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**THE EFFECTS OF VARIOUS TYPICAL AND ATYPICAL
ANTIPSYCHOTICS ON COGNITIVE FUNCTIONS IN SCHIZOPHRENIA –
STUDIES IN ZEBRAFISH AND HUMAN PATIENTS**

SUMMARY OF THE DOCTORAL THESIS

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LIST OF ABBREVIATIONS

SGA – Second-generation antipsychotics

FGA – First-generation antipsychotics

AVLT – Auditory Verbal Learning Test

BACS – Brief Assessment of Cognition in Schizophrenia

BPRS – Brief Psychiatric Rating Scale

BVMT-R – Brief Visuospatial Memory Test–Revised

CGI-S – Clinical Global Impressions Scale

CLO+AMI – Clozapine and Amisulpride

CLZ – Clozapine

CPT – Continuous Performance Test

CPT-IP – Continuous Performance Test – Identical Pairs

CTR – Control

DIEPSS – Drug-Induced Extrapyrmidal Symptoms Scale

DRT – Delayed Response Task

DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

DST – Digit Symbol Test

SEM – Standard Error of the Mean

GABA – Gamma-Aminobutyric Acid

GAF – Global Assessment of Functioning

HAL – Haloperidol

HVLT-R – Hopkins Verbal Learning Test–Revised

ICD-10 – International Classification of Diseases, 10th Revision

KET – Ketamine

LNS – Letter–Number Sequencing

MCCB – MATRICS Consensus Cognitive Battery

MCI – Mild Cognitive Impairment

MCQ-30 – Metacognitions Questionnaire-30

MET – Methionine

MMSE – Mini-Mental State Examination

MoCA – Montreal Cognitive Assessment

MOT – Monotherapy

MSCEIT – Mayer–Salovey–Caruso Emotional Intelligence Test

NDMC – N-Desmethylozapine

NMDA – N-Methyl-D-Aspartate

NOR – Novel Object Recognition

OLA – Olanzapine

PANSS – Positive and Negative Syndrome Scale

PIT – Polypharmacy

PTSD – Post-Traumatic Stress Disorder

RBANS – Repeatable Battery for the Assessment of Neuropsychological Status

RIS – Risperidone

RIS+ARI/QUE – Risperidone and Aripiprazole or Quetiapine

ROCF – Rey–Osterrieth Complex Figure

RTC – Resistance to Clozapine Treatment

RWT – Regensburger Wortflüssigkeitstest

SAM – S-Adenosylmethionine

SCZ – Schizophrenia

SF-36 – Short Form Survey-36

SOPT – Subject-Ordered Pointing Task

CBT – Cognitive Behavioral Therapy

TESS – Treatment Emergent Symptom Scale

TMT – Trail Making Test, Parts A and B

OCD – Obsessive-Compulsive Disorder

UKU – UKU Side Effect Rating Scale

VLMT – Verbal Learning and Memory Test

VLNOR – Visual Lateralization Novel Object Recognition

VTA – Ventral Tegmental Area

WMS-III – Wechsler Memory Scale, Third Edition

Keywords: Schizophrenia; Antipsychotics; Zebrafish; Cognition; Metacognition; Patients; Memory; Social behavior; Novel Object Recognition; Ketamine; Methionine; T-maze; Social preference; Clozapine; Amisulpride; Haloperidol; Risperidone; Olanzapine; MMSE; MoCA; MCQ-30; PANSS.

CHAPTER I. CURRENT STATE OF KNOWLEDGE IN THE FIELD

1.1 General concepts regarding schizophrenia

Schizophrenia represents a major psychiatric disorder characterized by severe and constant impairment of a person's quality of life. The etiology of the disease is complicated and incompletely elucidated, and the manifestations differ notably from one individual to another [1]. Disorders within the schizophrenia spectrum differ from other disorders through basic and consistent cognitive and perceptual deficits, as well as through affective flattening, avolition, and social withdrawal. Although certain cognitive deficits may appear over time, self-awareness and intellectual capacity are frequently preserved by patients [2].

Schizophrenia is characterized by symptoms such as delusions, hallucinations, thought disorders, and cognitive deficits. Schizophrenia affects certain basic cognitive processes, such as perception, emotion, and judgment. Data increasingly convincingly support the notion that schizophrenia is a subtle disorder of development and neuroplasticity [1].

Disorganized thinking (formal thought disorder) is primarily assessed based on the individual's speech. It may manifest through frequent shifts from one subject to another (derailment or loosening of associations), and responses may be only vaguely related or completely unrelated to the questions asked (tangentiality). In rarer cases, speech may become so disorganized that it becomes almost impossible to understand, resembling receptive aphasia due to extreme incoherence ("word salad"). However, because mild forms of speech disorganization are relatively common and nonspecific, this symptom must be sufficiently severe to significantly impair communication. Assessing severity may be difficult, especially when the evaluator and the person being assessed have different linguistic backgrounds. Also, during the prodromal and residual phases of schizophrenia, thinking and speech may present a lower degree of disorganization [3].

Dysfunctions of working memory, attention, processing speed, visual and verbal learning, with substantial deficits in reasoning, planning, abstract thinking, and problem-solving, have been extensively documented in schizophrenia. According to some estimates, almost 98% of patients suffering from schizophrenia have such impairments and do not achieve the predicted cognitive functioning based on premorbid intelligence and parental educational levels. The neurocognitive impairment profile of patients with schizophrenia describes the underlying disruption of cortico-cerebello-thalamo-cortical circuits. A large retrospective study showed that cognitive deficits are among the earliest signs in individuals who were subsequently diagnosed with schizophrenia [4]. Cognitive deficits, therefore, denote a trait marker for schizophrenia. In addition, evidence of cognitive deficits in both the prodromal phases and throughout the course of schizophrenia justifies the inclusion of cognitive decline as a central element of this disease. A significant correlation is highlighted between the functional outcome of the deficits and that of employment, independent living, cognition, and social and community functioning. Cognitive deficits are prominent despite clinical stability in patients with schizophrenia and are probably associated with disability [4].

Psychotic symptoms in schizophrenia generally appear between the end of adolescence and the middle of the fourth decade of life, with onset before adolescence being rare. Typically, the first psychotic episode occurs in the early to middle part of the third decade in men and toward the end of the same period in women.

Onset may be either abrupt or gradual; however, in most cases, significant clinical manifestations develop slowly and progressively [3]. In the clinical assessment of schizophrenia, positive symptoms, negative symptoms, and general psychopathology must be taken into consideration [3].

Positive symptoms are usually represented by the following:

- hallucinations (most frequently auditory);
- disorganized thinking and speech;
- psychomotor agitation and delusions.

Negative symptoms are mainly represented by the following:

- affective flattening;
- social withdrawal;
- stereotyped thinking;
- anhedonia, avolition, and difficulties with abstract thinking.

Regarding general psychopathology, clinicians and psychiatrists should look for symptoms such as somatic preoccupation, anxiety, depression, feelings of guilt, as well as cognitive deficits (including, but not limited to, unusual thinking, disorientation, attention deficit, poor impulse control, and anosognosia) [3].

Environmental factors, such as season of birth, have been correlated with the risk of developing schizophrenia, with incidence being higher among individuals born in late winter or early spring in certain regions, while summer births have been associated with deficit forms of the disease. The risk is also higher in individuals raised in urban environments and within certain ethnic minority groups [3].

Genetic factors make a significant contribution to vulnerability to schizophrenia, even though the majority of diagnosed individuals do not have a family history of psychosis. Risk is determined by the presence of a large number of alleles, either common or rare, each having a small but cumulative effect on overall predisposition. Many of these genetic variants are also involved in other psychiatric disorders, such as bipolar disorder, depression, and autism spectrum disorders [3].

First-generation antipsychotics (FGAs), commonly referred to as "typical antipsychotics," were first developed in the 1950s. SGAs, often called "atypical antipsychotics" or second-generation antipsychotics, became available during the 1980s. Regarding their mechanism of action, first-generation antipsychotics function by inhibiting

dopaminergic neurotransmission (with the observation that they are most effective when they block approximately 72% of the brain's D2 dopamine receptors), as well as cholinergic, noradrenergic, and histaminergic activity.

Second-generation antipsychotics act by blocking D2 dopamine receptors, as well as through the antagonistic action of serotonin receptors, with the 5-HT_{2A} subtype being the most frequently involved [5]. Although atypical antipsychotics (including paliperidone, ziprasidone, and lurasidone) have shown certain cognitive benefits, high doses of antipsychotics are associated with a series of cognitive deficits in patients with schizophrenia [6–9]. There is evidence that first-generation antipsychotic medications generate extrapyramidal side effects, such as akathisia, dystonia, parkinsonism, and tardive dyskinesia [10,11]. Despite the fact that second-generation antipsychotics (SGAs) have increasingly replaced first-generation antipsychotics (FGAs) in industrialized countries, the latter are still the most frequently prescribed medications in many other regions of the world, particularly in poor countries where they are significantly more accessible [10,11].

The available symptomatic treatment is only partially successful. Antipsychotics or neuroleptics are a group of medications used in the treatment of severe psychiatric diseases. In the study conducted by Goldberg et al., participants selected to receive olanzapine and risperidone did not present differences regarding the cognitive measure at the beginning of the study, both in the initial data with nonsignificant effects of the medication group and in the *post-hoc* analysis of the differences between groups at baseline [12]. In the study conducted by Cuesta et al., in patients suffering from chronic schizophrenia, the atypical antipsychotic medication was associated with slight differential improvements over time in attention, verbal memory, and executive functions compared with typical neuroleptic medications. No differential improvements were found in social functioning, verbal fluency, non-verbal domains of memory, or visuospatial abilities [13]. Comparisons between antipsychotics, mainly regarding cognitive differences in affected individuals, have resulted in limited and contradictory data.

1.2 Metacognition in schizophrenia

Metacognition is higher-order thinking that involves the active control of cognitive processes involved in learning [14]. Research has indicated that many individuals with schizophrenia spectrum disorders experience metacognitive deficits. These include deficits regarding social cognition, working memory, reduced processing speed, visual and verbal learning dysfunctions, as well as significant deficits in reasoning, planning, abstract thinking, and problem-solving [15]. Social cognition is also impaired, that is, the ability to appropriately incorporate information and apply it to develop appropriate responses under certain circumstances. Cognitive deficits and social functioning are closely interrelated, as patients with schizophrenia present metacognitive deficits, which are reflected in lower performance on tests evaluating these abilities. They have difficulties in forming complex ideas about themselves and others, present many memory disorders, are generally not aware of their condition, and present a significant impairment of their overall cognitive capacity. Considering that metacognition is important for learning and training, investigating metacognitive activity and metacognitive development becomes an essential condition for

identifying how individuals with neurodegenerative diseases could be taught to use cognitive resources through metacognitive control [14,15].

4. Zebrafish as an animal model for schizophrenia

Pharmacological models of schizophrenia are generally based on three main sources of evidence: neurochemical and molecular alterations identified in patients with schizophrenia, the pharmacodynamic properties of antipsychotics, and the observation that certain psychotomimetic substances can induce psychotic states in healthy individuals or exacerbate existing symptoms in patients diagnosed with schizophrenia [16–18]. The use of psychotomimetic compounds in pharmacological modeling has been based particularly on dysregulation of glutamatergic and dopaminergic systems, as well as the involvement of other neurotransmitter systems, especially serotonin [19]. The imbalance between excitatory and inhibitory neurotransmission may persist for prolonged periods or even permanently, contributing to the development and maintenance of neurobehavioral alterations relevant to schizotypal behavior [20]. Consequently, the effects of acute, chronic, or early-life exposure to psychoactive substances have been extensively investigated in animal models [21].

