The Restoration of Social Defects in Schizophrenic Mice by Green Plant Exposure

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The Restoration of Social Defects in Schizophrenic Mice by Green Plant Exposure

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Abstract

Schizophrenia is a serious psychotic disorder caused by both individuals' genetic background and their living environment. However, how negative and cognitive symptoms can be treated is still a challenge since most anti-psychotic drugs are only effective for positive symptoms. Previous epidemic studies have demonstrated that green plant exposure could decrease the risk of schizophrenia and shorten the length of psychiatric hospital admissions for patients. However, it is still unknown whether green plant exposure could improve cognition-related defects in schizophrenia, with no related animal studies. In the present study, we first induced a schizophrenia mice model by giving mice long-term (2 weeks) injections of the antagonist of the NMDA receptor: MK801. We then raised the animals in environments containing plants (4 weeks) including rosemary and *Epipremnum aureum*, and tested their locomotive, anxious and social behaviors. We found that green plant exposure did not change the behaviors of wild-type animals, but significantly reduced the schizophrenia-related social and anxious behaviors in the schizophrenic animals. In addition, we tested the expression of c-fos in animals exposed to green plants after social behavioral testing and found that the deficits in c-fos expression in both the hippocampus and prefrontal cortex were partially

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rescued after green plant exposure. These results indicate green plant exposure will be a new tool to improve the clinical deficits of schizophrenia.

Keywords: schizophrenia, green plant exposure, social interaction, mice

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Table of Contents

2024 S.-T. Yau High School Science Award
仅用于2024丘成桐中学科学奖论文公示

Introduction

Schizophrenia, a severe mental disorder, impacts 0.5-1% of the global population, significantly disrupting the lives of individuals and potentially leading to social disaters[1,2.] Through advancements in genetic and molecular research, schizophrenia has been associated with certain genetic mutations and environmental influences, including those that affect individuals before birth. Despite the diverse origin of schizophrenia, individuals with the disease share several common symptoms that can be grouped into 3 clusters: positive, negative, and cognitive dysfunction[1,2]. Positive symptoms typically relate to symptoms associated with a "break from reality", such as delusions, hallucinations, incoherent speech, and erratic behavior. Negative symptoms are typically identified by amotivational syndrome, which includes lack of drive, affective flattening, social avoidance, anhedonia, and diminished energy. The cognitive symptoms of schizophrenia manifest as a broad set of cognitive dysfunctions[1]. Though many antipsychotic medications have been developed in the past three decades to manage the clinical manifestations of schizophrenia, most are only effective in alleviating positive symptoms. Although cognitive symptoms also significantly impair patients' daily lives, few treatment methods have been clinically available until now.

Under natural conditions, humans and animals live together with many different kinds of plants, forming symbiotic relationships. Plants perform photosynthesis, producing oxygen and synthesizing glucose. Additionally, plants also emit different smells and visual stimuli, which can improve the sensory, emotional, and social behaviors of humans and animals. In return, animals provide organic manure and help to shape plant morphology. So, green plant exposure is beneficial for both animals and plants. Previous studies have indicated that abnormal environments during pregnancy and childhood development could be one of the key factors in the development of

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schizophrenia. People residing in urban cities are at a higher risk of developing schizophrenia compared to those living in rural regions.[3]. A contributing factor to this phenomenon may be that urban areas contain fewer plants than rural areas. Several previous studies have indicated that green space exposure decreased the risk of schizophrenia in a dose-dependent manner to some extent[4 5-7] and can shorten the length of psychiatric hospital admissions [8]. So, we hypothesize that enough green plant exposure may be able to rescue the cognitive symptoms of schizophrenia. In the present study, we raised schizophrenic mice in environments containing plants (4 weeks) including rosemary and *Epipremnum aureum*, then tested their locomotive, anxious and social behaviors. We found that green plant exposure significantly improved schizophrenia-related social and anxiety deficits. The c-fos expression deficits in both the prefrontal cortex and hippocampus of schizophrenic mice were partly rescued after green plant exposure in mice. These findings indicate a possible novel approach for treating the cognitive and negative symptoms associated with schizophrenia.

Methodology

Subjects

Adult male C57BL/6 mice (8-10 weeks,20-25g) were used for all experiments. The mice were housed in large plastic cages (0.7m x0.7m x0.6m), with 8-9 mice per cage. The room temperature was kept at 25 ± 1 °C, and lighting was set to a 12 h light/dark cycle. The humidity of the room is around 40 ± 5%. The Animal Use and Care Committee of Zhejiang University approved all experimental procedures.

Animal modeling

The schizophrenic mice were modeled based on previous studies[9,10], MK-801 (0.05mg/ml) was injected (I.p) to C57BL/6 mice at the dosage of 0.5mg/kg twice

2024 S.-T. Yau High School Science Award
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per day. The control mice were injected with saline twice per day at the same volume. The injections lasted for two weeks.

Groups

The animals were separated into 4 groups (**Fig. 1D**): wild type (WT) raised without plants (Sch- / Plant-, n=9), WT with plants (Sch- / Plant+, n=8), schizophrenia without plants (Sch+ / Plant-, n=8), schizophrenia with plants (Sch+ / Plant+, n=8).

