Involvement of regulation of the excitation:inhibition functional balance in the mPFC in the antidepressant-anxiolytic effect of YL-IPA08, a novel TSPO ligand

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Abstract

TSPO, an 18 kDa translocator protein, has received increased attention due to its antidepressant-anxiolytic effects. The balance between glutamatergic and GABAergic (E: I) in the medial prefrontal cortex (mPFC) is crucial for antidepressant-anxiolytic effects. However, no evidence is available to clarify the relationship between TSPO and E:I balance. In the present study, we used the TSPO global-knockout (KO) and TSPO wild-type (WT) mice to assess the effects of TSPO on antidepressant-anxiolytic effects of YL-IPA08 (a novel TSPO ligand) and the underlying neurobiological mechanism. Additionally, a multichannel electrophysiological technique was used to explore the effects of YL-IPA08 on pyramidal neurons and interneurons in mPFC. Open field test (OFT) and elevated plus maze (EPM) test revealed that a single dose of YL-IPA08 (0.3 mg/kg, i.p.) exhibited significant anxiolytic actions in WT mice except in KO mice. In only WT mice, significant antidepressant effects were observed in tail suspension test (TST) and forced swim test (FST). The multichannel electrophysiological technique demonstrated that YL-IPA08 significantly increased the firing rates of pyramidal neurons and decreased those of interneurons. Further studies illustrated that the firing rates of glutamatergic might be antagonized by PK11195 (a classic TSPO antagonist). Our results suggest that YL-IPA08 might regulate the E:I balance in mPFC, mediated by TSPO. In summary, TSPO regulates E:I functional balance in mPFC, play a critical role in antidepressant-anxiolytic effects of YL-IPA08, and provide a potential target site for the development of antidepressant and anxiolytic drugs.

1. Introduction

Major depressive disorder (MDD) is one of the common psychiatric disorders worldwide (Stallwood et al. 2021), and its lifetime prevalence is approximately 10–15% (Jin et al. 2013), imposing a severe social and economic burden. At present, first-line antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin, and norepinephrine reuptake inhibitors (SNRIs), are limited because of their delayed onset of action, partial response with residual symptoms, non-response, and undesirable side effects. Additionally, their therapeutic benefits can take several weeks to set in and are only effective in approximately two-thirds of the population with depression (Li 2020; Poleszak et al. 2019). Therefore, developing novel antidepressants with fast onset and low adverse effects is urgently required (Insel and Wang 2009).

TSPO is primarily located at the outer mitochondrial membrane of glial cells and mediates cholesterol entry into mitochondria to synthesize neurosteroids, the rate-limiting step of neurosteroid synthesis (Rupprecht et al. 2010). Clinical research has revealed that TSPO expression reduction is evident in patients suffering from depression with suicidal tendencies (Soreni et al.1999) or adult dissociative anxiety (Abelli et al. 2010; Chelli et al. 2008). TSPO ligands exhibited significant anxiolytic and antidepressant effects in several rodent models (Azeez et al. 2021; Da Pozzo et al. 2012; Kita and Furukawa 2008; Kita et al. 2004; Kita et al. 2009; Papadopoulos et al. 2015). YL-IPA08 is a novel TSPO ligand synthesized and obtained at our institute. Our previous study demonstrated that YL-IPA08 exhibited significant antidepressant and anxiolytic-like effects, completely blocked by TSPO antagonist

PK11195 (Yao et al. 2020; Zhang et al. 2014). Although our findings suggest that anxiolytic-like effects might be related to dendritic remodelling promotion, up-regulation of neurotrophic signalling pathway within the hippocampus, hippocampal morphological and functional plasticity maintenance, and HPA axis function regulation, the underlying neural regulation mechanism remains unclear (Li 2020; Zhang et al 2017).

The prefrontal cortex (PFC) is implicated in diverse emotion regulation in neuroanatomy (Shibuya-Tayoshi et al. 2008). Studies also suggested that the excitation: inhibition (E:I) functional balance (composed of excitatory glutamatergic pyramidal neurons and inhibitory γ-aminobutyric acid interneurons) in the mPFC is essential for the accurate execution of a series of PFC-dependent functions and might be a key target for regulating rapid antidepressant effect (Yin et al. 2021). The neurotransmitter systems of E:I balance might contribute to treating mood disorders (Kugaya and Sanacora 2005; Sanacora et al. 2003). Antidepressant studies suggest that E:I regulating mechanism play a key role in the rapid anxiolytic and antidepressant effect of scopolamine and ketamine (Krystal et al. 2002; Lener et al. 2017; Stone et al. 2012; Wilkinson and Sanacora 2019; Wohleb et al. 2016). Structural and functional neuroimaging studies have described volume reductions in cortical and limbic structures due to neuronal atrophy of glutamatergic in patients with depression (Fogaca and Duman 2019), whereas signal changes of GABAergic neurons might be involved in the pathogenesis of depression (Gunduz-Bruce et al. 2019). GABA normalization was associated with the relief of depressive symptoms (Godfrey et al. 2018).

