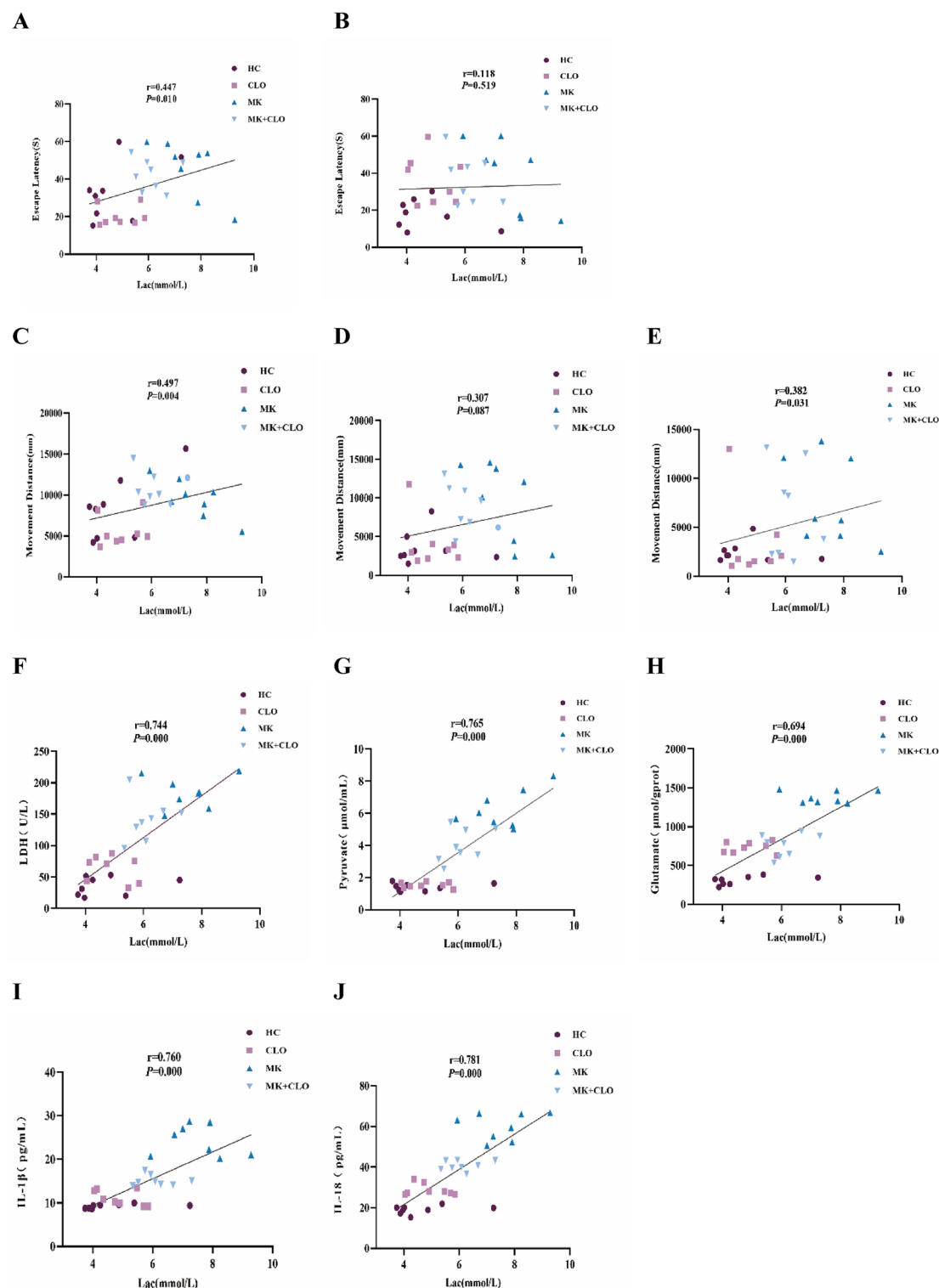


Fig. 7. Correlation analysis of serum lactate with the escape latency on the day 2 (A) and day 5 (B), the movement distance on the day 2 (C) and day 5 (D) and day 7 (E), serum LDH activity (F), serum Pyruvate (G), serum Glutamate (H), serum IL-1 β (I) and serum IL-18 (J). Abbreviations: healthy control group, HC; clozapine group, CLO; MK-801 group, MK; MK-801 + clozapine group, MK + CLO.