Green plant exposure

In this study, we built environments containing plants to address whether green plant exposure can alleviate social-related symptoms in schizophrenia. Two kinds of plants including rosemary and *Epipremnum aureum* were chosen. *Epipremnum aureum* is one of the most popular home pot plants, with strong leaves and great survivability. Rosemary (*Salvia rosmarinus*) is a small woody shrub that includes benefits such as antimicrobial and antioxidant properties and is used as an alternative method to treat mental fatigue, etc. In the present study, we put both rosemary and *Epipremnum aureum* inside the plastic cages. Because of the shape of the plants, the animals could run between and hide in the plants and even eat some parts of the plants. In addition, we observed that the mice exposed to green plants became wild and had a stronger willingness to interact with the environment and other animals. By raising the mice in environments containing plants for 4 weeks, we aimed to dissect whether green plant exposure could improve social-related defects in schizophrenic mice.

Behavior test

In this present study, we evaluated emotion-related behaviors using the open field test and elevated plus maze test, and assessed social behaviors with the three-chamber social test. The animals were first introduced to the experimental room, where they spent 10 minutes daily for 3 consecutive days to acclimate to the environment and become familiar with handling by the experimenter. For the open field test, each mouse was placed in the center of the field at the start

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of each trial and allowed 8 minutes of free exploration. In the elevated plus maze, each mouse was initially placed on one of the open arms and allowed 8 minutes of free exploration. The three-chamber social test was carried out in three phases: habituation, social ability testing, and social novelty testing, each phase lasting 10 minutes. In the habituation phase, no animals were placed inside the pen cups, and the tested mouse was allowed to explore the three-chamber freely. Then, in the second phase (social ability), a novel conspecific Stranger 1, who is the same sex and strain as the subject, is placed in cup 1. In the third phase (social novelty), a second novel conspecific Stranger 2, who is also the same sex and strain, is placed under cup 2. Stranger 1 remains in cup 1. Between each trial or phase of every test, a 75% alcohol solution was sprayed to remove any traces of scent left by previous test subjects. All behavioral video was recorded at 60 frames per second with a high-definition camera (1080p, Ordro, version D395).

Histology

Immunohistochemistry was performed as described in a previous study[11]. To quantify the c-fos positive cells, we sampled sections every 80-μm from the whole brain of mice from each of the 4 groups. Brain regions were outlined and compared according to the reference atlas (the Allen Mouse Brain Atlas). And c-fos positive cells were quantified using ImageJ.

Statistical Analyses

The bar graphs are presented as the mean plus the standard error of the mean (SEM). Statistical comparisons between groups were performed using either one-way or two-way ANOVAs (analysis of variance), followed by a rank-sum test for multiple comparisons.

Results

Green plant exposure does not change the locomotive behaviors in either

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wild-type or schizophrenic mice

To evaluate whether green plant exposure could improve behavioral deficits in animals with mental disorders, we first induced a schizophrenia mice model by giving the mice long-term (2 weeks) injections of the antagonist of the NMDA receptor: MK801(i.p.,0.5mg/kg, 2 weeks). We then raised the mice in environments containing two species of plants, rosemary and *Epipremnum aureum*, for at least two weeks (**Fig. 1A-C**). As they were raised, the mice could actively interact with plants, including hiding inside, running between, and even eating some parts of the plants (data not shown). The animals were separated into 4 groups (**Fig. 1D**): wild type (WT) raised without plants (Sch- / Plant-, n=9), WT with plants (Sch- / Plant+, n=8), schizophrenia without plants (Sch+ / Plant-, n=8), schizophrenia with plants (Sch+ / Plant+, n=8). Then, we assessed the social, anxious and locomotive behaviors (Fig. 1E).

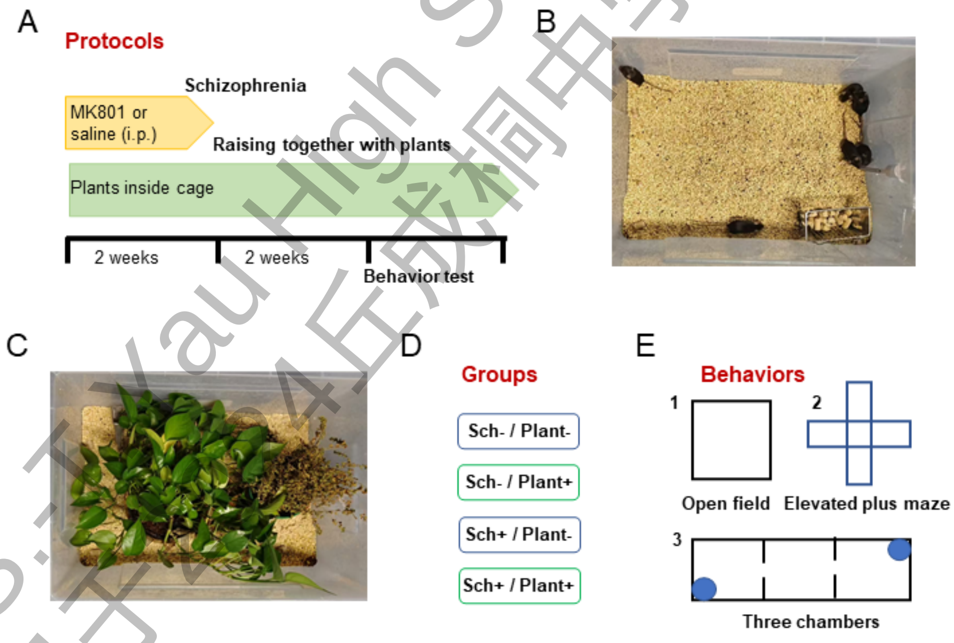

Figure 1. Green plant exposure protocol

(A) The protocol for green plant exposure. Upper panel: to model schizophrenia, MK801 (i.p., 0.5 mg/kg) or saline was administered to WT (C57B/L6) mice via intraperitoneal injection (i.p.) over a two-week period. Middle, the timeline for raising the animals together with plants (4 weeks). Bottom, the timeline for modeling schizophrenic animals, green plant exposure and behavioral testing.