We first used the TSPO KO and WT mice to assess the effects of TSPO deletion on antidepressant and anxiolytic effects of YL-IPA08. Then, a multichannel electrophysiological technique (in vivo) was used to explore the effects of YL-IPA08 on pyramidal neurons and interneurons in PFC. In conclusion, Our present study investigated the relationship between TSPO and E:I functional balance of antidepressant- and anxiolytic-like action using multichannel electrophysiological technique and behavioural tests to provide experimental evidence for developing the potential antidepressants and anxiolytics in the future.

2. Materials And Methods

2.1. Animals

Male Wistar rats (250 ~ 310g) were purchased from SPF (Beijing) Biotechnology Co. The TSPO heterozygous (HZ, +/−) mice with a C57BL/6J background were provided by Professor Jian-Min Zhang of the Chinese Academy of Medical Sciences (Wang et al. 2016). Both male TSPO wild-type (WT; +/+) mice and KO (−/−) mice used for the experiments (offspring of the TSPO HZ mice) weighed between 22 g and 26 g. The genotyping of the TSPO was like that of Chao Shang (Shang et al. 2020). Animals were housed in groups of 3 to 5 mice per plastic cage at constant temperature (24℃), in a 50% ± 5% humid environment and exposed to a light/dark cycle of 12 hours (lights on from 07:00 to 19:00). After one week of adaptive feeding, experimental animals were tested with food and water freely available. Experiments were performed in compliance with the National Institutes of Health Guide for the Care and

Use of Laboratory Animals. The experimental procedures were approved by the institutional committee on animal care and use.

2.2. Drug and dose

YL-IPA08 was synthesized by the Department of Medicinal Chemistry at our institute (purity ≥ 99.6%). PK11195 was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). YL-IPA08 was dissolved in saline, and PK11195 was suspended in a saline solution containing 2% DMSO and 0.8% Tween 80. A 3 mg/kg dose of PK11195 was administered based on previous studies. The optimal dose of YL-IPA08 in the behavioural experiment was 0.3 mg/kg, but in the electrophysiological experiments, we used the optimal (0.3 mg/kg, i.p.) and suboptimal (0.1 mg/kg, i.p.) doses of YL-IPA08 to explore the E:I balance in the mPFC (Zhang et al. 2014). Both the drugs were administered by intraperitoneal injection (i.p.) in a volume of 20 ml/kg (mice) or 2 ml/kg (rats).

2.3 Experimental instrument

The multichannel electrophysiologic recording instrument was purchased from Plexon Inc. (United States). The data was recorded using the neural data acquisition system Plexon®OmniPlex®(Omniplex System). The recording software was Omniplex Release 20.0, and the electronically isolated digital amplifier was a 16-channel amplifier, MiniDigi™. The sampling rate per channel was 40kHZ, and the principle applied was the same as that of Yong-Yu Yin (2021) at our institute. The recording electrodes featured the 16-channel Linear Arrays, made of platinum/iridium alloy wire from the US company Plexon, with a total length of 15mm, with a 50μm space between the two channels, and the amplified signal of the electrodes was digitized at a sampling rate of 40kHz.

2.4 Behavioural experiments

Different behavioural experiments (OFT, TST, FST, and EPM) were used to evaluate the antidepressant and anxiolytic effects of YL-IPA08 (0.3 mg/kg, i.p.) after 60 min of administration in TSPO WT and KO mice, with regular testing hours of between 9:00–16:00 each day.

2.4.1 Open field test (OFT)

The OFT experiment was adapted from Walsh and Cummins (1976). The head of each mouse was directed towards a fixed corner of the open field (60 × 60 × 16 cm^3), and the mice were allowed to explore the apparatus freely for 5 min, and the experiment was videotaped with a camera mounted above the apparatus. The bottom of the open field was equally divided into 16 grids, and the central field was defined as the central area (30 × 30 cm^2). The rearings and the total distance were recorded to assess locomotor activity. The total number of entries and the total distance covered into the central area were recorded to assess the anxiety-like behaviour of the mice.