reduced expression of mitochondria-related genes in pyramidal neurons, and defects in neuron-specific energy metabolism in the SCZ brain can lead to impaired neuronal lactate utilization, when abnormally activated astrocytes compensatorily produce excess lactate, which can lead to lactate accumulation³³. However, Beasley CL et al. supported that cerebral white matter hypermetabolic activity in patients with schizophrenia can alter

ANLS and lead to lactate being depleted³⁸. The discrepancy between the views of Sullivan CR et al. and Beasley CL et al. suggests that ANLS and impaired lactate metabolism in the SCZ brain may be progressive. Huang X et al. interpreted elevated lactate levels in venous blood of schizophrenic patients as gastric acid retention and tissue-derived hypoxia³⁶. Interestingly, astrocyte survival in hypoxia decreased in a time-dependent manner⁴⁵. In addition, study by Srivastava R et al. has demonstrated that mitochondrial defects in schizophrenic neurons are dynamically changing⁴⁶. Importantly, we have adequately addressed in a published article that disturbances in lactate metabolism in SCZ may perhaps change dynamically with disease progression: Schizophrenic neurons have impaired utilization of lactate, and activated astrocytes compensatorily produce higher levels of lactate. However, It's a dynamic process, and as the disease progresses the body may enter a state of decompensation, ultimately leading to severe destruction of brain energy and further neuronal damage, which in turn exacerbates impaired brain energy utilization¹⁸.

Furthermore, Yesilkaya UH et al. found that patients with schizophrenia had significantly higher fasting blood glucose levels compared to the HC group⁴⁷. Roosterman D et al. discovered that glucose metabolism was disturbed in the cerebrospinal fluid (CSF) of schizophrenia patients⁴⁸. Regenold WT et al. clarified that glucose in CSF is significantly and positively correlated with lactate levels ($r = 0.435$, $P = 0.003$)²⁷. Interestingly, our study found no significant positive correlation between serum lactate and blood glucose in patients with schizophrenia ($r = 0.157$, $P > 0.05$). However, a study by Bryll A et al. confirmed that nitrosative, oxidative, or sulfuric damage to enzymes of tricarboxylic acid cycle and glycolysis, as well as calcium transport and adenosine triphosphate (ATP) biosynthesis might lead to impaired bioenergetics function in the brain⁴⁹. This could explain the initial symptoms of schizophrenia, such as mild cognitive impairment. In addition, TianY et al. found that the prevalence of comorbid obesity was 16.4% among 633 Chinese hospitalized schizophrenic patients. Plasma levels of glucose, LDL, triglycerides, cholesterol, and apolipoprotein B were higher in obese patients. Meanwhile, HDL levels were significantly lower in obese patients compared to non-obese patients⁵⁰. We found a significant positive correlation between serum lactate and triglycerides in SCZ patients ($pr = 0.313$, $P < 0.001$). Interestingly, the G-protein-coupled receptor 81 (GPR81) has been shown to promote fat storage in adipocytes, and this process is active in the neocortex and hippocampus of the mammalian brain⁵¹. Lactate, as an agonist of the GPR81, can exert its effects through both autocrine and paracrine mechanisms⁵². In the autocrine pathway, lactate produced by cancer cells activates GPR81 on the surface of the cancer cells. In the paracrine pathway, lactate secreted by cancer cells activates GPR81 on endothelial cells, immune cells, and adipocytes within the tumor stroma.