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(B) The animals were housed in a 0.7m x 0.7m plastic box without plants.
(C) The animals were housed in a 0.7m x 0.7m plastic box containing plants.
(D) The different groups of animals. Sch- / Plant-, wild type (WT) raised without plants; Sch- / Plant+, WT with plants; Sch+ / Plant-, schizophrenia without plants; Sch+ / Plant+, schizophrenia with plants.
(E) The behavioral tests conducted, including 1. the open field test, 2. the elevated plus maze, and 3. the three-chamber social test.

MK801 is an NMDA receptor antagonist and may greatly change the locomotive behavior of the mice after long-term administration. Therefore, we evaluated their mobility by analyzing their movements in the open filed test (**Fig. 2A**). The mice's running paths were recorded and overlain onto the open field map. As shown in Figure 2A, the mice ran across almost all of the positions in the open field. Then we analyzed the averaged running distance (**Fig. 2B**) and speed (**Fig. 2C**) over 8 minutes of testing for each of the 4 groups of mice. We found that green plant exposure did not change the locomotive behaviors in either wild-type or schizophrenic mice in the open field (**Fig. 2B-2C**).

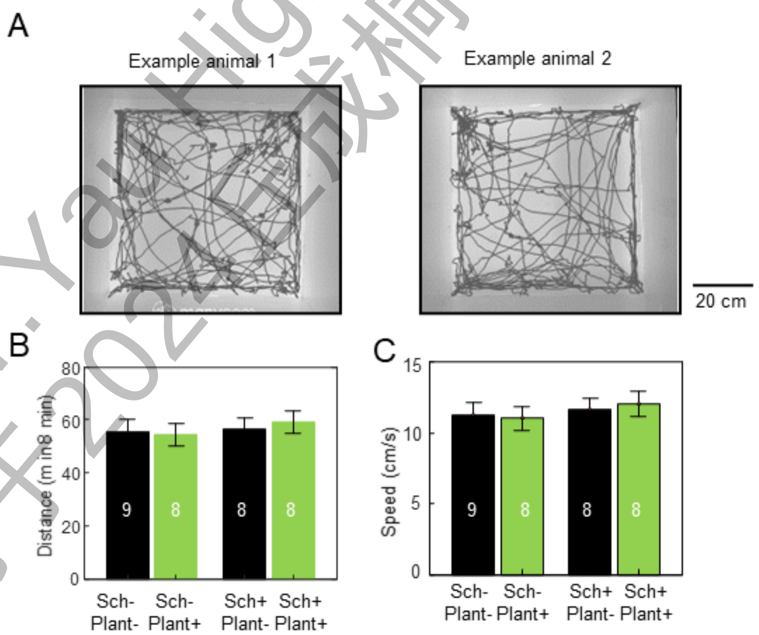

Figure 2. Green plant exposure does not change the locomotive behaviors in either WT or schizophrenic mice

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(A) The 8-minute walking paths of two representative mice in the open field test, with movement trajectories covering most areas of the open field.
(B) The average running distance of the four groups of animals over 8 minutes.
(C) The average running speed of the four groups of animals over 8 minutes.