2.4.2 Elevated plus-maze test(EPM)

The EPM experiment was adapted from the experiment by Lister (Lister 1987). The EPM comprised two open arms (30 × 5 cm^2) and two closed arms (30 × 5 × 15 cm^3) made of black Plexiglas, elevated 40 cm above the ground. The open and the closed arms were arranged in a plus shape. At the start of each race, the mice were placed in the central area of the apparatus facing the closed arm, and the mice were allowed to explore the apparatus freely for 5 min and videotaped with a camera mounted above the maze. The time spent in the apparatus and the number of entries into the open and enclosed arms were recorded to assess locomotor activity. The preference for the open arms (preference for open arms = open arm time or entries/total arm time or entries × 100%) were recorded to assess the anxiety-like behaviour of the mice.

2.4.3 Tail suspension test(TST)

The TST experiment was adapted from Steru et al. (1985). The tail of each mouse was fixed on the top of the apparatus using adhesive tape and was videotaped with a camera for 6 min. The cumulative immobility time (in seconds) was recorded in the last 4 minutes to assess the depression-like state of the mice.

2.4.4 Forced swimming test (FST)

The FST was adapted from the experiment by Porsolt et al. (1978). The mice were placed in an open glass cylinder (20 cm high, 14 cm diameter, and 10 cm deep) filled with water (24℃ ± 1℃). The mice were allowed to swim freely for 6 min and videotaped with a camera. The cumulative immobility time (in seconds) was recorded in the last 4 minutes to assess the depression-like state of the mice.

2.5 Electrophysiological experiments

We performed unicellular extracellular recordings in anaesthetized rats to analyze the changes in the firing rate of pyramidal neurons and interneurons in the layer V of mPFC. Rats were given an initial anaesthetic dose of 2% pentobarbital sodium (40 mg/kg, i.p.) before electrophysiological experiments and supplemented as required to maintain general anaesthesia. The rats were fixed in a stereotaxic instrument and then implanted with a Linear Array 16-channel electrode. According to the diagram of Paxinos and Watson, the stereotaxic coordinates with bregma as the origin were determined (AP + 3.2 to + 3.4 mm, ML -0.5 to -1 mm, DV -1 to -4 mm) (Llado-Pelfort et al. 2012; Paxinos and Watson 2007). Each electrode digitized the amplified signal at a 40kHz sampling rate. The recorded spike sorting was imported into the Offline Sorter (Plexon) to perform spike-wave classification through an automatic and manual sorting technique to remove noise (signal-to-noise ratio greater than 3:1) from the discrete waveform-based clustering. The discrete unit group with stable characteristic waveform and absolute refractory period greater than 1.1 ms was regarded as a single unit identified by the peak interval histogram. In the electrophysiological experiment, we focused on recording two firing units in mPFC, glutamatergic and GABAergic units, and identified them according to the aforementioned characteristics (Yin et al., 2021; Zhang et al., 2014). The characteristic waveforms are shown in Fig. 5a and Fig. 5e, respectively.

The electrodes recording target neurons were stabilized for 30 min, and neuronal firing activities were recorded for 10 min as the mean baseline. YL-IPA08 was injected intraperitoneally immediately after the mean baseline recording to study whether a single acute intraperitoneal injection of YL-IPA08 affected the E/I balance in mPFC, followed by recording every 30min for 10 min each to obtain experimental data, totalling 90 min of recording. At the end of the mean baseline recording, the antagonists PK11195 and YL-IPA08 were injected simultaneously (to explore the antagonism of TSPO antagonist PK11195 on the agonism YL-IPA08), followed by recording every 30 min for 10min each to obtain experimental data, totalling 90 min of recording. The obtained data were analyzed according to the above descriptions to obtain corresponding results. The waveforms remained stable before and after administering the drugs in the electrophysiological experiment.

2.6. Data and statistical analysis

All data were expressed as Means ± SEM. GraphPad Prism 8.0.1 (GraphPad Software Inc., San Diego, CA, USA) was used to analyze and plot. The behavioural data and the dose-time relationship of electrophysiology were analyzed using a two-way analysis of variance (ANOVA). The effect of the electrophysiological antagonist PK11195 on pyramidal neurons was investigated using one-way analysis of variance (ANOVA), followed by Dunnett's test. For all tests, $P < 0.05$ denoted statistical significance between data.