MK-801 induces cognitive dysfunction in animal models of SCZ

It is now generally confirmed that MK-801 causes cognitive and social deficits in model rats. Previous studies have shown that acute and subchronic MK-801 administration have different effects. Lee J et al. found that acute injection of MK-801 impaired working memory, but did not affect attentional function in rats⁵³. Wang X et al. found that acute MK-801 treatment (0.4 mg/kg) caused cognitive deficits and anxiety in SD rats⁵⁴. Unal G et al. showed that male rats injected with MK-801 subchronically experienced cognitive deficits in both the novel object recognition experiment and the MWM test⁵⁵. Maleninska K et al. found that MK-801-induced cognitive deficits related to space and time were dose-dependent. Both MK-801 doses (0.1 mg/kg and 0.12 mg/kg) significantly impaired timing strategies and increased locomotor activity in the dark⁵⁶. However, only the 0.1 mg/kg dose, not the 0.12 mg/kg dose, impaired spatial avoidance strategies in the light. Our results showed that after chronic injection of MK-801 (0.2 mg/kg/d for 14 days) in SD rats, the escape latency was significantly longer than HC group in the MWM test on day 5 ($P < 0.01$). In addition, on days 5 and 7, the distance traveled was significantly longer in the MK group than in the HC and CLO groups ($P < 0.05$). This suggests that MK-801 significantly weakened spatial learning ability in these rats compared to those in the HC and CLO groups. However, the pathological mechanism of MK-801-induced cognitive impairment in rats remains unclear. In the MK-801-induced SCZ rat model, NMDA receptor dysfunction led to severely impaired metabolism of neurotransmitters. This affected the glutamatergic, dopaminergic, and GABAergic systems in the cortico-striato-thalamo-cortical loop⁵⁷. Our study found that serum and FCX glutamate levels were significantly higher in the MK group than in the HC group ($P < 0.001$). Glutamate, an important excitatory neurotransmitter, plays a key regulatory role in the pathogenesis of schizophrenia⁵⁸. Dysfunction of glutamate-gated ion channels (NMDAR) has been demonstrated in schizophrenia. NMDAR antagonists significantly increase prohibitin 2 (PHB2) levels in the prefrontal cortex. Interestingly, PHB2 was significantly elevated in SCZ patients and was significantly negatively correlated with cognitive impairment^{59,60}.

In our experiments, rats in the MK + CLO group had a shorter escape latency on day 5 compared to the MK group; however, we found that this difference was not statistically significant ($P > 0.05$). In addition, there was no statistically significant difference between the distance traveled of rats in the MK + CLO group and the MK group on day 5 and day 7 ($P > 0.05$). Interestingly, we found that CLO treatment significantly reduced the abnormally high glutamate levels in FCX of rats induced by MK-801 ($P < 0.001$). Zhou X et al. showed that clozapine mildly activates NMDA receptors and is effective in treating negative and cognitive symptoms of schizophrenia. They found that clozapine combined with the neuroprotectant pyrroloquinoline quinone improved MK-801-induced schizophrenia-like behaviors⁶¹. Regulation of NMDA receptors can reduce the expression levels of NMDAR1 and GluR3, leading to a decrease in tau protein hyperphosphorylation in the hippocampus of rats, and inhibit cell apoptosis through modulation of the Akt/GSK-3 β signaling pathway. In addition, two weeks of treatment with 10 mg/kg quetiapine significantly improved cognitive dysfunction and cerebrovascular white matter damage in a mouse model induced by MK-801 (1 mg/kg/day, 1 week, i.p.), and the mechanism may be related to the PI3 K/Akt signaling pathway⁶². Studies have shown that modulation of the Akt and GSK-3 β signaling pathways partially alleviates MK-801-induced impairments in prepulse inhibition, social interaction, and cognitive function⁶³.

In the MK-801-induced SCZ model, lactate metabolism and glycolytic processes were disrupted