Green plant exposure decreases the anxiety of schizophrenic mice in open field

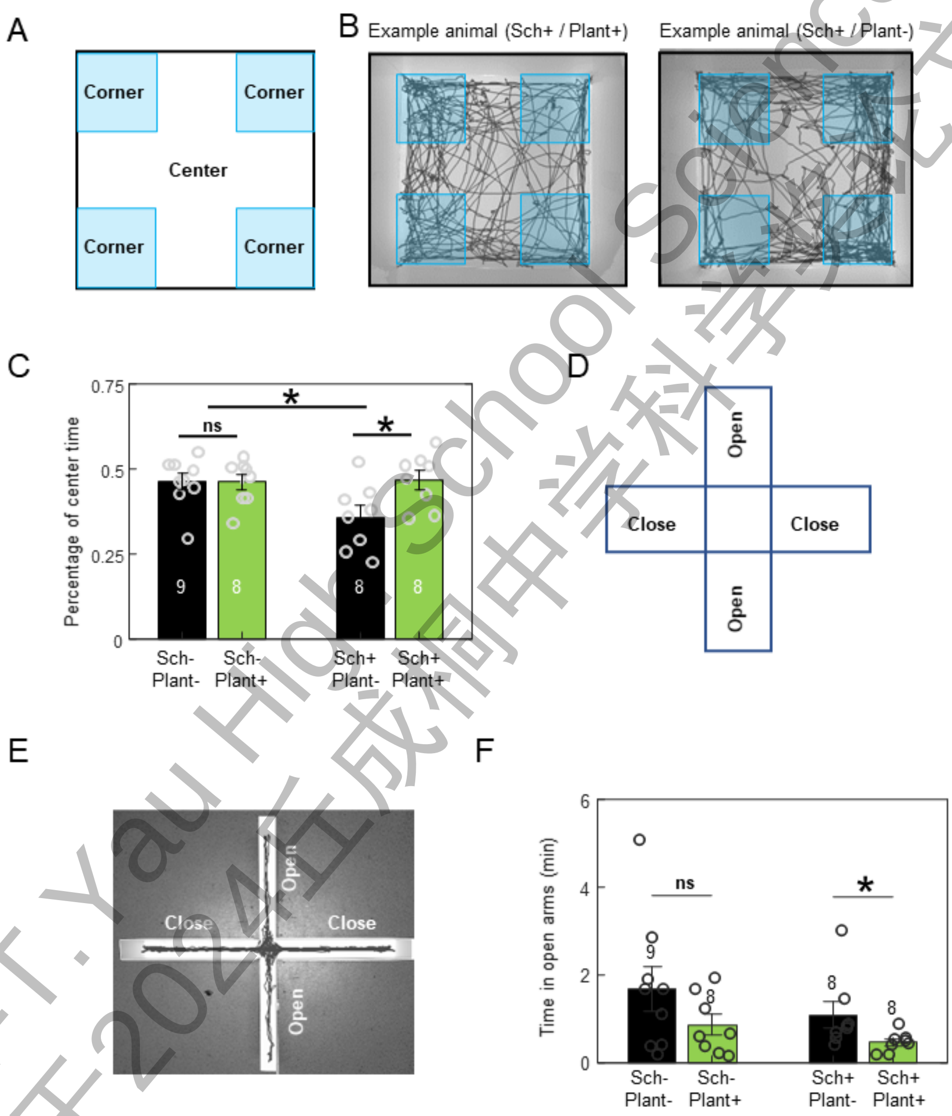

Figure 3. Green plant exposure decreases the anxiety of schizophrenic mice in open field
(A) A diagram showing the corner and center segments of the open field which were used to define the anxious behavior.
(B) The 8-minute walking paths of two representative mice in the open field test. The cyan-shaded areas label the corner segments of the open field. Left, a schizophrenic animal with green plant exposure; Right, a schizophrenic animal without green plant exposure.

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(C) The percentage of time mice from each of the 4 groups spent running in the center segment. The schizophrenic mice showed significantly less time spent in the center segment than that of WT animals, and green plant exposure increased the time spent in the center.
(D) A diagram showing the elevated plus maze.
(E) The walking trace of a representative mouse in the elevated plus maze.
(F) The time mice from each of the 4 groups spent in the open arms of the elevated plus maze.

As we know, most mental diseases including schizophrenia will induce anxiety[12-14]. To test whether green plant exposure can rescue anxiety deficits caused by MK801-induced schizophrenia, we first analyzed the time each mouse spent running in the center and corner segments of the open field (**Fig. 3A and 3B**). We observed that the schizophrenic mice spent less time running in the center of the field in comparison to the WT mice (**Fig. 3C**). Importantly, green plant exposure significantly increased the running time in the center compared with the animals raised without plants (**Fig. 3C**). However, the increase in center running time was not shown in the WT animals with green plant exposure (**Fig. 3C**). So, green plant exposure significantly decreased the schizophrenia-related anxious behavior of mice in the open field test, and had little effect on WT mice's anxiety.

The anxiety level of rodents was also evaluated using the elevated plus maze test, where anxious individuals usually spend more time in the closed arms than in the open arms. So, we also tested the anxiety changes of schizophrenic mice that were exposed to green plants using the elevated plus maze (**Fig. 3D-3E**). Surprisingly, the anxiety level of schizophrenic mice was marginally lower compared with WT animals. In addition, the animals of both WT and schizophrenic with green plant exposure spent significantly less time exploring in the open arms (**Fig. 3F**). So, green plant exposure did not decrease the anxiety of schizophrenic mice in the elevated plus maze. As the mice exposed to green plants were raised, we observed them frequently running or

2024 S.-T. Yau High School Science Award
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hiding within the plants. This may have led them to develop a preference for enclosed environments, resulting in these behavioral results which could not explained by increasing anxiety. Therefore, green plant exposure reduced anxious behavior of schizophrenic mice only in the open field condition, but not in the elevated plus maze.

Green plant exposure ameliorates the social interaction deficits of schizophrenic mice

Most individuals with schizophrenia show social-related symptoms, such as social withdrawal and reduced communication, etc. Therefore, we also evaluated the social interaction of the 4 groups of mice via the three-chamber social test. The test includes 3 phases: habituation, social ability and social novelty (**Fig. 4A and 4B**). In the habituation phase, there is no mouse in either pen cup, and the test subject is allowed to freely explore the empty three-chamber box. The habituation phase is usually used to test whether the tested mouse has a preference for either of the two cups in opposite corners of the three-chamber box. In the social ability phase, one mouse is put in pen cup 1 and no mouse in pen cup 2. This phase is used to test whether the tested mouse has a stronger motivation to interact with other mice instead of the object (pen cup 2). In the social novelty phase, a new mouse is then introduced into pen cup 2. The WT usually has a stronger motivation to interact with the new mouse which is called social novelty (**Fig. 4A**).