3. Results

3.1 TSPO-mediated the anxiolytic- and antidepressant-like effects of acute YL-IPA08

An effective dose of YL-IPA08 (0.3 mg/kg, i.p.) or saline was intraperitoneally injected one hour before the behavioural tests to TSPO WT or KO mice.

In the OFT (Fig. 1), neither TSPO KO nor drug treatment affected the number of rearings and the total distance of movements by the mice (Fig. 1a, Fig. 1b). Dunnett's tests revealed that YL-IPA08 treatment increased the total number of entries and distance of movement into the central area in TSPO WT without affecting the locomotor activity of mice ($P < 0.05$; Fig. 1), suggesting that the anxiolytic effect of YL-IPA08 could be antagonized by TSPO knockout.

In the EPM test (Fig. 2), neither TSPO KO nor drug treatment affected the total duration and a total number of entries into either arm by the mice (Fig. 1a, Fig. 1b). Dunnett's tests demonstrated that YL-IPA08 treatment increased the percentage of time spent and the number of entries of movement into open arms in TSPO WT without affecting the locomotor activity of mice. However, the effect was antagonized by TSPO KO ($P < 0.05$; Fig. 1), consistent with OFT. OFT and EPM results indicated that the anxiolytic effect of YL-IPA08 was mediated by TSPO activation, consistent with our previous studies.

In the FST (Fig. 3a) and TST (Fig. 3b), Dunnett's tests analysis of FST and TST results exhibited that YL-IPA08 treatment decreased the immobility time in TSPO WT, but the effect was antagonized by TSPO KO ($P < 0.05$; Fig. 3). FST and TST results suggested that TSPO mediated the antidepressant-like effects with a single dose of YL-IPA08 (0.3 mg/kg, i.p.).

3.2 Effect of YL-IPA08 on E: I balance in mPFC

We primarily recorded two crucial types of neurons in mPFC: glutamatergic pyramidal neurons and GABAergic interneurons and analyzed the electrophysiological effects of a single acute intraperitoneal injection comprising different doses of YL-IPA08 (0.1–0.3 mg/kg, i.p.) in a dose-time effect manner. The classifications of neurons were based on the waveform, valley-peak space, and firing rate (Homayoun and Moghaddam 2007; Yin et al., 2021). In the present study, we observed that the waveform characteristics of the two neurons remained stable after drug treatment, manifested as the change of firing rate of action potentials.

The electrophysiological results suggested that YL-IPA08 (0.3 mg/kg, i.p.) significantly increased the firing rate of glutamatergic pyramidal neurons after 60 min (Fig. 4b and c). However, a dose of 0.1mg/kg significantly increased the firing rate of pyramidal neurons only after 90 min of the treatment (Fig. 4b and c). The effect of such increase on pyramidal neurons in these two different doses continued at 90 min ($F(3, 100) = 12.95$, $P < 0.05$; compared with basal; Fig. 4. b).

Effect on the firing rate of GABAergic interneurons: YL-IPA08 (0.3 mg/kg, i.p.) significantly decreased the firing rate of GABAergic interneurons after 60 min (Fig. 4e and f). However, a 0.1mg/kg dose did not significantly affect GABAergic interneurons, but it exhibited an evident trend of increase (Fig. 4e and f). The effect of such decrease on GABAergic interneurons in these two different doses continued at 90 min ($F(3, 40) = 9.154$, $P < 0.05$; compared with basal; Fig. 4e). These findings suggested that the effects of different doses of YL-IPA08 were consistent in electrophysiological and behavioural experiments. Further analysis revealed that YL-IPA08 could significantly regulate the E:I functional balance in the mPFC, consistent with our expectations.

To investigate the physiological mechanism of the potential antidepressant-anxiolytic, like the effect of YL-IPA08, we recorded the effect of PK11195 (a TSPO antagonist) on the firing rate of the glutamatergic neuron. As shown in Fig. 5, according to the results of the dose-to-time curve (Fig. 4b and e) and the previous behavioural results, the optimal doses of YL-IPA08 and PK11195 were selected as 0.3mg/kg and 3mg/kg, respectively. The electrophysiological results suggested that the intensification of firing rate of pyramidal neurons induced by YL-IPA08 (0.3 mg/kg, i.p.) was antagonized by PK11195 (3 mg/kg, i.p.) after 60 min, simultaneous injection of YL-IPA08 and PK11195 (Fig. 4B, $F(3, 100) = 12.99$, $P < 0.05$; compared with YL-IPA08 + YL-IPA08 & PK11195), consistent with our previous findings (Zhang et al., 2014). Based on the results mentioned above, we speculated that YL-IPA08 might regulate E:I balance through TSPO in the prefrontal cortex.