Exposure to psychotomimetic compounds is often combined with other experimental strategies, such as brain lesions, either to test increased sensitivity to such substances during adolescence or to strengthen the construct validity of the model used [22]. In this context, zebrafish represent a valuable experimental model because they possess all major neurotransmitter and neuromodulatory systems found in mammals, allowing the development of pharmacologically relevant models. However, because of differences in brain morphology between zebrafish and mammals, certain specific features of neurotransmitter-system organization must also be considered [23]. Pharmacological substances may be administered to rodents and zebrafish through intraperitoneal, intramuscular, or subcutaneous injections. In zebrafish, substances can additionally be administered non-invasively by dissolving them in the water of the experimental aquarium, a method that represents an important practical and methodological advantage [24].

CHAPTER II. RESEARCH HYPOTHESIS AND OBJECTIVES OF THE THESIS

The general research hypothesis was that different classes of antipsychotics differentially influence cognitive functions implicated in schizophrenia, and that these effects can be demonstrated both clinically, using standardized cognitive assessment instruments, and experimentally, using validated behavioral paradigms in an animal model. It was also hypothesized that the pharmacodynamic profile of each antipsychotic compound differentially modulates processes such as memory, exploration, novelty processing, and social behavior, depending on the underlying neurobiological mechanisms and the pharmacological model used to induce schizophrenia-like characteristics.

To test this general hypothesis, a series of primary and secondary objectives were pursued, structured according to the four studies included in the thesis.

- Primary objective 1: To evaluate the influence of antipsychotic treatment on cognitive and metacognitive functions in human patients diagnosed with schizophrenia by comparing antipsychotic monotherapy and polypharmacy.
 - Secondary objective 1: To analyze overall cognitive performance using the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).
 - Secondary objective 2: To evaluate differences between patients receiving monotherapy and polypharmacy in domains such as orientation, attention, short-term memory, language, abstract thinking, and executive functions.
 - Secondary objective 3: To investigate metacognitive characteristics using the Metacognitions Questionnaire-30 (MCQ-30), as well as potential relationships between pharmacological regimen and maladaptive metacognitive beliefs, with emphasis on self-reflexivity, thought control, and the role of worry in maintaining psychological distress.
- Primary objective 2: To investigate the effects of haloperidol and risperidone on memory, learning, and exploratory behavior in an experimental model of ketamine-induced schizophrenia in adult zebrafish.
 - Secondary objective 1: To evaluate the impact of ketamine exposure on locomotor behavior and cognitive functions using specific behavioral tests.
 - Secondary objective 2: To compare the effects of a typical and an atypical antipsychotic on performance in memory and learning tasks.
 - Secondary objective 3: To analyze behavior in the novel object test, social preference task, and spatial memory paradigms, and to assess the value of the *Danio rerio* model for investigating pharmacological effects on cognition in schizophrenia.

- Primary objective 3: To analyze the effects of amisulpride and clozapine administered at two different doses on memory, exploration, and social behavior in a methionine-induced schizophrenia-like model in zebrafish.
 - Secondary objective 1: To evaluate the effects of methionine on novelty processing, cognitive flexibility, and social interaction.
 - Secondary objective 2: To investigate the effects of amisulpride and clozapine on locomotor, motivational, and cognitive parameters in the context of methionine exposure, including behavior in the T-maze memory test, novel object test, and social preference test.
- Primary objective 4: To compare the effects of amisulpride and clozapine administered at two distinct doses in order to identify potential differences dependent on pharmacological mechanism and dose.

CHAPTER III. RESEARCH METHODOLOGY

3.1 Cognitive tests in zebrafish

3.1.1 Novel Object Recognition Test in the aquarium

Impaired learning and memory are common symptoms of neurodegenerative and neuropsychiatric disorders. Several behavioral tests are currently used to assess cognitive functions in animal models, including the Novel Object Recognition (NOR) test, which is widely used. The NOR test is considered to reflect certain aspects of human declarative memory, and its universal nature makes it similar in several respects to memory tests used in humans [25]. However, few animal models allow the study of mechanisms involved in learning and memory in relation to brain asymmetry [26]. The test can be completed within a short period so that animals experience minimal stress and provides valuable information about recognition capacity after a single trial, which represents an advantage over other methods of memory assessment [25].

3.1.2 T-maze test

Zebrafish habituation to the maze without colored sleeves was performed as described in the literature before testing. Training was conducted for six consecutive days by feeding the fish daily in the right arm. On the test day, each fish was placed in the starting box for 30 seconds, and the divider was raised and then replaced after the fish left the starting area. Once the fish entered either of the short arms of the T-maze, another divider was closed to prevent the fish from re-entering the long arm. The fish were then observed for 4 minutes. The number of entries into each colored arm and the open central area (colorless “blank” compartment), the time spent in each zone, and the first-entry preference were recorded [27].

3.1.3 Social preference test

Social preference may be defined as the individual's tendency to live in a shoal with other fish [28]. In zebrafish, this social behavior is assessed by observing how an individual responds to or interacts with a social stimulus. Social preference tests have been developed for zebrafish that are similar to those used in rodents [29]. Social preference tests consist of two operational phases. The first is the habituation phase, during which the tested zebrafish is left alone in one compartment of the testing tank to explore the novel environment. The second is the interaction phase, which begins with the introduction of the social stimulus, consisting of one or two small groups of zebrafish. Alternatively, the social stimulus may consist of a pre-recorded video of live fish displayed on a computer monitor, computer-animated images of zebrafish [30], or virtual-reality systems [31]. To test behavior toward a single stimulus, a rectangular tank divided into two compartments by a transparent barrier may be used. To simultaneously test preference for one of two social stimuli, a rectangular tank divided into three compartments is recommended (Fig. 3.2). Alternatively, a T-maze adapted for this purpose may be used; in this case, divider doors are used to seal the horizontal section of the maze, which is then divided into three compartments (Fig. 3.3). The T-maze is a laboratory apparatus commonly used to test cognitive abilities in rodents [32,33].

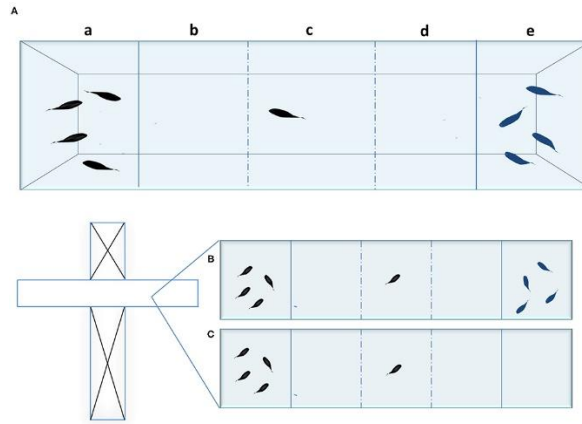


Figure 3.2 and 3.3. Social preference test using a cross-shaped maze [33].

3.2 Study 1

3.2.1 Mini-Mental State Examination (MMSE)

Folstein et al. [34] originally developed the MMSE to differentiate psychiatric patients from healthy individuals. They explicitly stated that MMSE scores were useful for quantitatively estimating cognitive impairment and documenting cognitive changes over time. The MMSE was not, in itself, designed to provide a diagnosis of a specific disorder. It consists of different questions and tasks, has a maximum score of 30 points, and generally takes 10–15 minutes to administer. The questions are grouped into seven categories, each representing a different cognitive domain/function: temporal orientation (5 points); spatial orientation (5 points); registration of three spoken words (3 points); attention and calculation (5 points); recall of three words (3 points); language (8 points); and reproduction of a drawing (1 point) [35].

3.2.2 Montreal Cognitive Assessment (MoCA)

The Montreal Cognitive Assessment (MoCA) was developed by Dr. Ziad Nasreddine in Montreal, Canada, in 1995 for the detection of mild cognitive impairment (MCI) by healthcare professionals. MCI is a syndrome defined as cognitive decline greater than expected for an individual's age and educational level but which does not consistently interfere with activities of daily living [36]. The prevalence of MCI in population-based epidemiological studies ranges from 3% to 19% among adults over 65 years of age, and more than half progress to dementia within 5 years. The MoCA may be useful to psychological and psychiatric professionals for detecting MCI or early dementia, particularly as the workforce ages. The test, together with its instructions, normative data, references, frequently asked questions, permissions, and updates, is available online free of charge [37].

The assessment consists of questions and tasks evaluating cognitive abilities, with a maximum score of 30 points, and can be administered within 10–15 minutes. A score of 26 or higher is generally considered normal. A score below 25 points suggests the need for further specialist evaluation [37]. The MoCA evaluates several cognitive domains: visuospatial/executive function, naming, memory, attention, language, abstraction, delayed recall, and temporal-spatial orientation. Many tasks are similar to those included in other cognitive-function assessment tests.

3.2.3 Metacognitions Questionnaire-30 (MCQ-30)

The Metacognitions Questionnaire (MCQ-30) measures individual differences in a selection of metacognitive beliefs, judgments, and monitoring tendencies considered important within the metacognitive model of psychological disorders. Construct validity has been demonstrated through confirmatory and exploratory factor analyses. Exploratory factor analysis of the short version of the instrument identified five factors that were highly similar to the original factors. These five factors are: lack of confidence in one's own thoughts, positive beliefs about worry, cognitive self-consciousness, negative beliefs about thoughts concerning uncontrollability, and the need to control thoughts. The MCQ-30 has demonstrated good internal consistency and convergent validity, as well as good test-retest reliability. Positive correlations between this questionnaire and questionnaires measuring worry provided additional support for its convergent validity [38–40].

Participants

Participants were recruited from the “Socola” Institute of Psychiatry in Iași according to the following inclusion and exclusion criteria.

Inclusion criteria:

- Patients diagnosed with schizophrenia and under clinical follow-up;
- Age between 18 and 65 years;
- Stable medication during the previous 3 months;
- Unchanged residence during the previous 3 months.

Exclusion criteria:

- Presence of other neurological disorders;
- Delusional ideation;
- Substance abuse during the previous 3 months;
- Cranial trauma during the previous 3 months.

3.3 Study 2

Animal model used in the study (*Danio rerio*)

A total of 65 wild-type zebrafish were used for these experiments. The fish were obtained from a local breeder and treated according to European standards for laboratory animals, with approval from the relevant ethics committees.

The experimental groups were as follows:

Group 1 – Control (15 fish)

Group 2 – Ketamine (15 fish)

Group 3 – Ketamine and Risperidone (15 fish)

Group 4 – Ketamine and Haloperidol (15 fish)

3.3.1 Ketamine administration

Ketamine exposure was performed at the end of the 5-day training period. On administration days, the fish were removed from their respective aquaria and placed in 50-mL tubes containing 0.1% ketamine solution for 5 minutes. A concentration of 0.1% was selected because the concentration reported in the literature (0.2%) [41] produced an anesthetic effect during the initial phase. The solution was prepared on the day of administration from veterinary ketamine intended for horses, cattle, sheep, goats, pigs, dogs, and cats (Pasteur Institute, Romania; 1 mL injectable solution = 100 mg ketamine hydrochloride). After 5 minutes of exposure to the test substance, the fish were returned to the aquaria containing the antipsychotics under investigation [42].