As shown in Figures 4A and 4C, wild-type (WT) animals not exposed to green plants spent similar amounts of time interacting with both cups during the habituation phase. They showed a preference for the cup containing the animal during the social ability phase and favored the cup with the novel animal during the social novelty phase (**Fig. 4A and 4C**). WT animals exposed to green plants displayed similar behavior, preferring the cup with the animal during the social ability phase and the cup containing the novel animal during the social novelty

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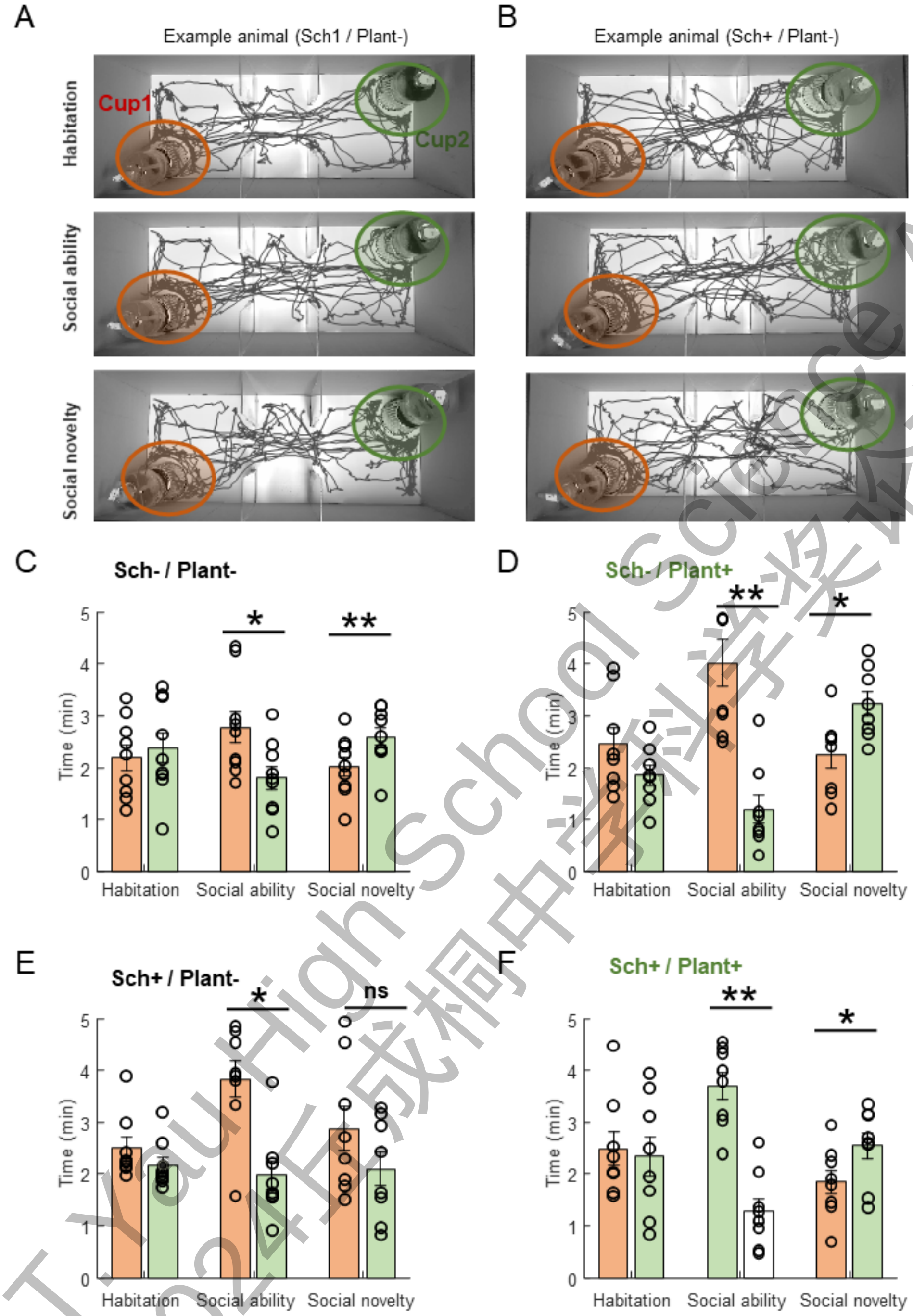

Figure 4. Green plant exposure ameliorates the social interaction deficits of schizophrenic mice

(A) The 10 min walking trace of one example mouse (a WT animal with green plant exposure) in the three-chamber box. The test includes 3 phases: habituation (Upper), social ability (Middle) and social novelty (Bottom).

(B) The 10 min walking trace of 1 example mouse (a schizophrenic animal without green plant exposure) in the three-chamber box.

(C-F). The social behaviors of the 4 groups of animals. C, Sch-/plant-; D, Sch-/plant+; E, Sch+/plant-; F, Sch+/plant+. The interaction time of the testing mouse with the mice in the

2024 S.-T. Yau High School Science Award
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cup or the cup itself was measured by the time in the shade regions. Green plant exposure improves the social novelty behaviors of schizophrenic mice.

phase (**Fig. 4D**). Importantly, The WT animals with green plant exposure showed a larger interaction time difference between the animal and the empty cup in the social ability phase (**Fig. 4C**) in contrast to WT animals raised withoutplants (**Fig. 4D**). Similar to previous studies, the schizophrenic mice without green plant exposure showed normal social ability, indicated by a preference for the animal rather than the empty cup in the social ability phase, while also demonstrating abnormal social behavior in the social novelty phase, indicated by similar interaction times between the novel animal and old animal (**Fig. 4E**). Interestingly, the schizophrenic mice with green plant exposure showed both normal social ability and social novelty behaviors in the three-chamber social tests (**Fig. 4F**). Additionally, schizophrenic mice with green plant exposure also showed a larger interaction time difference between the animal and the empty cup in the social ability phase (**Fig. 4F**) when compared to the schizophrenic mice that were not exposed to plants (**Fig. 4E**). So, green plant exposure consistently improves the social interaction of mice in the social novelty phase and rescues the social novelty deficit in the schizophrenic mice.