Disturbances in lactate metabolism may contribute to the development of core symptoms of schizophrenia, such as cognitive deficits and negative symptoms⁶⁴. A study by Eyjolfsson EM et al. found reduced lactate concentrations in the FCX, parietal cortex, thalamus, hippocampus, striatum, and nucleus accumbens in rats injected with MK-801 (0.5 mg/kg/day) for 6 days⁵⁷. However, a significant reduction in glycolysis and impairment of mitochondrial enzymes was observed only in FCX, which is thought to be related to the duration of exposure to MK-801. However, our study found that lactate levels in both serum and FCX were significantly higher in the MK group compared to the HC group ($P < 0.001$). Similarly, lactate levels in both serum ($P < 0.01$) and FCX ($P < 0.001$) were significantly higher in the MK group than in the CLO group. These results suggest that chronic MK-801 intervention can abnormally increase endogenous lactate levels in the serum and FCX. Our findings are consistent with previous research. In the MK-801-induced SCZ mouse model, glycolysis and lactylation were enhanced in hippocampal tissues. 2-deoxy-D-glucose, a glycolysis inhibitor, effectively prevented the abnormal behaviors induced by MK-801⁶⁵. Furthermore, lactic acid accumulated in MK-801-treated primary hippocampal neurons. The lactate receptor (HMGB1) levels were abnormally elevated in the culture medium, inducing apoptosis in primary hippocampal neurons. We also found that chronic MK-801 intervention increased pyruvate levels and LDH activity in serum and FCX ($P < 0.001$). These changes suggest that energy metabolism processes related to glycolysis and the tricarboxylic acid cycle were disturbed in the MK-801-induced SCZ model. Kolar D et al. suggested that repeated inhibition of NMDA receptor activity in specific regions can lead to progressive metabolic dysregulation⁶⁶. They found that three days after a single injection of MK-801, LDH activity in the striatum was decreased. However, two hours after repeated injections of MK-801, LDH activity in the striatum was increased. Pinacho R et al. suggested that in schizophrenia, abnormal NMDA-dependent glycogenolysis causes a temporary energy deficit at the glial-neuronal junction, and this deficit leads to disturbed lactate metabolism in schizophrenic patients⁴⁴. They demonstrated that this is a dynamic process. In the MK-801-induced model of SCZ, glycogen phosphorylase (PYGM) levels were abnormally elevated, leading to the breakdown of glycogen in the brain into lactate for energy. Interestingly, PYGM levels were found to correlate with the duration of schizophrenia. The longer the duration of the disease, the lower the PYGM levels. A study by Halim ND et al. found that chronic intraperitoneal injection of both clozapine and haloperidol in healthy adult male SD rats significantly increased lactate levels in the postmortem prefrontal cortex²⁸. Another study showed that clozapine significantly reduced glucose-related metabolism in several brain regions. This effect was especially pronounced in the cerebral cortex of healthy Wistar rats⁶⁷. In our study, there was no significant difference in lactate levels in the serum or FCX between the CLO group and the HC group ($P > 0.05$). This suggests that clozapine itself did not cause abnormal lactate metabolism in the serum or FCX. Additionally, we found that lactate levels in the FCX of rats in the MK + CLO group were significantly lower than those in the MK group ($P < 0.001$). On day 4 of MK-801 treatment (0.2 mg/kg/d for 4 days), the extracellular lactate concentration in the medial prefrontal cortex of adult male Wistar rats was elevated above baseline levels⁶⁸. High-dose tandospirone reduced abnormally elevated lactate levels and improved cognitive deficits in the SCZ.

Aberrant activation of NLRP3/Caspase-1/IL-1 β inflammatory pathway in the MK-801-induced SCZ model

Zhao J et al. performed RNA sequencing of the prefrontal cortex (PFC) in male mice exposed to MK-801, and they found that differentially expressed genes in the SCZ model were associated with immunomodulatory processes as well as impaired postsynaptic transmission⁶⁹. Behavioral changes in mice after acute injection of MK-801 (0.1 mg/kg) were significantly correlated with microglia density in the HPC and PFC. Depletion of microglia improved schizophrenia-like behavior in mice, which was significantly associated with genes related to the regulation of brain immunity, including NLRP3⁷⁰. Interestingly, glutamate excitotoxicity leads to upregulation of key proteins that activate the NLRP3 inflammasome, including NLRP3, pro-Caspase-1, cleaved Caspase-1, and IL-1 β ⁷¹. Our study found that glutamate levels in serum and FCX of rats in the MK group were significantly higher than those in the HC group ($P < 0.001$). The protein expression levels of NLRP3 and Caspase-1 in FCX of rats in the MK group were significantly higher than those in the HC group ($P < 0.01$). In addition, the levels of IL-1 β and IL-18 in serum and FCX were significantly higher in the MK group compared with the HC group ($P < 0.05$). Kim JY et al. found that MK-801 treatment induced an increase in phosphorylated-NF- κ B expression, which in turn increased the levels of IL-6 and TNF- α expression in the PFC⁷². Zhang Z et al. suggested that neuroinflammation and cognitive deficits induced by ketamine were associated with NLRP3/Caspase-1 axis-dependent hippocampal apoptosis⁷³. Our results showed that NLRP3 protein expression was not significantly elevated in the FCX of SD rats in the CLO group compared with the HC group ($P > 0.05$), while Caspase-1 protein expression was abnormally elevated ($P < 0.01$). Consistent with our findings, clozapine induced NLRP3 inflammation-dependent Caspase-1 activation and IL-1 β release in undifferentiated and differentiated human monocytes⁷⁴. In addition, we found that Caspase-1 protein expression in the FCX of rats in the MK + CLO group was significantly lower than that in the MK group ($P < 0.01$). This suggests that clozapine, while slightly increasing Caspase-1 protein levels in the FCX of healthy rats, significantly reduced the MK-801-induced overexpression of Caspase-1 in the FCX. Giridharan VV et al. found that clozapine reduced the expression of the NLRP3 inflammasome by 57% in Poly(I: C)-activated microglia⁷⁵. They suggested that clozapine could exert anti-inflammatory effects by inhibiting NLRP3 inflammasome. Importantly, clozapine had a better anti-inflammatory effect than haloperidol and risperidone.