3.3.2 T-maze test

The fish were acclimatized to the apparatus by placing a transparent liftable barrier; removing the barrier allowed movement from one compartment to another. This was done to ensure that the fish learned about the existence of two different compartments in the apparatus. The fish were allowed to explore the apparatus for five minutes. The apparatus used was a T-maze with red and green sleeves attached to its arms.

The cross-maze test was performed by acclimatizing the groups before treatment for a period of 7 days. On the first day, 10 zebrafish were initially placed in the maze and allowed to swim for 30 minutes. During the following two days, groups of five zebrafish were placed in the maze for 30 minutes. During the next two days, pairs of fish were tested for 10 minutes. Days four and five of training consisted of training two fish at a time for 5 minutes. During the final two days, individual subjects were trained for 5 minutes. Each day, the fish had access to the established stimulus (food).

3.3.3 Social preference test

The social preference test was performed according to the model of R. B. Jones et al. (1999), in which a T-maze was adapted to assess social preference in chicks. We adapted this test for zebrafish. Three zebrafish were placed in the left arm and hermetically enclosed behind a transparent divider. The tested zebrafish was then allowed to leave the starting box. The fish was tested for 5 minutes, and behavior was recorded using EthoVision.

3.3.4 Novel Object Recognition Test in the aquarium

The protocol included one day of fish acclimatization, followed by one training day during which the fish were exposed to one of the novel objects for 10 minutes. On the final day, both objects (an aluminum ball and a colored plastic ball) were placed in the aquarium, and behavior was recorded and the number of interactions with the novel object was quantified. Video recordings were used in this test to assess distance traveled, mean velocity, turning angle, and angular velocity. These parameters were evaluated using EthoVision [43].

3.4 Study 3

A total of 90 wild-type zebrafish were used for this experiment. The fish were obtained from a local breeder and treated according to European standards for laboratory animals, with approval from the appropriate ethics committees.

The experimental groups were as follows:

Group 1 – Control (20 fish)

Group 2 – Methionine (20 fish)

Group 3 – Methionine and Clozapine (30 fish)

Group 4 – Methionine and Olanzapine (20 fish)

3.4.1 Substance administration

Methionine was administered for 7 days by introducing it directly into the water at a concentration of 4.5 mM. The solution was freshly prepared and changed daily throughout the treatment period. This concentration was selected because it has properties capable of inducing schizophrenia-like symptoms [44]. Following this period of methionine treatment, the antipsychotics were prepared on the day of administration. Clozapine (CLZ) was used at a concentration of 34 $\mu\text{L/mL}$ [45], while olanzapine (OLA) was used at concentrations of 0.7 $\mu\text{L/mL}$ and 1.4 $\mu\text{L/mL}$ [46]. These doses were selected from standard human treatment regimens and converted according to zebrafish body mass, a widely used approach in this type of research [47]. Individual zebrafish were placed in 50 mL of antipsychotic solution for 5 minutes.

3.4.2 Novel Object Recognition test (NOR)

The protocol consisted of one day of fish acclimatization, followed by one training day during which the fish were exposed to one of the objects for 10 minutes. On the final day, the objects (a yellow-colored ball and a red-colored ball) were fixed to the walls of the aquarium using suction cups (Figure 3.7), and behavior was recorded and the number of interactions with the objects was quantified.

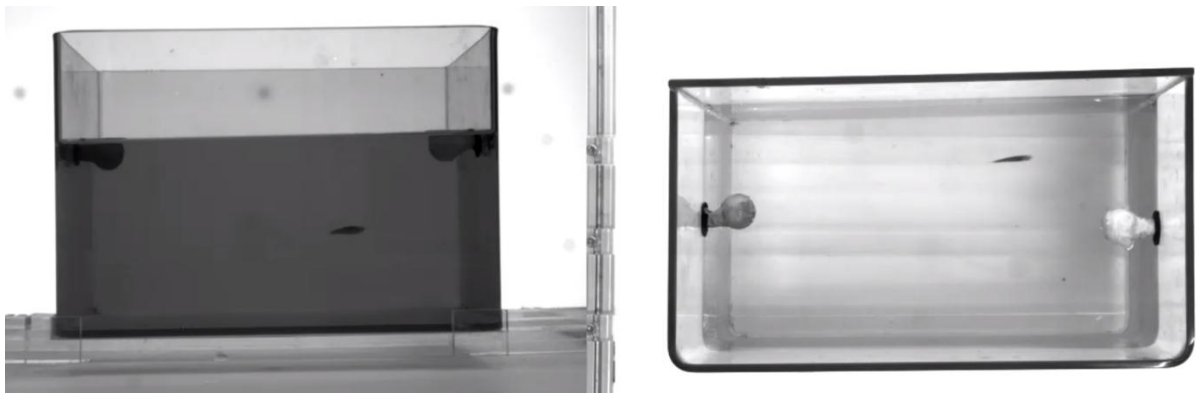


Figure 3.7. Method of fixing floating objects in the environment.

3.4.3 T-maze test

The cross-maze test was performed by acclimatizing the groups before treatment for a period of 7 days. On the first day, 10 zebrafish were initially placed in the maze and allowed to remain for 30 minutes. On days two and three, groups of five zebrafish were tested for 15 minutes. On days four and five, pairs of fish were tested for 10 minutes. Day six consisted of training two fish at a time for 5 minutes, while on day seven one subject at a time was tested for 5 minutes. Each day, the fish had access to the established stimulus (food).

3.5 Study 4

Animal model used in the study (*Danio rerio*)

A total of 80 wild-type zebrafish were used for these experiments. The fish were obtained from a local breeder and treated according to the specific European standards imposed for the treatment of laboratory animals, with approval from the relevant ethics committees. Several consecutive memory tests were performed.

The experimental groups were as follows:

Group 1 – Control (20 fish)

Group 2 – Methionine (20 fish)

Group 3 – Methionine and Amisulpride (20 fish)

Group 4 – Methionine and Clozapine (20 fish)

3.5.1 Substance administration

Methionine was administered for 7 days by introducing it directly into the water at a concentration of 4.5 mM. The solution was freshly prepared and changed daily throughout the treatment period. This concentration was selected because it has properties capable of inducing schizophrenia-like symptoms [44]. Following the methionine treatment period, the antipsychotics were freshly prepared. Amisulpride (AMI) was used at a concentration of 10 μ L/mL, the dose used in the study conducted by Leggieri et al. [48], whereas clozapine (CLZ) was used at

concentrations of 17 $\mu\text{L/mL}$ and 34 $\mu\text{L/mL}$ [46]. These doses were selected from standard human treatment regimens and converted according to zebrafish body mass, a widely used approach in this type of research [47]. On the day of administration, individual zebrafish were placed in 50 mL of antipsychotic solution for 5 minutes.

3.5.2 T-maze test

The fish were acclimatized to the apparatus by placing a transparent liftable barrier; removing the barrier allowed movement from one compartment to another. This was done to ensure that the fish learned about the existence of two different compartments in the apparatus. The fish were allowed to explore the apparatus for five minutes. The apparatus used was a T-maze with red and green sleeves attached to its arms.

3.5.3 Social preference test

The social preference test was performed according to the model of R. B. Jones et al. (1999), in which a T-maze was adapted to assess social preference in chicks. We adapted this test for zebrafish. Three zebrafish were placed in the left arm and hermetically enclosed behind a transparent divider.

3.5.4 Novel Object Recognition test

The protocol included one day of fish acclimatization, followed by one training day during which the fish were exposed to one of the objects for 10 minutes. On the final day, both objects (a blue ball and a yellow-colored ball) were placed in the aquarium, and behavior was recorded and the number of mouth contacts with the objects was quantified. The balls were fixed at the water surface using thread.

CHAPTER IV. RESULTS

4.1 Experiment 1

4.1.1 Mini-Mental State Examination (MMSE)

Regarding the results obtained for short-term memory (2A), differences were recorded only between the CTR group and the other groups, namely the PIT group ($p = 0.030$) and the MOT group ($p < 0.001$). However, no statistically significant differences were recorded between the two antipsychotic-treatment groups. For the writing task (2B), the PIT group showed statistically significant differences compared with CTR ($p = 0.019$). The results obtained for the copying task (2C) showed differences between PIT and MOT ($p = 0.013$), as well as differences between MOT and CTR ($p < 0.001$) (Fig. 4.2).

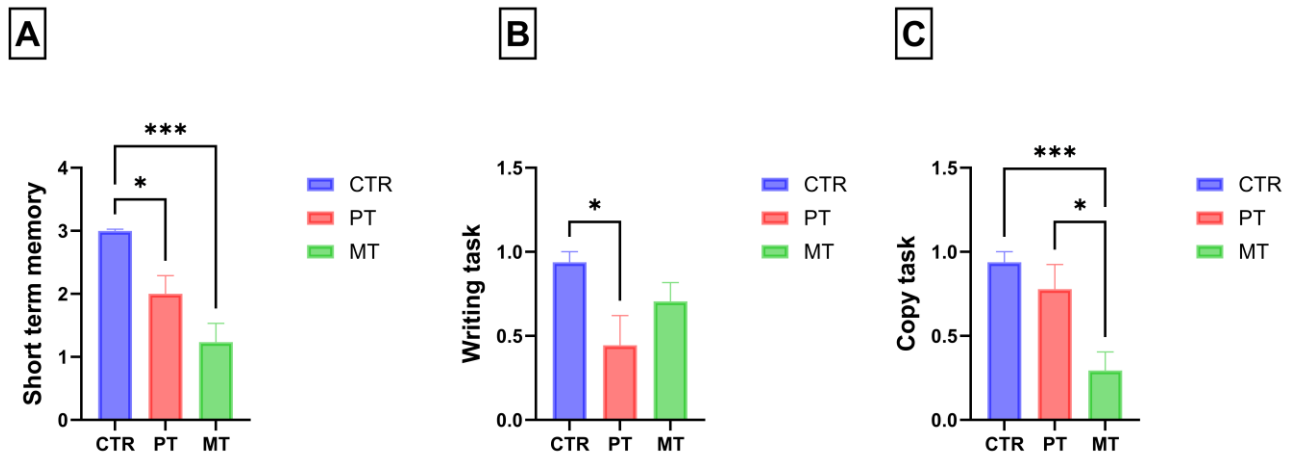


Figure 4.2. Graphical representations of the results for short-term memory (A); results for the writing task (B); results for the copying task (C). Data are presented as mean $\pm$ SEM for CTR ($n = 16$), PIT ($n = 9$), and MOT ($n = 18$); “*” indicates statistical significance at $p < 0.05$ for one-way ANOVA.

4.1.2 Montreal Cognitive Assessment (MoCA)

The attention parameter (4A) was significant for the comparisons CTR vs. PIT ($p = 0.031$) and CTR vs. MOT ($p < 0.001$). Another significant result was the difference between the two treatment groups ($p = 0.007$). Similarly, for the drawing task (4B), differences were significant between CTR and both treatment groups ($p < 0.001$). For the third parameter presented graphically, namely visuospatial-executive function (4C), CTR and PIT showed significantly higher values than MOT ($p < 0.001$). For spatial orientation (4D), differences were recorded between CTR and MOT ($p = 0.002$) (Fig. 4.4).