The c-fos expression deficits in the hippocampus and prefrontal cortex of schizophrenic mice were partially restored by green plant exposure

Previous studies have shown that abnormal prefrontal cortex[15-17] and hippocampus[18,19] circuits contribute to the abnormal cognitive behaviors of schizophrenia. To determine whether exposure to green plants could reverse abnormalities in the prefrontal cortex and hippocampus, we performed c-fos staining after mice from each of the 4 groups completed all three phases of the three-chamber social test. (**Fig. 5**).

2024 S.-T. Yau High School Science Award
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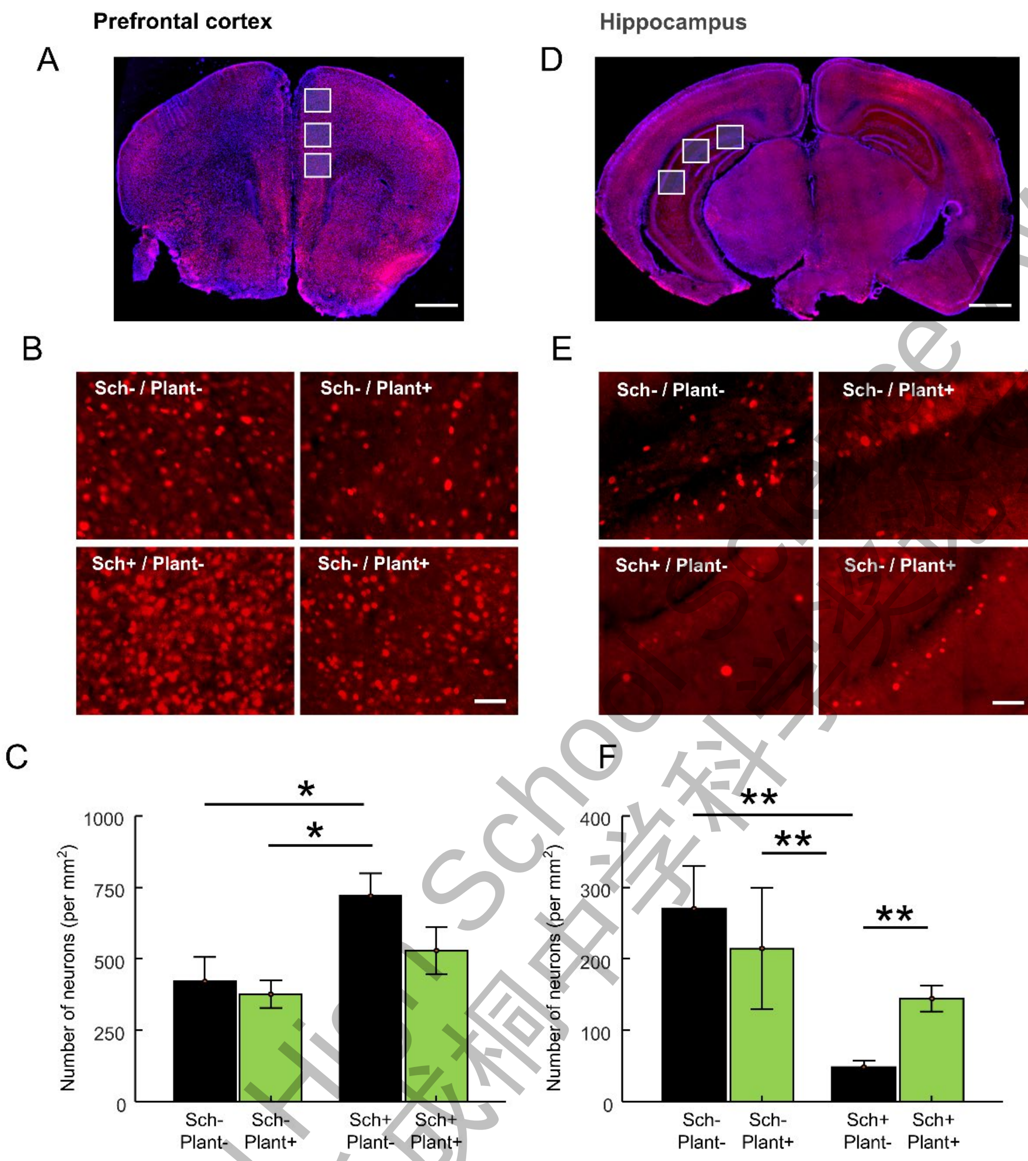

Figure 5. Green plant exposure partially rescues c-fos expression deficits in the hippocampus and prefrontal cortex of schizophrenic mice.

(A) and (D), example images showing the overall expression of c-fos in the whole coronal section, including the prefrontal cortex(A) and hippocampus(B) after three-chamber social tests. scale bar, 1mm.

(B) and (E), example images showing the expression of c-fos in the 4 groups of animals in the prefrontal cortex(B) and hippocampus(E). scale bar, 25 um.