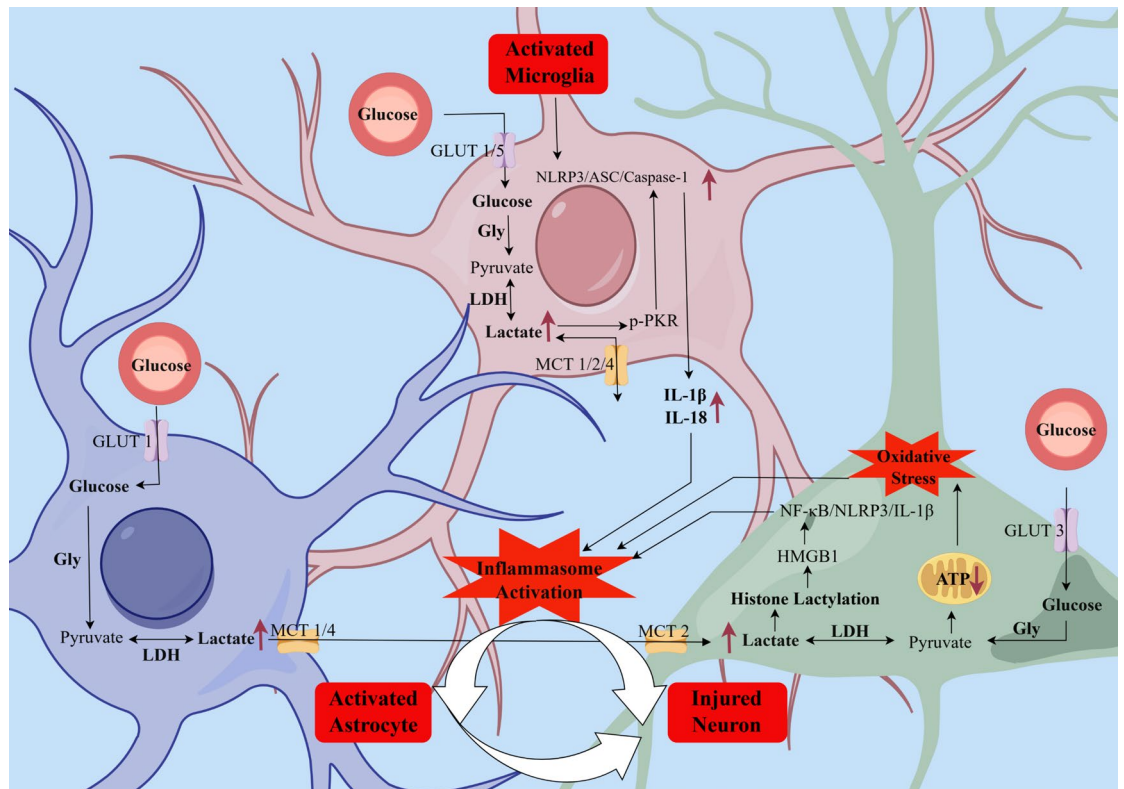


Fig. 8. Pathological mechanisms of the interaction between lactate metabolism disorders and neuroinflammation in the SCZ brain. Abnormal activation of microglia in SCZ can trigger the activation of NLRP3 inflammasomes. Additionally, the abnormal accumulation of lactate in these cells can activate NLRP3 inflammasomes through protein kinase R phosphorylation (p-PKR). In SCZ neuronal cells, excessive lactate accumulation can lead to NLRP3 activation via histone lactylation, facilitated by LDHA. Mitochondrial dysfunction in SCZ induces oxidative stress and activates neuroinflammation, which damages neurons and astrocytes. This damage further disrupts lactate metabolism. Microglial activation also increases astrocyte activity, contributing to central nervous system damage. This process represents a dynamic progression from compensation to decompensation^{20,70,79,82}. This figure was created by Figdraw2.0, <https://www.figdraw.com>.