4. Discussions

In the present study, we discovered that the antidepressant and anxiolytic behavioural effects of YL-IPA08 were reversed in the TSPO KO mice, and the E:I balance in mPFC was involved in the antidepressant, anxiolytic behavioural effects of YL-IPA08. Based on the above studies, we speculated that TSPO functions as the critical protein mediating neurosteroid synthesis. The TSPO ligand YL-IPA08 might exert antidepressant and anxiety effects by regulating neurosteroid synthesis and subsequently regulating E:I balance, one of the critical neurobiological mechanisms of YL-IPA08.

In the behavioural tests, OFT and EPM were performed to assess the locomotor activity of animals, widely used in evaluating anxiety-related behaviours (Griebel and Holmes 2013). FST and TST were used to evaluate antidepressant actions, reflected by decreased immobility duration, mature indicators for evaluating antidepressants with extremely high reliability (Powell et al. 2012). The results revealed that a single acute intraperitoneal injection of YL-IPA08 (0.3 mg/kg, i.p.) after 60 min in TSPO WT mice exhibited significant anxiolytic behaviours in OFT and EPM tests and antidepressant-like behaviours in TST and FST in TSPO WT mice, except in KO mice, suggesting that the anxiolytic and antidepression actions of YL-IPA08 were mediated by TSPO, consistent with our previous studies (Zhang et al. 2014).

The multichannel electrophysiological technique is widely used in basic research of pharmacology and neuro mechanisms to acquire, record, and analyse specific brain regions' neural firing signals in living animals and examine associations between electrical activities and specific behaviours in animals. The current results suggested that YL-IPA08 significantly increased the firing rates of pyramidal neurons and significantly decreased the firing rates of interneurons after a single administration of YL-IPA08. YL-IPA08 might regulate the E:I functional balance in the mPFC, consistent with the research findings of the rapid-acting antidepressant mechanism of ketamine (Yin et al. 2014). Clinical and preclinical research have demonstrated that depression and chronic stress decrease glutamate and GABA neurotransmitter systems and reduce PFC volume (Duman et al. 2016). Recently, some studies have suggested that currently available first-line antidepressants might eventually exert antidepressant and anxiolytic effects by affecting the functional balance between Glu and GABA. The ratio of Glu: GABA is often used as an objective index to assess the excitation and inhibition of central nervous system function (Li et al. 2018). Some researchers have speculated that E:I imbalance may directly cause MDD (Li et al. 2018). Therefore antidepressants that can quickly regulate E:I functional balance in mPFC carry great potential for a rapid-acting effect in treating depression (Luscher and Fuchs 2015; Yin et al. 2014).

TSPO is an important molecule that mediates neurosteroid synthesis in mPFC, and it is mainly located in pyramidal neurons, microglia and astrocytes (Rupprecht et al; 2010). Clinical studies have shown that some neurosteroids can alleviate depression and anxiety symptoms in postpartum depression and MDD (Deligiannidis et al. 2019; Frieder et al. 2019). Clinical evidence has also demonstrated that Glu is associated with the pathophysiology of MDD: The abnormal reductions in Glu concentrations found in severe and moderate naive patients with MDD (Hasler et al. 2007; Michael et al. 2003). There is a direct effect of mPFC E:I balance on antidepressant and anxiolytic of YL-IPA08 by Combined behavioural and

electrophysiological evidence, which might be mediated by TSPO. To confirm our suspicions, we recorded the effect of PK11195 (a TSPO antagonist) on the firing rate of glutamatergic neurons to explore further the physiological mechanism of the potential antidepressant-anxiolytic like the effect of YL-IPA08. As expected, further studies revealed that the firing rate of Glutamatergic may be antagonized by PK11195, a classic TSPO antagonist, indicating that TSPO mediated the electrophysiological mechanism of YL-IPA08, which is consistent with the behavioural results of TSPO KO. It is further suggested that the activation of TSPO contributed to the acting antidepressant-anxiolytic effect of YL-IPA08, following the regulation of E:I balance in mPFC.