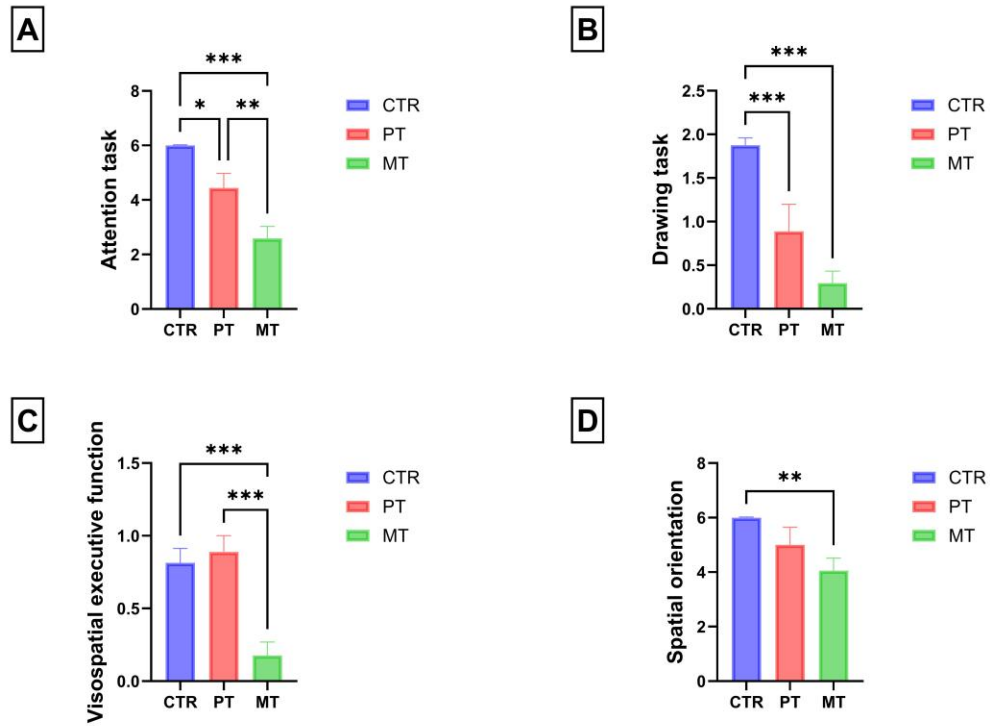


Figure 4.4. Graphical representations of the attention task (A); results for the drawing task (B); visuospatial-executive function (C); and spatial orientation (D). Data are presented as mean $\pm$ SEM for CTR ($n = 16$), PIT ($n = 9$), and MOT ($n = 18$); “*” indicates statistical significance at $p < 0.05$ for one-way ANOVA.

4.1.3 Metacognitions Questionnaire-30

For confidence (Fig. 6A) and cognitive self-consciousness (Fig. 6C), similar effects were observed in participants with schizophrenia and healthy participants. However, positive beliefs about worry (Fig. 6B) were more pronounced among individuals treated with RIS+ARI/QUE compared with controls ($p = 0.001$), and a difference was also observed between the CLO+AMI and RIS+ARI/QUE groups ($p = 0.028$) (Fig. 4.6).

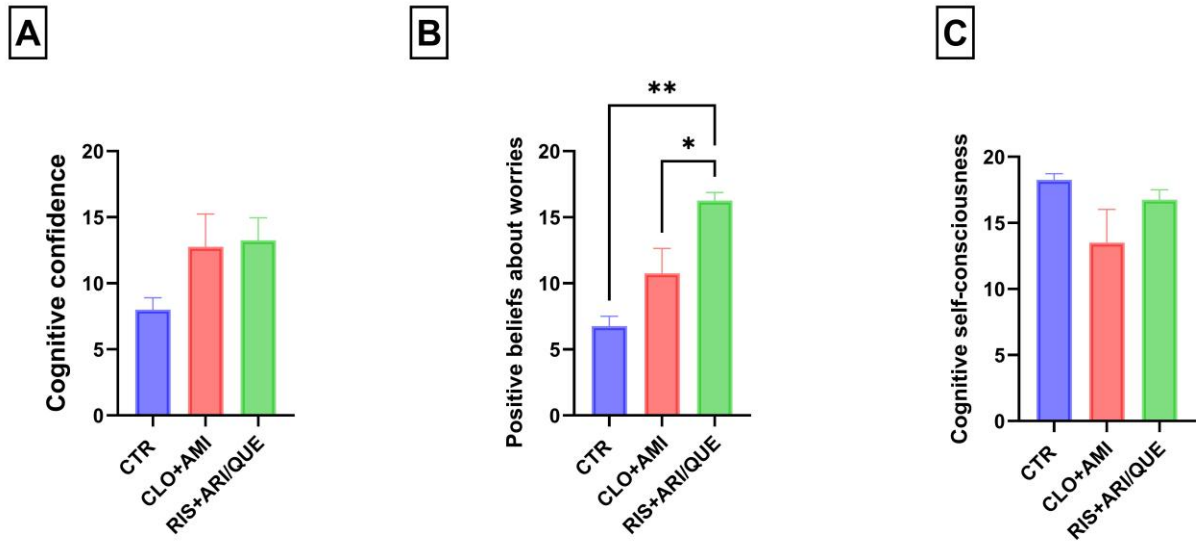


Figure 4.6. Graphical representation of MCQ-30 scores for the cognitive confidence subscale (A); positive beliefs about worry (B); and cognitive self-consciousness (C). Data are presented as mean $\pm$ SEM for CTR ($n = 4$), CLO+AMI ($n = 4$), and RIS+ARI/QUE ($n = 4$); “*” indicates statistical significance at $p < 0.05$ for one-way ANOVA [40].

4.2 Study 2

4.2.1 T-maze memory test

Regarding the cognitive-specific parameters, swimming latency to the decision point was significantly greater in KET+RIS compared with the RIS-treated group ($p = 0.005$) (Fig. 9A). However, no statistically significant values were recorded for the frequency of leftward rotations (Fig. 9B). Both RIS ($p = 0.012$) and KET+RIS ($p = 0.012$) spent significantly less time in the right arm of the maze compared with the CTR group (Fig. 9C), while the KET group ($p = 0.050$) spent significantly less time compared with the HAL group. No differences were recorded between these groups for time spent in the left arm (Fig. 9D) (Fig. 4.9) [42].

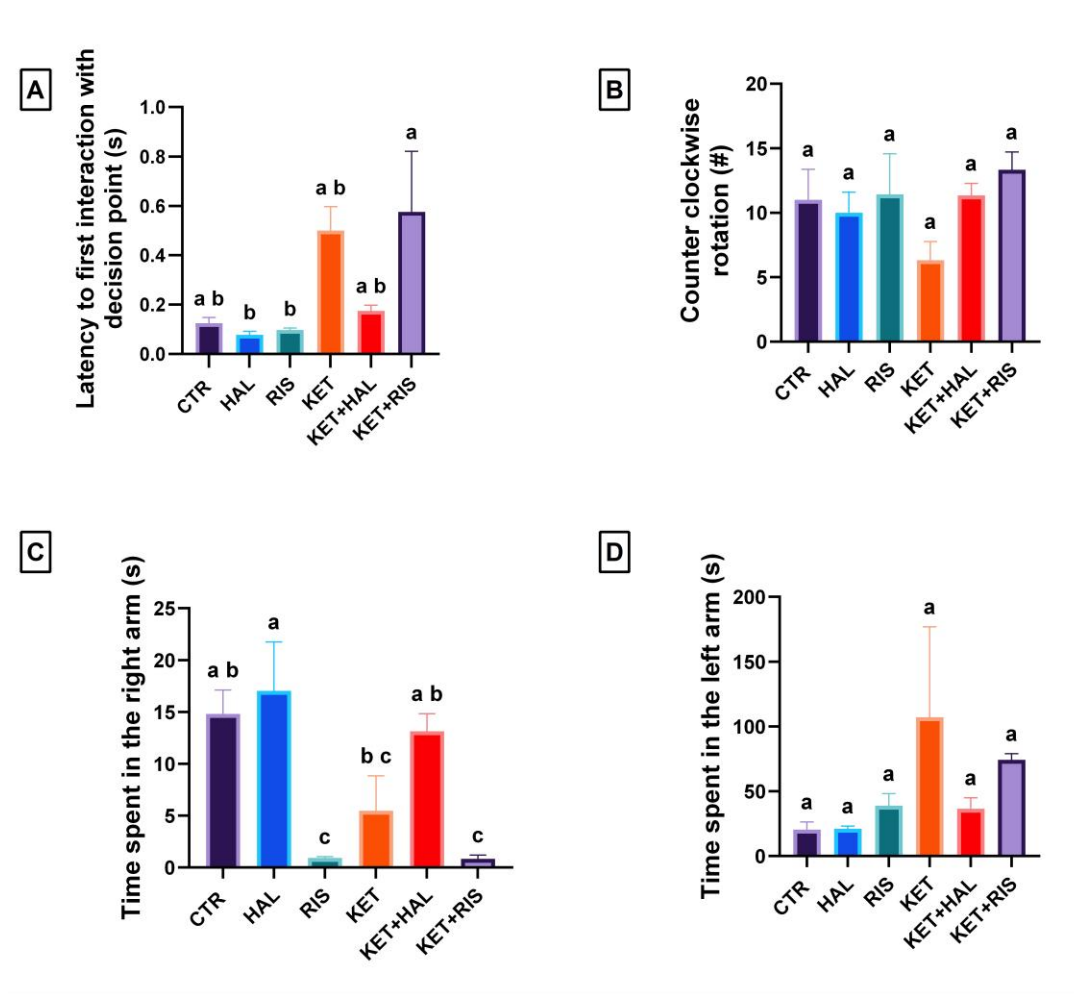


Figure 4.9. Results of the behavioral T-maze test for cognitive parameters (HAL = 0.13 mg/L; RIS = 0.08 mg/L; KET = 0.1 mg/mL). During a 5-minute test, cognitive and swimming parameters including (A) swimming latency to the decision point (s), (B) frequency of leftward rotations (#), (C) time spent in the right arm (s), and (D) time spent in the left arm (s) were recorded and analyzed. Results are presented as means $\pm$ SEM ($n = 5$ animals/group; a–c, Tukey's HSD test) [42].

4.2.2 Floating novel object test

Regarding the behavioral parameters describing interaction with the objects, we observed that the time spent showing interest in spatial stimuli (body contact with the objects) was significantly increased after HAL and RIS treatment in healthy controls (CTR vs. HAL, $p < 0.001$; CTR vs. RIS, $p < 0.001$), whereas the opposite effect was observed in the KET-treated groups (CTR vs. KET, $p < 0.001$; CTR vs. KET + HAL, $p < 0.001$; CTR vs. KET + RIS, $p < 0.001$) (Fig. 11A).

We also observed that interest in the silver ball was significantly higher in the KET + HAL group compared with CTR ($p < 0.001$) and HAL ($p < 0.001$). At the same time, RIS treatment induced lower interest in the silver ball compared with HAL in KET-treated groups (KET + HAL vs. KET + RIS, $p < 0.001$) (Fig. 11B). Proximity to the silver object was significantly greater after KET + RIS treatment compared with KET ($p = 0.004$) and KET + HAL (p

< 0.001). Conversely, interaction with the red ball was significantly less frequent in the RIS- and KET-treated groups compared with CTR ($p_{RIS} = 0.019$) and HAL ($p_{RIS} = 0.002$, $p_{KET} = 0.021$) (Fig. 11C) [42].