Interaction between SCZ lactate metabolism disorders and aberrant activation of NLRP3/Caspase-1/IL-1β inflammatory pathways

In the lipopolysaccharide (LPS)-induced maternal immune activation model, alterations in the CD200-CD200R and/or CX3 CL1-CX3 CR1 pathways may be associated with the etiology of SCZ⁷⁶. Interestingly, LPS-induced early inflammatory changes promote glycolysis, including increased glucose uptake and lactate release⁷⁷. Microglial cells exert a pro-inflammatory role in the brain, which can influence cerebral energy metabolism. LPS can stimulate astrocytes, thereby activating the TLR4 signaling pathway (NLRP3/Caspase-1). This process leads to an increased release of cytokines such as IL-6 and TNF- α , heightened tissue energy demands, and enhanced lactate production⁷⁸. Our study found a significant positive correlation between serum lactate and serum IL-1 β ($r = 0.760$, $P = 0.000$). A similar correlation was observed between serum lactate and IL-18 ($r = 0.781$, $P = 0.000$). Additionally, serum lactate showed a significant positive correlation with LDH activity ($r = 0.744$, $P = 0.000$) and pyruvate levels ($r = 0.765$, $P = 0.000$). It has been shown that activation of NLRP3-inflammasome requires glycolysis to produce lactate rather than pyruvate oxidation. Interestingly, phosphorylated protein kinase R (p-PKR) can act as a bridge between NLRP3-inflammasome and lactate (see Fig. 8). And inhibition of LDH not only reduces glycolytic lactate production but also decreases PKR and Caspase-1 activity and IL-1 β maturation⁷⁹. In rat microglia, p-PKR activates inflammatory signaling via NF- κ B⁸⁰. Additionally, lactate and pyruvate can be interconverted by LDHA/B. LDHA not only drives the conversion of pyruvate to lactate, but also mediates histone lactylation and induces inflammation and sepsis by targeting HMGB1 (see Fig. 8). Interestingly, HMGB1 was significantly elevated in SCZ patients compared to healthy controls⁸¹. Abnormally elevated HMGB1 can produce neurotoxic effects, including effects on the WNT/ β -catenin signaling pathway and the BBB. In cells treated with oxygen-glucose deprivation/reoxygenation, LDHA, lactate, histone lactylation, and HMGB1 were upregulated. Knockdown of LDHA decreased IL-1 β and IL-18 levels and inhibited Caspase-1 cleavage⁸². LDHB promotes the conversion of lactate to pyruvate. LDHB (-/-) mice show upregulated expression of apoptotic mediators, such as Caspase-3 and cytochrome C. In addition, LDHB deficiency induces neurogliosis, increases inflammatory mediators (e.g., NF- κ B, TNF- α), and leads to cognitive deficits in mice⁸³. Interestingly, the inflammatory factor IL-2 promotes aerobic glycolysis, strongly inducing lactate and LDH production⁸⁴. We

found a significant positive correlation between serum lactate and glutamate ($r = 0.694$, $P = 0.000$). It has been shown that aberrant glutamate stimulation in SCZ can contribute to LDH release, mitochondrial damage, and a loss of mitochondrial membrane potential. This stimulation also increases the number of cells containing autophagic lysosomes, ultimately leading to a decrease in cell viability and an increase in the rate of apoptosis⁸⁵.

Among the genes upregulated in early-onset schizophrenia, genes related to the LPS and NF- κ B signaling pathways were enriched. In contrast, genes associated with glucocorticoids (GCs) were downregulated⁸⁶. However, GCs may undergo dynamic changes during the pathogenesis of schizophrenia. Goldwasser EL et al. found elevated long-term GCs in schizophrenia, which were associated with cerebral white matter deficits⁸⁷. Zhang Q et al. also demonstrated that PANSS scores in patients with schizophrenia were correlated with disease duration and GCs \times IL-8 \times TNF- α , suggesting that abnormally elevated glucocorticoids and aberrant immune activation may have an impact on cognitive deficits in SCZ patients⁸⁸. Interestingly, excess GCs can impair mitochondrial oxidation and energy production, leading to increased lactate production⁸⁹. Furthermore, dysregulation of the classical WNT/ β -catenin pathway is responsible for altered metabolic thermodynamics, interestingly, up/down-regulation of this pathway can lead to altered glucose aerobic glycolysis. Upregulation of the WNT/ β -catenin pathway promotes glucose aerobic glycolysis. However, down-regulation of the pathway leads to oxidative stress and cell death through inactivation of LDHA, glucose transporter, monocarboxylate transporter-1, pyruvate kinase M2, pyruvate dehydrogenase kinase 1 and activation of the pyruvate dehydrogenase complex⁹⁰. The interaction between oxidative stress and neuroinflammation is regulated by the WNT/ β -catenin pathway, which is downregulated in schizophrenia and interacts with the NF- κ B pathway⁹¹. Taken together, the above studies suggest a possible link between disturbances in endogenous lactate metabolism and aberrant activation of the NLRP3/Caspase-1/IL-1 β inflammatory pathway in schizophrenic. And, PKR phosphorylation, histone lactylation, abnormalities in LDHA/B and glucocorticoids, dysregulation of the WNT/ β -catenin pathway, and mitochondrial dysfunction may serve as a regulatory bridge between the two.