Our preliminary studies have also demonstrated that YL-IPA08 can increase the concentration of neurosteroids (e.g. pregnenolone) in rats' mPFC or explant astrocytes (Zhang et al. 2014). YL-IPA08 exerted significant antidepressant-like actions in a rat chronic unpredicted stress model. To some extent, the discovery of antidepressants has shifted from traditional monoamine targeting to regulating the TSPO activity and E:I functional balance. The evidence of rapid-acting antidepressant effects in depression treatment may encourage the ongoing development of a new field of rapid-acting antidepressant drugs (O'Leary et al. 2015). Based on these results of the electrophysiological tests and all the above, the slew of experimental results aforementioned are reasonably linked together, and we have deduced a clear set of antidepressant and anxiolytic mechanisms mediated by TSPO.

To sum up, YL-IPA08, as an effective ligand of TSPO, affects the synthesis of neurosteroids by activating TSPO proteins. Neurosteroids regulate the action potential discharge of Glutamatergic pyramidal neurons and GABAergic interneurons by binding to $GABA_A$ receptors, and ultimately maintain a dynamic balance of E:I functional system in mPFC, exert antidepressant- and anxiolytic-like effects, which might represent a common mechanism of a series of TSPO-ligands based antidepressant and anxiolytic drugs. YL-IPA08 is a novel antidepressant-anxiolytic drug dependent on TSPO and lays a theoretical foundation for future development of TSPO-ligands based antidepressant-anxiolytic drugs opening research and development prospects for innovative treatment of psychiatric and cerebral diseases.

Declarations

Data Availability

All the original numbers supporting the conclusions of this paper are provided by authors without undue reservation.

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Competing Interests

The author has no relevant financial or non-financial conflicts of interest.

Author Contributions

Jin Yuan: Investigation, Performed multiple tests, Data acquisition and analysis, Writing-Original Draft, Visualization; **Jun-Qi Yao**: Performed the TSTand FST tests, data acquisition and analysis; **Xin-Xin Fang**: Revised the manuscript; **Wei Dai**: Methodology; Supervised the experiments; **Yun-Hui Wang**: Revised the manuscript; **Li-Ming Zhang**: Conceptualization, Writing-review and editing; **Yun-Feng Li**: Resources, Conceptualization, Supervision, Project Administration; and all participants agreed to publish the studies data.

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Figures

Figure 1

Effect of TSPO knockout on the behaviour of acute YL-IPA08 *(0.3 mg/kg, i.p.)* treatment in OFT. a and **b** Neither a single dose of YL-IPA08 *(0.3 mg/kg, i.p.)* nor TSPO KO affected the total number of rearings and a total distance of mice. **c** and **d** YL-IPA08 *(0.3 mg/kg, i.p.)* could significantly increase the total number of entries and the total distance into the central area in TSPO WT mice only. **e** Represents the trail map of mice in OFT. $^{*}P < 0.05$, $^{**}P < 0.001$; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$; n = 7 ~ 10 / group. TSPO: translocator protein; KO: knock-out; OFT: open-field test.