(C) and (F), Bar graphs display the average number of c-fos positive cells in the prefrontal cortex(C)and hippocampus(F)across the 4 groups of animals.

Compared the wild-type animals, schizophrenic mice showed significantly higher c-fos expression in the prefrontal cortex (**Fig. 5A-5C**). Exposure to green plants did not alter c-fos expression in wild-type mice. However, it did slightly lower the c-fos expression in the prefrontal cortex of schizophrenic mice,

2024 S.-T. Yau High School Science Award
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compared to the schizophrenic mice without plants (**Fig. 5A-5C**). Compared to WT mice, the c-fos expression in the hippocampus of schizophrenic mice was significantly lower. (**Fig. 5D-5F**). More importantly, green plant exposure completely rescued the c-fos expression deficit in the hippocampus of schizophrenic mice (**Fig. 5F**). So, green plant exposure can partly lower the high c-fos expression in the prefrontal cortex and rescue the c-fos expression deficit in the hippocampus of schizophrenic mice, which may contribute to the behavioral rescue.

Discussion

In the present study, we first raised the mice in environments containing two kinds of plants to induce green plant exposure. Then, we investigated whether exposure to green plants could mitigate negative or cognition-related deficits in individuals with schizophrenia by employing a range of behavioral tests. We found green plant exposure can improve animals' social (**Fig. 4**) and emotional related deficits (**Fig. 3**), but do not influence the locomotive behaviors in schizophrenic mice. In addition, we tested the c-fos expression in the hippocampus and prefrontal cortex of schizophrenic mice and found green plant exposure can partly lower the high c-fos expression in the prefrontal cortex and rescue the c-fos expression deficit in the hippocampus of schizophrenic mice, which may contribute to behavioral rescue.

Genetic and environmental factors are two key factors in the etiology of schizophrenia [20]. Schizophrenia is highly heritable, especially indicated by twin studies[21,22] in which the risk of schizophrenia increased from 1% (in normal population) to 20%-45% (in twins)[22-24]. With the development of genetic tools, many risk genes have been identified including DISC1[25-27], reelin[28-30], Neuregulin[28], COMT[28], etc. It is worth noting that schizophrenia is a multigene disease, which was confirmed by a transcriptome study from the prefrontal cortex (PFC)[17]. In this study, the authors found there are 50 risk genes which organized as a network to function in neuronal migration, transcriptional

2024 S.-T. Yau High School Science Award
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regulation, transport, synaptic transmission and signaling[17]. Apart from the risk genes, abnormal environments in prenatal or early development are also one of the major factors of schizophrenia[31]. For example, schizophrenia is associated with some infections before birth[32] where the infections affect fetal brain development[33]. Additionally, individuals living in urban environments have a higher risk of developing schizophrenia compared to those in rural areas. Recent research has highlighted that both environmental and genetic factors are crucial in the development of schizophrenia and often interact with one another.[31]. Although having different etiology, all cases of schizophrenia share similar symptoms[20,24,28], and how schizophrenia can be treated is still a challenge, especially for negative and cognitive symptoms.

In the last 2-3 decades, many antipsychotic drugs such as SGAs, clozapine, lumateperone, risperidone, olanzapine, and quetiapine have been developed to treat schizophrenia[34,35]. Most of these drugs interact with various neurotransmitter receptors including the dopamine receptor[36,37] and ion channels[15]. However, most of these drugs are mainly effective for treating the positive symptoms of schizophrenia[34,35] or emotion-related symptoms[13].

As individuals with schizophrenia frequently experience social challenges such as withdrawal from social interactions[1,2] several new treatment strategies have been implemented in clinical practice. These strategies include increasing community engagement, providing family psychoeducation, offering supported employment, and implementing social skills training[20]. However, many of these treatments have demonstrated only limited effectiveness. Under natural conditions, humans and animals live together with many different kinds of plants, forming symbiotic relationships. Several previous studies have suggested that exposure to green spaces can reduce the risk of schizophrenia to some extent in a dose-dependent manner[4 5-7], and may also potentially shorten psychiatric hospitalizations[8]. Clinically, some patients showed decreased anxiety and depression after green plant exposure[8,38-40]. However, all of the studies are

2024 S.-T. Yau High School Science Award
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epidemiology investigations and could not address the mechanism of green plant exposure and mental disease. So, using an animal model to dissect the effect of green plant exposure on schizophrenia is very important. In the present study, we made a schizophrenia mice model using long-term administration of MK-801[9,10] and tested their social, emotional and locomotive behaviors. We found the mice displayed deficits of social and anxious behaviors after 2-3 weeks of recovery (**Fig. 3 and Fig.4**), but showed normal locomotor activity (**Fig. 2**). Importantly, social deficits observed in the three-chamber social test were eliminated following exposure to green plants (**Fig. 4**). Meanwhile, the increased anxiety of schizophrenic mice in the open field condition is completely rescued with green plant exposure (**Fig. 3**). However, the schizophrenic animals showed a small decrease in the time spent in the open arms compared to the control animals, and the animals with green plant exposure exhibited an overall decrease in the time spent open arms compared with the animals without plant exposure (**Fig. 3**). We observed the animal's behaviors as they were raised together with the plants, and found the animals often hid inside the green plants and some mice even dug in the soil. As mice's instinctive behavior, habits of staying in the hidden regions of their environment will become stronger during green plant exposure. Thus, the reduced time spent in the open arms could not be explained by the increased anxiety. An alternative explanation is that animals raised with plants may be more relaxed, but the contrast between their familiar environment and the elevated plus maze was heightened by the exposure to plants. Lastly, we examined c-fos expression in the hippocampus and prefrontal cortex of schizophrenic mice and found that green plant exposure partially reduced the elevated c-fos expression in the prefrontal cortex and restored the c-fos expression deficit in the hippocampus, which may contribute to the behavioral rescue (**Fig. 5**). So, green plant exposure could improve the social and emotion-related symptoms of schizophrenic mice.