Limitations of our study

Despite the findings of our study, there are still some aspects that need to be studied in depth and improved. Firstly, only patients with schizophrenia who were hospitalized in Yunnan Province Psychiatric Hospital in between April and December 2022 were included in this study. In order to improve the generalizability of the study results, our team will collaborate with several other psychiatric hospitals. A multicenter, big data clinical study will be conducted by collecting, organizing, and analyzing the hospitalization data of SCZ patients from various hospitals throughout the year 2024 and monitoring the dynamic changes of these parameters during the course of treatment. Secondly, this study reveals an association between dysregulated lactate metabolism, aberrant activation of the NLRP3/Caspase-1/IL-1 β inflammatory pathway, and SCZ, but direct causality validation experiments are lacking. We will observe their subsequent effects on SCZ-related symptoms and neuropathological changes by directly modulating lactate levels or the NLRP3 inflammatory pathway. Additionally, disturbed lactate metabolism in schizophrenia may be linked to astrocyte-neuron lactate shuttling disorders, as lactate is a key energy source for the brain. However, our experiments were conducted solely in vivo. The mechanism of lactate metabolism dysfunction in schizophrenia will be further explored using in vitro cellular experiments. Finally, we summarized potential molecular mechanisms for the interaction between the aberrant NLRP3 inflammatory pathway and SCZ lactate levels, however, no validation of these mechanisms was performed. Our team will validate the interactions between these mechanisms through molecular biology approaches to further support causal evidence for the interactions between these hypotheses.

Conclusions

In conclusion, serum lactate levels were significantly higher than the normal range in patients with schizophrenia, and they were significantly and positively correlated with both length of hospitalization and serum triglyceride levels. In addition, the MK-801-induced SCZ animal model exhibited impaired cognitive function, abnormally elevated levels of lactate, pyruvate, glutamate, IL-1 β , IL-18, and LDH activity in serum and FCX, and significantly abnormally elevated levels of NLRP3 and Caspase-1 proteins in FCX. There may be a link between elevated serum lactate in schizophrenia and aberrant activation of the NLRP3/Caspase-1/IL-1 β inflammatory pathway. And, mediators between the two may include protein kinase R phosphorylation, histone lactylation, abnormal LDHA/B and glucocorticoid levels, dysregulation of the WNT/ β -catenin pathway, and mitochondrial dysfunction. In future studies, modulation of serum/brain lactate levels and the NLRP3/Caspase-1/IL-1 β pathway in SCZ patients may serve as potential targets for improving cognitive impairment in SCZ.

Data availability

Data for this study is provided within the manuscript or supplementary information files.

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Figures 1 and 8 use Figdraw software.

Author contributions

Yingying Zhang, Yanjun Wang and Haoran Xing share the first authorship. Yingying Zhang, Yanjun Wang and Haoran Xing wrote the main manuscript text. Mier Li, Haiqiang Zhao and Luanmei Ding assisted in the experiment. Yuncheng Bai, Weiwei Wang and Tianhao Bao provided research design and guidance. Corresponding Author: Weiwei Wang* (Email: doctor_b@whu.edu.cn) and Tianhao Bao* (Email: doctor@kmmu.edu.cn). All authors revised the paper critically for intellectual content and gave final approval of the version to be published.

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Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Ethical Review Committee for Animal Experiments of Kunming Medical University and conformed to the standards (No. kmmu20230164).

Competing interests

The authors declare no competing interests.

Additional information

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