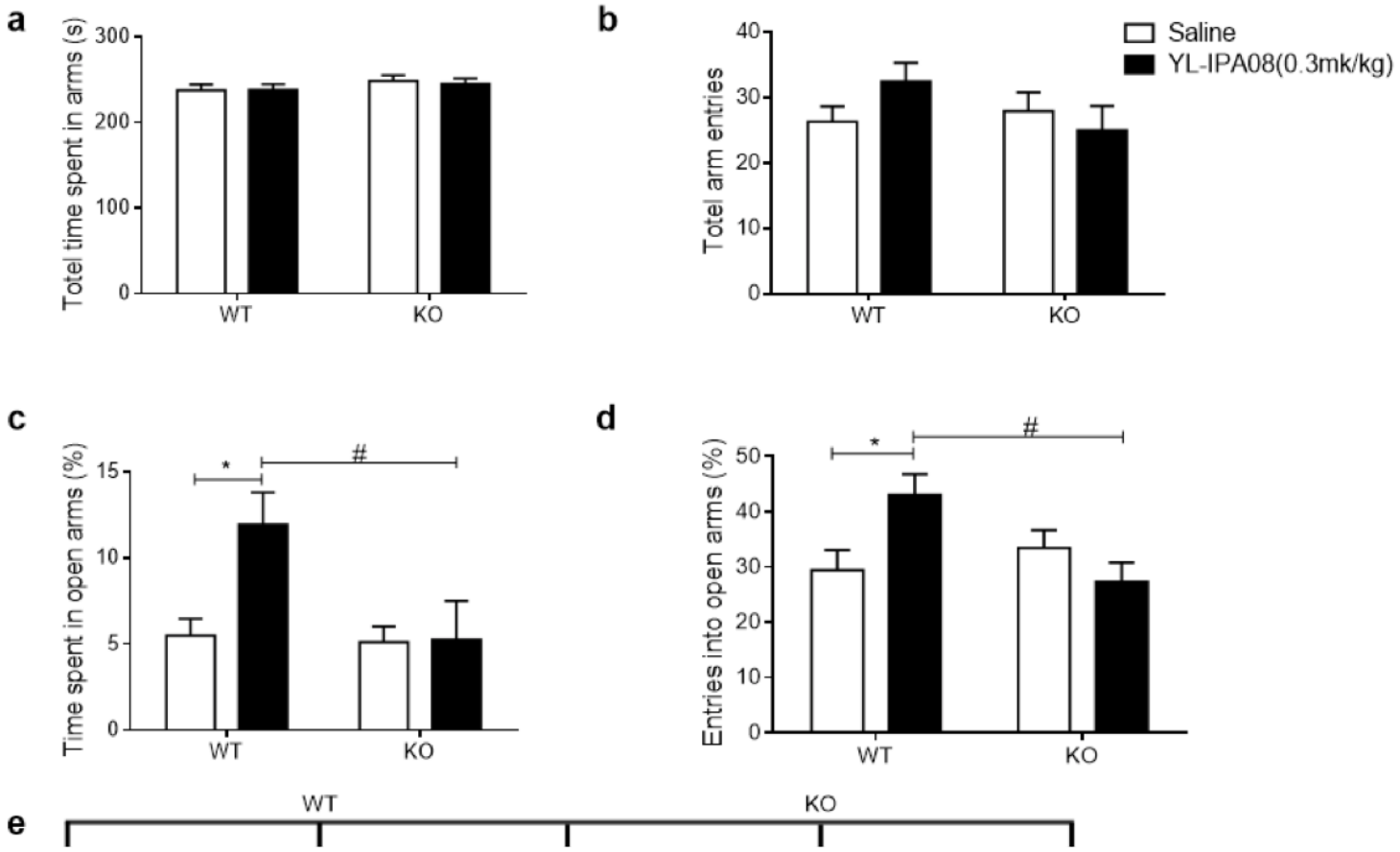

Figure 2

Effect of TSPO knockout on the behaviour of acute YL-IPA08 *(0.3 mg/kg, i.p.)* treatment in the EPM test. a and **b** Neither a single dose of YL-IPA08 *(0.3 mg/kg, i.p.)* nor TSPO KO affected the total duration and a total number of entries into both arms in EPM. **c** and **d** YL-IPA08 *(0.3 mg/kg, i.p.)* could significantly increase the percentage of time spent and the number of entries into the open arms in TSPO WT mice only. **e** Represents the trail map of mice in EPM. $^{*}P < 0.05$, $^{**}P < 0.001$; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$; n = 11 ~ 14 / group. EPM: elevated plus-maze.