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Although our results indicated that green plant exposure can be a viable method to treat clinical symptoms of schizophrenia, there are several limitations in the present study. First, there are several types of mouse models for schizophrenia, including developmental, drug-induced, lesion-induced, and genetic manipulation models. So, it is still unknown whether green plant exposure could also treat schizophrenia in other kinds of animal models. Second, there are different kinds of plants and we only tested the rosemary and *Epipremnum aureum* in the present study. Other plant information including flowers, grass and xylophyta etc. will form different green plant exposure environments and should be considered in the future. Finally, because the issue of time, deep mechanism studies including studies of neural activity difference and gain and loss of function are still ongoing. Despite these limitations, the present work provides a new method to treat the symptomatic deficits in schizophrenia and this method may be generalized to other psychotic diseases.

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2024 S.-T. Yau High School Science Award
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Acknowledgments

Background

I live in a household of neuroscience research, so I have always been interested in the brain and mental diseases. In early 2023, I participated in a study where we investigated whether the overuse of risperidone will induce cognitive deficits during treatment of schizophrenia. Then, in the middle of 2023, my parents arranged for me to study for a while at Hangzhou Seventh People's Hospital, to get the whole view of the mental disease. In the hospital, I observed the behavior of patients and learned how the disease can be treated from the doctors. In addition, I talked with patients and realized how schizophrenia causes both physical and mental damage. Importantly, I noticed the sterile environment, the white walls and the empty space in the hospital. This was unsettling for me, and I wondered if the hospital environment would negatively affect the patients' anxiety or social abilities. So, I searched for literature and discussed my ideas with Professors Xinjian Li and Han Xu. Together we decided to simplify the experiment to investigate whether green plant exposure could improve the symptoms of schizophrenia and conduct the experiment using a mouse model.

Relationship between instructor and student

The instructor is my father. He is a neuroscientist researching spatial navigation and motor control. He is very strict with me in all parts of this investigation, including in creating the research idea, experimentation, data analysis, and writing. He told me that I must list all the persons who had contributed to this manuscript. In addition, he asked me to follow the rules of academic integrity and animal welfare. So, I was fully trained to be an academic researcher from this project.

Whether the instructor is paid:

The instructor was not paid for his contributions to this investigation.

Division of labor

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The selection of the research topic was initiated by Mingxuan Li. Mingxuan Li, Prof. Xinjian Li, and Prof. Han Xu had several rounds of deep discussion and finalized the design.

Xiangyu Zhang and Mingxuan Li made the schizophrenic mice model. Mingxuan Li and Xiangyu Zhang set up the green plant exposure environments. We thank Shanshan Liu's help in teaching how to carry out the behavior testing and Yuyu Wu's help in teaching how to carry out histology. Behavioral testing, including the open field test, elevated plus maze, and three-chamber social testing was carried out by Mingxuan Li with help from Mingda Li. Histology was carried out by Mingxuan Li and Kai Zhou.

Mingxuan Li carried out double-blind data analysis with teaching and guidance from Prof. Xinjian Li. Mingxuan Li and Xinjian Li discussed the data and Mingxuan Li wrote the first manuscript draft. Two further drafts were completed by Mingxuan Li with the guidance of Xinjian Li. Xinjian Li finalized the proofreading of the manuscript.

Obstacles and solutions

At first, I planned to conduct the investigation at Professor Xu's laboratory who is a neuroscientist in social behavior and mental disease. I discussed the experimental design with Professors Xu and Li. However, Professor Xu's laboratory lacked many conditions for this experiment, for example, raising mice with plants requires a large space which could not be performed in Professor Xu's laboratory. Finally, we decided to conduct the experiment at Professor Li's laboratory.

One of the greatest obstacles we faced in this investigation was creating the schizophrenia mouse model. Initially, we wanted to use a genetic model. The laboratory where I conducted the experiment had a genetic schizophrenic model, but their paper was still ongoing so they didn't allow me to use their model. We also contacted other laboratories and companies, but we weren't able to find this kind of model anywhere else. So, we looked into literature and

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found schizophrenia can be induced by different methods including using some drugs. So, we chose an antagonist of NMDA receptor: MK801 to induce schizophrenic model.

Another obstacle we faced was during the preliminary experiment of the behavioral tests. We were using a new room in which all the equipment and experimental setup had to be calibrated from scratch. After a few trials in the preliminary test, we found that the lighting in the recordings was uneven on the open field and three-chamber box. So, we had to change the positioning of the test environments relative to the lights and use a black curtain to block light from other lights. We also created custom floorboards for the test environments to increase contrast, which at the same time made the test environments easier to be cleaned by alcohol.

Another obstacle was the start of the school semester. I began the experiment in February and carried out the experiments on weekends and during the summer break. By the time school started in September most of the experiment was completed, but there was one batch of histology that was still ongoing. So, this batch was completed by Kai Zhou.

DECLARATION OF INTERESTS

The authors declare that they have no competing financial interests.

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