Latency to interaction with the silver object was significantly greater in the RIS-treated group compared with CTR ($p_{RIS} < 0.001$), HAL ($p_{RIS} = 0.001$), and KET ($p_{RIS} < 0.001$). KET-treated animals also showed greater interest in the silver ball compared with HAL-treated animals ($p = 0.039$), whereas RIS treatment appeared to reduce latency to interaction with the silver ball ($p < 0.001$). Conversely, for the red ball (the novel object), latency to interaction was significantly greater after RIS treatment compared with CTR ($p < 0.001$), KET ($p < 0.001$), and KET + RIS ($p < 0.001$) (Fig. 11D) [42].

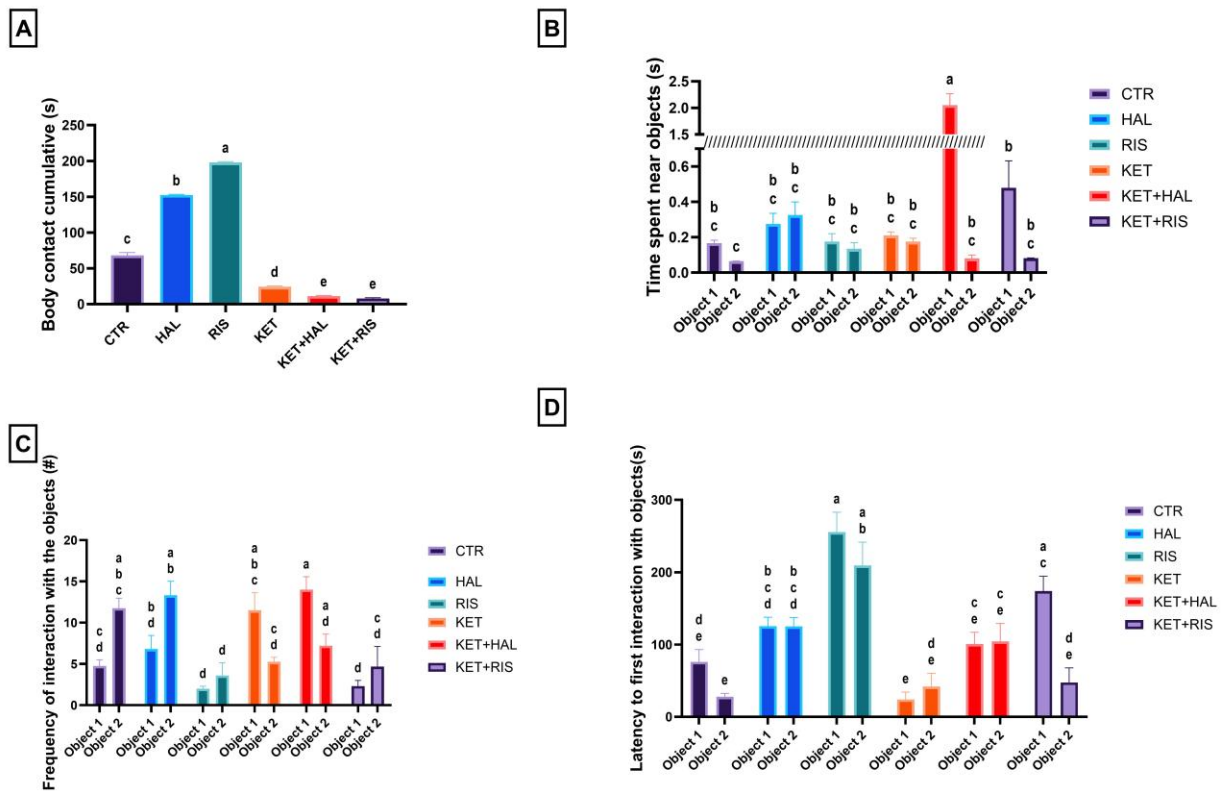


Figure 4.11. Graphical representations of memory and learning parameters measured using the novel object test (HAL = 0.13 mg/L; RIS = 0.08 mg/L; KET = 0.1 mg/mL). During a 5-minute test, memory and learning parameters including (A) duration of contact between fish (s), (B) time of interaction with objects (s), (C) frequency of interaction with objects (#), and (D) latency to interaction with objects (s) were recorded and analyzed. Results are presented as means $\pm$ SEM ($n = 5$ animals/group; (A) a–c, Tukey's HSD test; (B–D) a–e, Šídák test for multiple comparisons) [42].

4.2.3 Social interaction test

Regarding the parameters specifically describing social interaction behaviors, we observed that time spent at the decision point increased significantly in all KET-treated groups compared with the control group (CTR *vs.* KET, $p < 0.001$; CTR *vs.* KET + HAL, $p < 0.001$; CTR *vs.* KET + RIS, $p < 0.001$). Both HAL and RIS reduced decision time compared with the model group (KET *vs.* KET + HAL, $p = 0.015$; KET *vs.* KET + RIS, $p = 0.001$), but it remained significantly increased compared with the untreated control group (KET + HAL *vs.* CTR, $p < 0.001$; KET + RIS *vs.* CTR, $p < 0.001$) (Fig. 13A).

Similarly, cumulative time spent in the left arm, containing the social stimuli, decreased significantly in all KET-treated groups compared with untreated controls (CTR *vs.* KET, $p = 0.009$; CTR *vs.* KET + HAL, $p = 0.004$; CTR *vs.* KET + RIS, $p = 0.005$). HAL and RIS did not influence preference for social stimuli in either KET-naïve or KET-treated animals (Fig. 13B).

The frequency of clockwise rotations decreased following KET treatment compared with untreated controls (CTR *vs.* KET, $p < 0.001$), suggesting a potential anxiety-like effect. HAL and RIS also induced similar effects in healthy animals (CTR *vs.* HAL, $p < 0.001$; CTR *vs.* RIS, $p = 0.018$), although the effect was less pronounced than in the KET group for RIS treatment (RIS *vs.* KET, $p < 0.001$). HAL, but not RIS, significantly attenuated anxiety-like behavior in KET-treated animals (HAL *vs.* KET + HAL, $p = 0.045$), although not to levels comparable with CTR (CTR *vs.* KET + HAL, $p = 0.018$) (Fig. 13C).

The frequency of counterclockwise rotations was significantly reduced by KET treatment and remained significantly decreased following RIS treatment compared with untreated controls (CTR *vs.* KET, $p = 0.041$; CTR *vs.* KET + RIS, $p = 0.016$) (Fig. 13D).

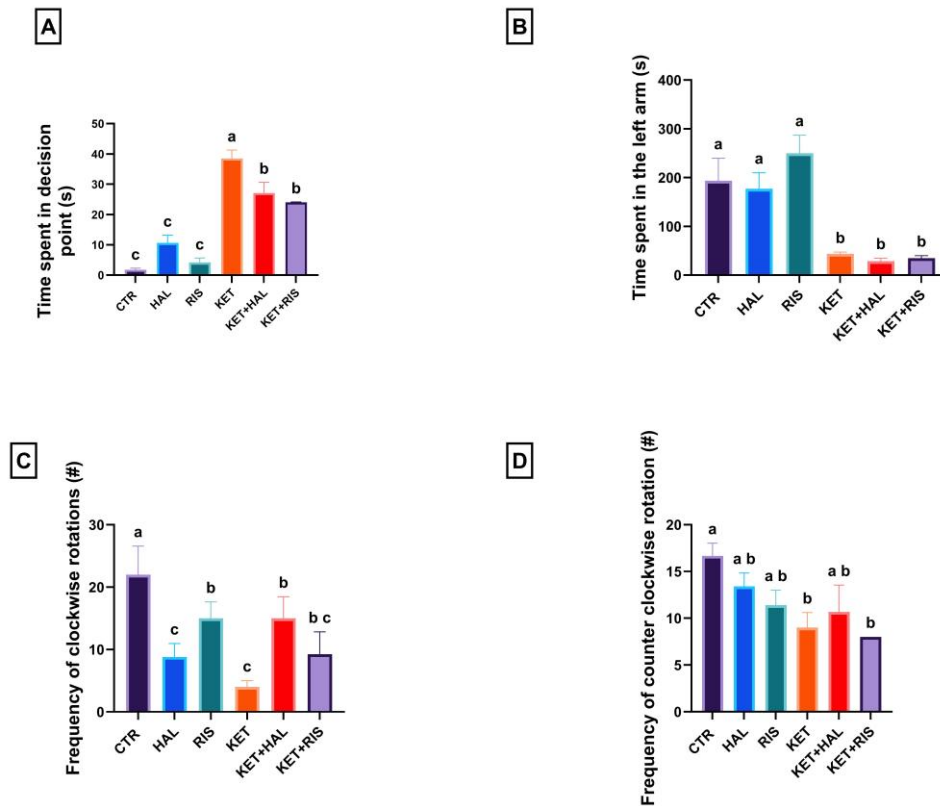


Figure 4.13. Behavioral assessment of sociability as evaluated using the social interaction test (HAL = 0.13 mg/L; RIS = 0.08 mg/L; KET = 0.1 mg/mL). During the 5-minute test, locomotor and anxiety-like behavioral parameters, including (A) decision time between the two arms (s), (B) time spent in the left arm (s), (C) frequency of rightward rotations (#), and (D) frequency of leftward rotations (#), were recorded and analyzed. Results are presented as means $\pm$ SEM ($n = 5$ animals/group; a–c, Tukey HSD test) [42].

4.3 Study 3

4.3.1 T-maze memory test

For the first interaction with the stimulus arm (14A), differences were observed between the MET+OLA₂₅ group and CTR ($p = 0.011$), as well as between MET+OLA₂₅ and MET ($p = 0.014$). For the frequency of entries into the stimulus arm (14B), the values for the MET group ($p = 0.009$) and the CLZ-treated group ($p = 0.001$) were lower than those of the healthy control group. Time spent in the stimulus arm (14C) did not show any statistically significant values (Fig. 4.14).