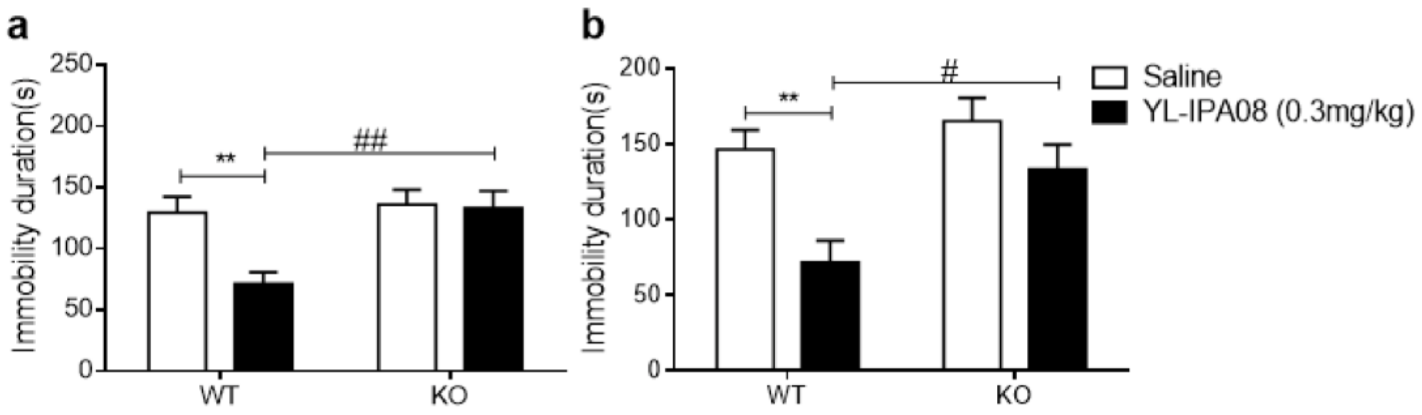

Figure 3

Antidepressant-like effect of TSPO knockout on the antidepressant effects of acute YL-IPA08 *(0.3 mg/kg, i.p.)* treatment in TST (a)and FST(b). YL-IPA08 *(0.3 mg/kg, i.p.)* treatment could significantly decrease the immobility duration of TSPO WT mice but did not exhibit effect on the immobility duration of TSPO KO mice. $^{**}P < 0.005$; $^{\#}P < 0.05$, $^{\#\#}P < 0.005$. n = 8 ~ 9 / group. FST: forced swim test.

Figure 4

Effect of acute intraperitoneal injection of YL-IPA08 on E:I functional balance in mPFC within 90 min. a and **d** represent the waveform characteristics of glutamatergic pyramidal neurons and GABAergic interneurons, respectively. **b** and **e** represent the dose-time effect of YL-IPA08 on the firing rate of action potentials in glutamatergic pyramidal neurons and interneurons, respectively. **c** and **f** show the Raster diagram of the change of firing rate of pyramidal neurons and interneurons, respectively. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$. n = 6 ~14 / group, compared with Basal.

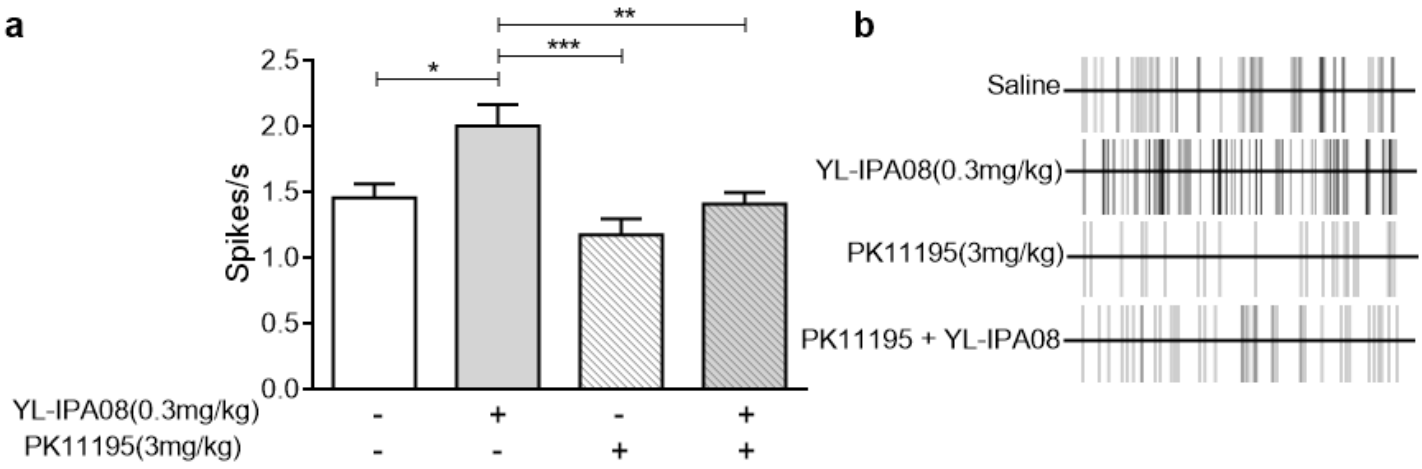

Figure 5

Effect of TSPO antagonist PK11195 on the action potentials of Glutamatergic pyramidal neurons after a single acute intraperitoneal injection of YL-IPA08. a The effect of PK11195 and YL-IPA08 combined on the firing rate of Glutamatergic pyramidal neurons in mPFC. **b** Raster diagram of a single Glutamatergic pyramidal neuron. n = 7 ~ 9/group, *P <0.05 **P <0.005, ***P < 0.001.