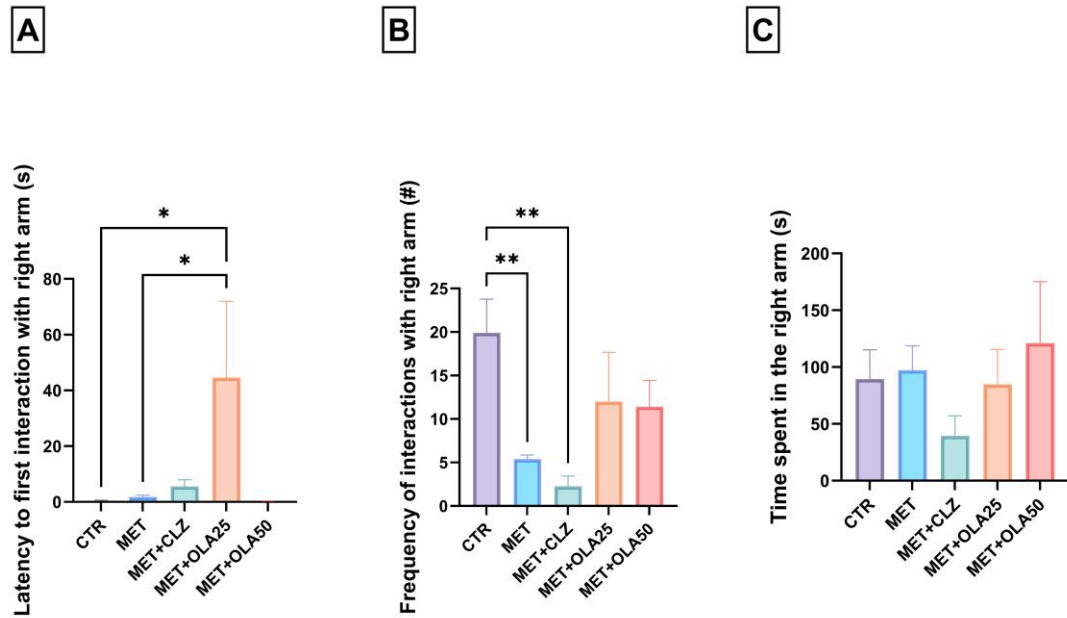


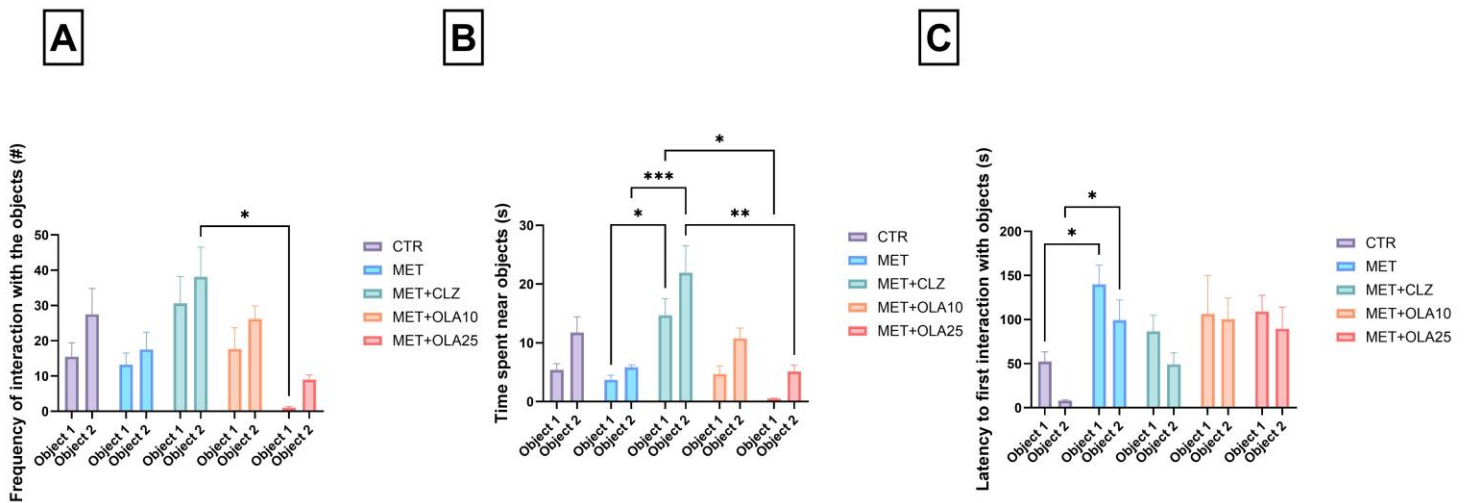
Figure 4.14. Results of the behavioral T-maze test for swimming parameters (MET = 0.674 mg/L; MET+CLZ = 34 μ L/mL; MET+OLA25 = 0.7 μ L/mL; MET+OLA50 = 1.4 μ L/mL). During a 5-minute test, anxiety- and memory-related parameters including (A) latency to the first interaction with the right arm (s), (B) frequency of entries into the right arm (#), and (C) time spent in the right arm (s) were recorded and analyzed. Results are presented as means $\pm$ SEM ($n = 10$ animals, one-way ANOVA).

4.3.2 Novel Object Recognition test

From the perspective of exploratory and memory-related behavior, a series of results were observed. First, for the frequency of direct interactions with the object (19A), differences were recorded between the red and yellow objects (MET+OLA25 vs. MET+CLZ, $p = 0.012$). For the time spent interacting with the two objects (19B), the MET+CLZ combination showed differences compared with the other groups for both objects: for object 1 (red), MET+CLZ vs. MET ($p = 0.042$) and MET+CLZ vs. MET+OLA₂₅ ($p = 0.022$); for object 2 (yellow), MET+CLZ vs. MET ($p < 0.001$) and MET+CLZ vs. MET+OLA₂₅ ($p = 0.002$). For the first interaction with the objects (19C),

increased latency to the first interaction was observed in MET compared with CTR for both objects: for the red object, CTR vs. MET ($p = 0.032$); and for the yellow object, CTR vs. MET ($p = 0.020$) (Fig. 4.19).

Figure 4.19. Results of the novel object recognition test in the aquarium for swimming parameters (MET = 0.674 mg/L; MET+CLZ = 34 μ L/mL; MET+OLA25 = 0.7 μ L/mL; MET+OLA50 = 1.4 μ L/mL). During a 5-minute test, memory and exploratory parameters including (A) frequency of interaction with both objects (#), (B) time spent



interacting with both objects (s), and (C) latency to the first interaction with both objects (s) were recorded and analyzed. Results are presented as means $\pm$ SEM ($n = 10$ animals, two-way ANOVA).

4.3.3 Social interaction test

For the memory parameters within the arm containing the stimulus of interest, the following phenomena were observed. No significant changes were identified in the frequency of entries into the left arm (21A). A decrease in interaction time with conspecifics (21B) was observed for MET+CLZ vs. CTR ($p = 0.0043$). Another statistically significant difference was observed between MET+OLA₂₅ and MET+OLA₅₀ ($p = 0.038$) for latency to the first interaction with the left arm (21C).

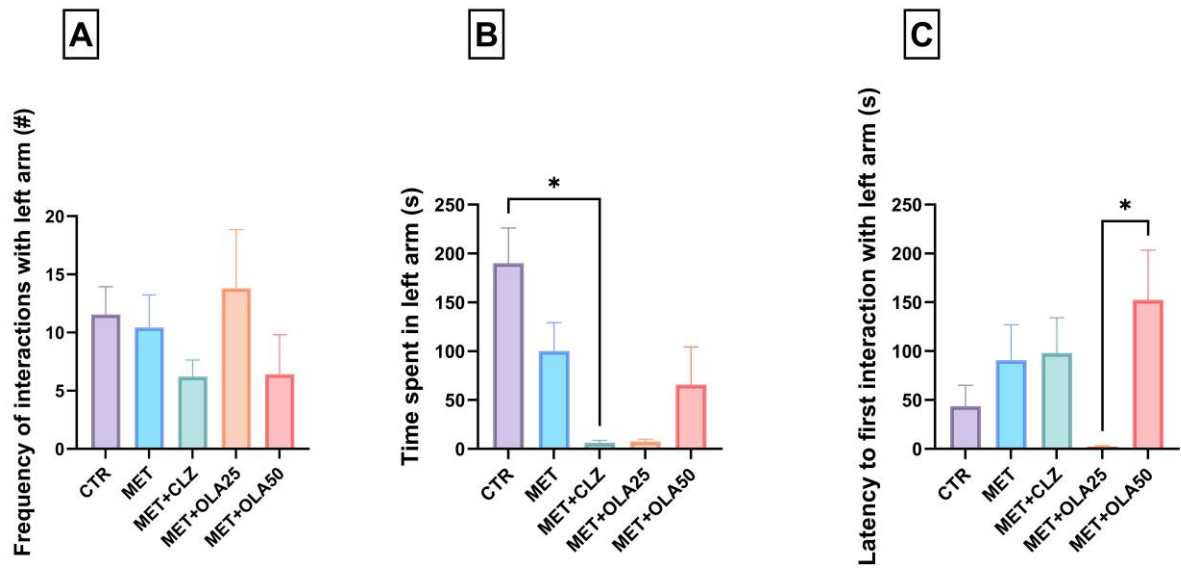


Figure 4.21. Results of the social preference test for swimming parameters (MET = 0.674 mg/L; MET+CLZ = 34 μ L/mL; MET+OLA25 = 0.7 μ L/mL; MET+OLA50 = 1.4 μ L/mL). During a 5-minute test, sociability parameters including (A) frequency of entries into the left arm (#), (B) time spent in the left arm (s), and (C) latency to the first interaction with the left arm (s) were recorded and analyzed. Results are presented as means $\pm$ SEM (n = 10 animals, one-way ANOVA).

4.4 Study 4

4.4.1 T-maze memory test

Regarding parameters associated with the stimulus arm, neither the distance traveled in the right arm nor the distance swum at the decision point (25A and 25C) showed significant differences between groups. However, for time spent in the right arm (25B), the amisulpride-treated group showed significant differences compared with the other study groups: MET+AMI vs. CTR (p = 0.003), MET+AMI vs. MET (p = 0.034), MET+AMI vs. MET+CLO₃₀₀ (p = 0.009), and MET+AMI vs. MET+CLO₆₀₀ (p = 0.004) (Fig. 4.25).

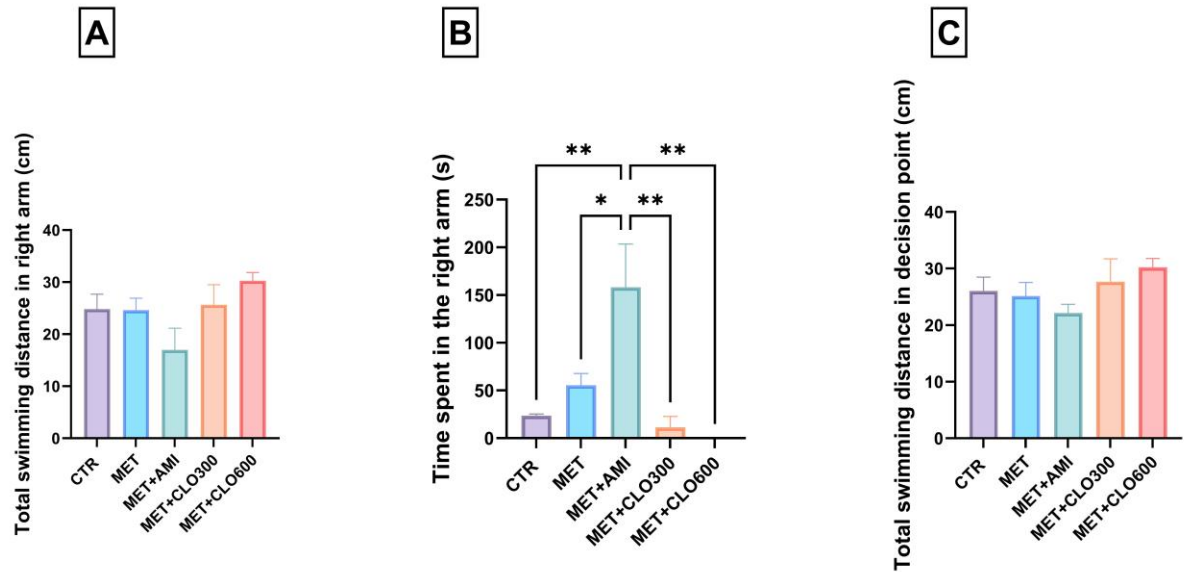


Figure 4.25. Results of the behavioral T-maze test for memory parameters ($MET = 0.674 \text{ mg/L}$; $MET+AMI = 10 \text{ } \mu\text{L/mL}$; $MET+CLO300 = 17 \text{ } \mu\text{L/mL}$; $MET+CLO600 = 34 \text{ } \mu\text{L/mL}$). During a 5-minute test, memory parameters including (A) distance swum in the right arm (cm), (B) time spent in the right arm (s), and (C) distance swum at the decision point (cm) were recorded and analyzed. Results are presented as means $\pm$ SEM ($n = 10$ animals, ANOVA test).

4.4.2 Novel Object Recognition test

Regarding parameters quantifying interaction with the yellow object, interaction time with the object (28A) in the CTR group showed significant differences compared with all other groups included in the study ($p_{MET} < 0.001$; $p_{MET+AMI} < 0.001$; $p_{MET+CLO300} = 0.028$; $p_{MET+CLO600} = 0.012$). No differences were recorded for interaction with the red object. For the yellow object, a difference was recorded between the AMI-treated group and CLO300 ($p = 0.033$). Time spent in the vicinity of the objects (28B) showed differences between CTR and all other study groups for the yellow object ($p < 0.001$), as well as a statistically significant difference between AMI and CLO600 ($p = 0.022$) (Fig. 4.28).

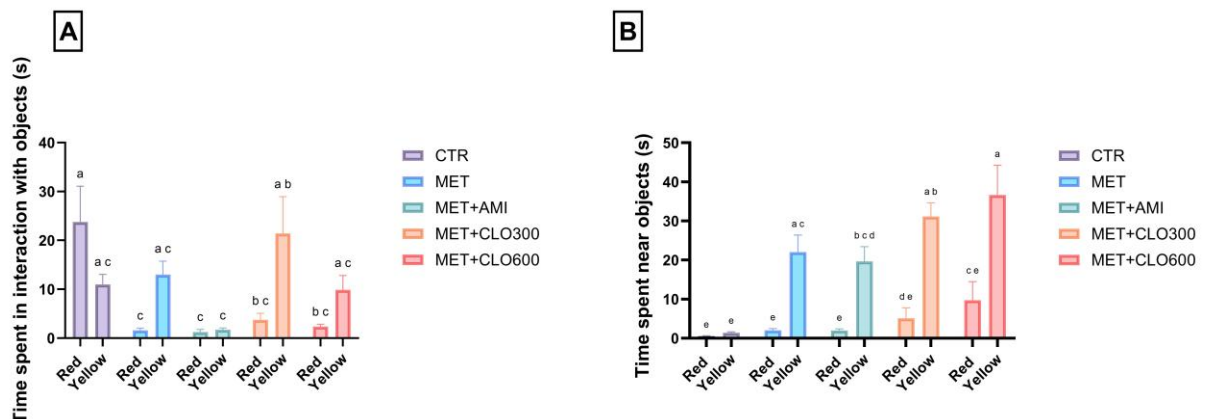


Figure 4.28. Results of the novel object recognition test in the aquarium for swimming parameters (MET = 0.674 mg/L; MET+AMI = 10 μ L/mL; MET+CLO300 = 17 μ L/mL; MET+CLO600 = 34 μ L/mL). During a 5-minute test, swimming parameters including (A) time of interaction with the objects (s) and (B) time spent in the vicinity of the objects (s) were recorded and analyzed. Results are presented as means $\pm$ SEM (n = 10 animals, Tukey HSD test; parameters sharing the same letter do not differ significantly, (A) a–c; (B) a–e).

4.4.3 Social preference test

Regarding the sociability parameters, neither the distance swum in the stimulus arm nor its mean value (31A and 31B) showed statistically significant differences. However, for the distance swum in the right arm (31C), differences were recorded for CLO₆₀₀ compared with CTR (p = 0.014) and AMI (p = 0.0045) (Fig. 4.31).

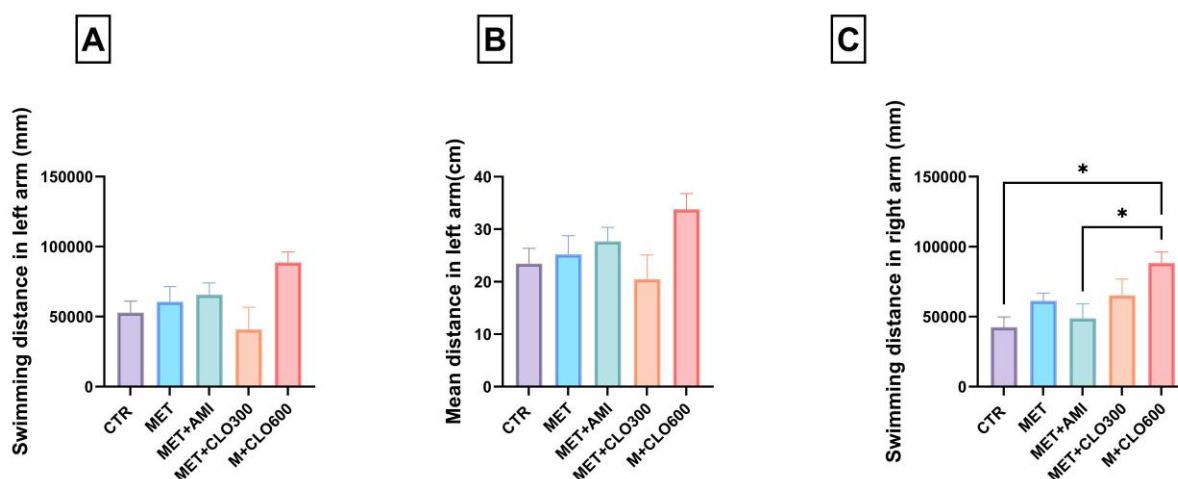


Figure 4.31. Results of the social preference test for swimming parameters (MET = 0.674 mg/L; MET+AMI = 10 μ L/mL; MET+CLO300 = 17 μ L/mL; MET+CLO600 = 34 μ L/mL). During a 5-minute test, swimming parameters including (A) total distance swum (cm), (B) swimming velocity (cm/s), and (C) distance swum at the decision point (cm) were recorded and analyzed. Results are presented as means $\pm$ SEM (n = 10 animals, one-way ANOVA).

CHAPTER V. DISCUSSION

5.1 Experiment 1

5.1.1 MMSE & MoCA

Honer et al. [49] conducted an experiment in which patients with schizophrenia received combination treatment (clozapine and risperidone) to evaluate reductions in positive and negative symptoms. Their results showed no significant differences between monotherapy and polypharmacy. This finding is comparable with the observations of our team, as no differences were observed between the monotherapy and polypharmacy groups with respect to short-term memory and the writing task.

Wang et al. [50] investigated the possible relationship between motor dysfunction and schizophrenia symptomatology, observing that reductions in negative symptoms were associated with increased motor capacity and general mobility. Results obtained by Lähteenvuo et al. demonstrated a similar phenomenon, namely a reduction in negative symptoms among patients receiving polypharmacy compared with those receiving monotherapy [51]. This aspect can also be observed in the results obtained by our team.

The drawing task in the MoCA test required participants to draw a clock indicating ten past nine. Bozikas et al. [52] applied a similar task and found that lower scores on this test correlated with higher values on the PANSS (Positive and Negative Syndrome Scale), suggesting that this type of task may be useful for assessing the patient's cognitive status. Although no significant differences were identified between monotherapy and polypharmacy, their results are comparable with those obtained by our team.

5.1.2 Metacognitions Questionnaire-30 (MCQ-30)

Although no significant differences emerged with respect to cognitive confidence, patients treated with combinations of risperidone with aripiprazole or quetiapine demonstrated stronger positive beliefs about worry compared with both the control group and patients treated with clozapine and amisulpride. This pattern may reflect an increased tendency toward cognitive rigidity or maladaptive appraisal of worry as a coping mechanism, a phenomenon frequently described in schizophrenia [53,54]. The amplification of such beliefs may contribute to rumination and intrusive thought processes that maintain psychological distress and symptom persistence.

Self-reflexive awareness (cognitive self-consciousness) was highest among healthy participants compared with those with schizophrenia, consistent with established evidence that schizophrenia is frequently associated with reduced introspective and metacognitive capacity [55,56]. Pharmacological factors may contribute: certain agents may affect cognitive flexibility through prefrontal dopaminergic mechanisms or secondary sedative effects [57,58]. Such medication-related influences on cognition may interact with disease-related deficits to further restrict metacognitive awareness.

5.2 Study 2

5.2.1 T-maze memory test

Our KET-based zebrafish model, previously characterized behaviorally and validated by our group [59], reflects some of the behavioral characteristics specific to SCZ, such as locomotor deficits suggesting dissociative effects, confusion, and impairment of cognitive decision-making processes. Across all behavioral assessment paradigms (T-maze memory task, NOR task, and social preference task), we observed that the principal locomotor parameters were significantly affected in KET-treated groups.

Vinnakota et al. [60] observed that, in a mouse model, KET induced alterations of the NMDA receptor at the GluN2D subunit, which is responsible for excitatory neurotransmission and synaptic plasticity, resulting in locomotor disturbances and anxiety-related behavior.

Furthermore, behavioral parameters associated with cognitive decision-making processes, such as latency to move toward the decision point, time spent at the decision point, clockwise and counterclockwise rotations, were significantly altered by KET exposure. Thus, behaviors such as excessive circling and increased swimming capacity were also observed in our model, as described in previous studies [61,62]. Another important characteristic successfully modeled by KET exposure may be related to associative learning impairments observed in patients with SCZ. Because of glutamatergic dysfunction, both patients with SCZ and KET-exposed zebrafish models have shown slower formation of spatial memory [63–65]. Our results showed that KET-exposed groups spent more time at decision points as a consequence of impaired spatial memory regarding the position of food stimuli, familiar or novel objects, and conspecifics.

5.2.2 Novel Object Recognition test

Numerous previous studies have demonstrated that impairment of dopamine pathways, particularly through D2 receptor antagonism by HAL, may correct KET-induced abnormal reward-related processing [66,67]. Conversely, previous research indicates that KET-induced NMDA receptor hypofunction may impair synaptic plasticity and memory encoding, thereby influencing the compensatory effects of antipsychotic treatment [68,69]. On the other hand, other studies [70] have reported that HAL treatment did not improve the ability of mice to retrieve learned information in memory tests. These conflicting reports may result from an incompletely understood drug-interaction mechanism [24].

Another important effect of combined KET-RIS treatment was obtained during the NOR test. In contrast to the original protocol of Gaspary et al. [71], Magyary [43] introduced a significant improvement by replacing static objects embedded in the environment with floating objects at the water surface to stimulate feeding-associated and exploratory behaviors. Our results suggested that combined KET-RIS exposure leads to significantly reduced exploratory behavior, most likely because of RIS-induced freezing behavior, as also observed in the T-maze memory task. Egan et al. [72] explained the occurrence of KET-induced freezing behavior by the anesthetic properties of KET; however, the current doses were selected to avoid sedative effects. Rogóz [73] and Samizadeh et al. [74] did not

associate reduced exploratory behavior with antipsychotic treatment in the NOR task; therefore, further studies should focus on elucidating these contradictory results.

5.2.3 Social preference test

Deficits in social interaction are also important components of the behavioral changes associated with SCZ. Both positive and negative symptoms of SCZ, as well as memory and learning deficits, may contribute to these alterations. Social detachment has previously been described as a typical behavior among patients with SCZ; however, little is known about the effects of antipsychotics in this respect. Our results suggested that KET-induced impairment of social interaction could not be attenuated by the doses of either typical or atypical antipsychotics tested in the present study. Marked avoidance of the arm containing social stimuli and the significantly increased presence of KET-treated animals at the decision point during the social preference task, regardless of antipsychotic treatment, may suggest that social withdrawal could be caused by memory and learning deficits. Several studies have previously described the association between social withdrawal in patients with SCZ and impaired attention, verbal memory, and working memory [75,76]. In addition, impaired social interaction has previously been documented in RIS-treated zebrafish larvae [77], consistent with some studies in patients with SCZ reporting impaired social behavior or no social benefit following antipsychotic treatment [78,79]. These changes may reflect convergence between NMDA receptor hypofunction, which disrupts cortical networks required for social cognition, and dopaminergic dysregulation, which may affect reward-related aspects of social interaction [68,80,81].

5.3 Study 3

5.3.1 T-maze memory test

The results of the present study highlight the behavioral effects of methionine (MET), clozapine (CLZ), and olanzapine (OLA), administered individually or in combination, on memory capacity. The results for latency to the first interaction with the right arm, frequency of entries, and time spent in the right arm suggest that these substances distinctly influence the initiation and dynamics of memory-related behavior.

Regarding latency to the first interaction with the right arm, the MET + OLA25 combination produced a significant increase in this parameter compared with the control and MET + CLZ groups. This delay may reflect reduced initiation of exploratory behavior and increased behavioral inhibition. From a pharmacological perspective, olanzapine has high affinity for histamine H1 receptors, and their antagonism is well known as a major mechanism underlying sedation and psychomotor slowing associated with newer-generation antipsychotics [82,83]. Analysis of the frequency of entries into the right arm revealed a significant decrease in the MET and MET + CLZ groups compared with controls, suggesting reduced learning and memory processes. Associative learning is closely related to activation of mesolimbic dopaminergic synapses, and their disruption represents a central mechanism in schizophrenia [67]. Methionine, through its effects on transmethylation and monoamine metabolism, may contribute to fragmented exploratory behavior characterized by relatively intact initiation but reduced frequency of re-entries.

5.3.2 Novel Object Recognition test

Analysis of time spent interacting with objects revealed robust differences in the MET+CLZ group compared with MET and MET+OLA25, for both the familiar and novel objects. Increased interaction time with objects in the MET+CLZ group suggests more consistent exploration and possible improvement in novelty processing, compatible with an ameliorating effect on methionine-induced deficits. The literature supports the interpretation that prolonged exploration of a novel object reflects efficient memory capacity and preserved discrimination between familiar and novel stimuli [25,84].

In contrast, the lower values observed in the MET and MET+OLA25 groups may reflect either impaired recognition memory, increased anxiety, or reduced exploratory behavior. Given the role of methionine in one-carbon metabolism and the regulation of transmethylation, the observed pattern may be associated with epigenetic and synaptic changes affecting hippocampal–perirhinal cortex circuits, which are important for object recognition [85–87].

Regarding latency to the first interaction with the objects, the significant increase observed in the MET group compared with controls for both objects suggests a delay in the initiation of exploration. This pattern is frequently interpreted as an indicator of increased anxiety or reduced interaction with novel stimuli rather than a memory deficit per se [88,89]. The fact that this increased latency was present for both objects supports the hypothesis of a generalized alteration in the initiation of exploratory behavior, potentially associated with changes in visual processing.

5.3.3 Social interaction test

Changes in latency and time spent at the decision point suggest impairment of behavioral-response selection processes and social exploration strategies. In T-maze paradigms used in zebrafish, increased time spent at decision points is frequently interpreted as an indicator of indecision or reduced capacity to process the environment rather than social avoidance itself [89,90]. In this respect, the data obtained suggest that the methionine-induced schizophrenia model may involve dysfunction of spatial and social information integration, affecting the organization of baseline behavior.

A particularly relevant finding is represented by changes in time spent in the stimulus arm and latency to the first interaction with it, indicating an influence of MET on spatial strategies and behavioral flexibility in a social context. Considering the role of methionine in one-carbon metabolism and SAM transmethylation, the literature suggests that these effects may reflect changes in the expression of genes involved in synaptic plasticity and cortico-limbic synaptic function, which are essential for social cognition [85–87].

The fact that antipsychotics partially modulated these variables suggests an ameliorating effect on certain components of social behavior, although without complete restoration. This observation is consistent with clinical data showing that, although antipsychotics may improve positive symptoms and certain affective components, benefits for social functioning are often limited and context-dependent [91,92]. Clozapine, in particular, has been described as having more favorable effects on certain functional domains compared with other antipsychotics; however, these effects do not translate uniformly into improvements in social behavior [93,94].

5.4 Study 4

5.4.1 T-maze memory test

Regarding parameters associated with the stimulus arm, the results indicate a selective behavioral profile characterized predominantly by changes in the duration of stationary behavior rather than spatially directed mobility. Thus, the distance traveled in the right arm and the distance swum at the decision point did not show significant differences between groups, suggesting that the treatments did not substantially affect orientation capacity. In contrast, time spent in the right arm was significantly increased in the MET+AMI group in the absence of a corresponding increase in locomotor activity, suggesting freezing-like behavior or difficulty initiating active exploratory behavior in the stimulus area. In behavioral paradigms, this pattern is interpreted as a dissociation between presence in an area and active exploration of that area, and is associated with dopaminergic inhibition or behavioral rigidity [88,89].

5.4.2 Novel Object Recognition test

An important methodological element of this test is the use of a floating object as the novel stimulus, introducing an additional component of perceptual and spatial complexity. Interaction with such a stimulus requires integration of sensory information and three-dimensional evaluation of the environment, processes known to be affected in schizophrenia, particularly within the cognitive symptom domain. Novel-object paradigms are frequently used to assess novelty processing and attention, including in the context of schizophrenia [95], and their adaptation to the zebrafish model contributes to the translational relevance of the results obtained.

Analysis of behavioral parameters quantifying interaction with objects revealed significant differences between experimental groups, suggesting relevant alterations in novel-stimulus processing and exploratory behavior in the methionine-induced model. In particular, time spent interacting with the yellow object was significantly higher in the control group compared with all other experimental groups, indicating intact exploratory behavior and a clear preference for the novel stimulus under physiological conditions. This profile is consistent with existing literature showing that zebrafish display robust investigation of novel objects, frequently used as an indicator of the integrity of cognitive and attentional processes [96].

In the methionine-treated group, the significant reduction in time spent interacting with the yellow object suggests impaired novelty processing and reduced ability to discriminate relevant environmental stimuli. Such behavioral changes are compatible with the hypothesis that methionine can induce a schizophrenia model characterized by cognitive deficits and disturbances in attentional processes without directly affecting swimming capacity. Recent literature supports this interpretation, emphasizing that pharmacological models used in schizophrenia tend to predominantly affect cognitive and perceptual dimensions of behavior [97].

Antipsychotic treatment modulated object-interaction behavior depending on the compound and dose. In the case of amisulpride, time spent interacting with the yellow object remained reduced compared with controls, but the differences observed between amisulpride- and clozapine-treated groups suggest distinct effects of the pharmacological mechanisms involved. Given the predominantly D2/D3 dopaminergic antagonist profile of

amisulpride, these results may reflect a more pronounced influence on motivational processes and initiation of exploratory behavior compared with clozapine, which has a polypharmacological mechanism [97].

Regarding time spent in the vicinity of the objects, the significant differences observed between the control group and all other groups, particularly for the yellow object, indicate that methionine treatment and subsequent pharmacological interventions influence not only active interaction with stimuli but also the tendency to approach and remain near them. This parameter is considered a sensitive indicator of exploratory behavior and environmental evaluation processes and is frequently used in this type of paradigm [95].

5.4.3 Social interaction test

Analysis of sociability parameters revealed a differentiated profile of social behavior depending on the behavioral measure analyzed. The absence of significant differences in distance swum in the stimulus arm and mean distance in this arm suggests that the general tendency to approach the social stimulus remains relatively stable. These results are consistent with literature showing that certain components of sociability, such as initial approach to conspecifics, may be more resistant to acute or subchronic pharmacological manipulations [89,98].

In contrast, distance swum in the right arm showed significant differences in the group treated with clozapine at a dose of 600 µg/mL compared with the control group and the amisulpride-treated group. Because this arm does not represent the stimulus area, increased activity in this region may reflect redistribution of spatial exploration or a change in exploration strategies rather than a direct alteration of social preference. Such effects have previously been described for compounds with complex pharmacological profiles that can simultaneously influence sensory integration, spatial decision-making, and behavioral organization [96].

The significant difference observed between the two clozapine doses for time spent in the left arm suggests a dose-dependent effect on persistence of behavior directed toward the social stimulus. This result indicates that higher doses may modulate maintenance of social interaction without necessarily affecting its initiation, an aspect also reported in clinical and preclinical studies describing differential effects of clozapine on social functioning [99].

CHAPTER VI. FINAL CONCLUSIONS OF THE THESIS

The data obtained in the clinical study complement and support the experimental observations. Cognitive assessments performed using the MMSE and MoCA indicated persistent deficits in domains such as orientation, attention, verbal memory, and abstract thinking, both under monotherapy and polypharmacy. The absence of consistent differences between monotherapy and combination treatments suggests that pharmacological intensification does not automatically translate into significant cognitive benefits. Furthermore, the results indicate that cognitive performance is influenced by the complex interaction between negative symptomatology, extrapyramidal effects, and the pharmacological profile of treatment, rather than by the number of antipsychotics administered.

The metacognitive analysis, performed using the MCQ-30, showed that complex antipsychotic treatments may be associated with more pronounced maladaptive metacognitive beliefs, particularly in the domains of worry and thought control. These results suggest that, although certain pharmacological combinations may improve components of positive or negative symptomatology, they may simultaneously contribute to the maintenance of rigid or maladaptive cognitive styles. Metacognition therefore emerges as an essential dimension of clinical variability in schizophrenia, relatively independent of improvement in classical psychotic symptoms.

By integrating the experimental and clinical results, the thesis highlights the translational value of Danio rerio models for investigating cognitive and behavioral mechanisms relevant to schizophrenia. The identified behavioral models, particularly those related to initiation of exploration, novelty processing, and cognitive flexibility, may be considered useful models for evaluating pharmacological effects and developing more targeted therapeutic strategies.

In conclusion, the results support the view that cognitive and metacognitive deficits represent central and relatively persistent components of schizophrenia that are not fully corrected by standard antipsychotic treatment. The marked differences among antipsychotics with regard to their impact on cognition and behavior emphasize the need for a personalized treatment approach that considers not only control of positive symptoms but also the patient's cognitive and metacognitive profile. Through its theoretical, experimental, and clinical contributions, the present thesis provides a solid framework for future research aimed at optimizing therapeutic interventions and developing integrated strategies targeting cognitive and social functioning in schizophrenia.

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