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Research Article

The role of TRPV4 in acute sleep deprivation-induced memory impairment: Mechanisms of calcium dysregulation and synaptic plasticity disruption

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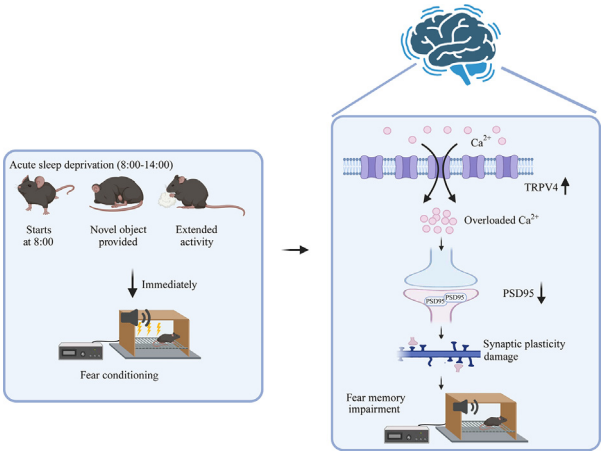
HIGHLIGHTS

- TRPV4 plays a significant role in ASD-induced memory impairment.
- ASD leads to Ca^{2+} overload and synaptic plasticity impairment.
- TRPV4 knockdown reduced Ca^{2+} concentration, enhancing synaptic plasticity.
- TRPV4 knockdown alleviated ASD-induced memory impairment.

GRAPHICAL ABSTRACT



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ABSTRACT

Acute sleep deprivation (ASD) impairs memory formation, but the underlying mechanisms remain unclear. In this study, we employed an ASD model combined with fear conditioning to investigate these mechanisms. mRNA sequencing revealed upregulated expression of Transient Receptor Potential Vanilloid 4 (TRPV4), a nonselective Ca^{2+}-permeable cation channel critical for calcium signaling, in mice with ASD-induced memory impairments.

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(TRPV4)
Postsynaptic density protein 95 (PSD95)
Synaptic plasticity

Notably, TRPV4 knockdown reversed ASD-induced memory deficits. ASD was associated with increased intracellular Ca^{2+} concentrations, reduced spine density, and decreased expression of postsynaptic density protein 95 (PSD95), a key regulator of synaptic plasticity. These findings suggest that ASD may cause Ca^{2+} overload, leading to disrupted synaptic plasticity and impaired learning and memory. Importantly, TRPV4 knockdown significantly reduced Ca^{2+} concentrations, mitigated synaptic plasticity impairments, and contributed to memory restoration. Together, these findings demonstrate a protective role of TRPV4 knockdown against ASD-induced memory deficits and highlight TRPV4 as a potential therapeutic target for memory impairment associated with ASD.

1. Introduction

Sleep is crucial for normal life activities, and regulates various brain physiological processes, including neuromodulation, neuronal activity, and neurotransmission (Puentes-Mestril & Aton, 2017). It plays an essential role in multiple cognitive processes, particularly in supporting memory formation (Ognjanovski et al., 2018). Sleep deprivation can lead to deficits in synaptic plasticity (Gao et al., 2023; Havekes et al., 2012), which is closely linked to learning and memory. Previous studies have demonstrated that sleep deprivation impairs memory consolidation in both clinical and animal studies (Kuriyama et al., 2010; Ravassard et al., 2016; Tiba et al., 2008). However, the precise mechanism underlying sleep deprivation-induced learning and memory impairment remains unclear.

Fear memory is closely associated with the development and occurrence of trauma-related and emotional disorders, particularly post-traumatic stress disorder (PTSD) (Shalev et al., 2017). The classical method to assess fear memory expression is fear conditioning, which relies on associative learning (Dong et al., 2020). Noxious unconditioned stimuli (US) can alter neuronal responses to conditioned stimuli (CS), resulting in specific fear behaviors. Fear conditioning is closely related to memory formation, particularly emotional memory, and provides a robust model for understanding how memories—especially those involving fear or trauma—are encoded, consolidated, and retrieved in the brain (Borgomaneri et al., 2020). Accumulating evidence highlights the critical role of long-term synaptic plasticity in the acquisition and encoding of fear memory (Luchkina & Bolshakov, 2019). Therefore, fear conditioning serves as an excellent behavioral paradigm to explore the mechanism underlying ASD-induced memory impairment.

The medial prefrontal cortex (mPFC) is a crucial region involved in the encoding and processing of memories (Klavir et al., 2017). The prelimbic (PL) region, a subdivision of the mPFC, is anatomically and functionally linked to fear-related behaviors (Dixsaut & Gräff, 2022). PL cortices regulate stimulus-response and action-outcome learning (Sun et al., 2018) and play an essential role in the establishment of fear memories. For example, neuronal activity in the PL is closely associated with freezing behaviors during fear conditioning (Burgos-Robles et al., 2009). Stimulation of PL neurons enhances freezing behaviors during fear memory expression (Vidal-Gonzalez et al., 2006). Moreover, the PL is also critically involved in the sleep-wake cycle. Previous research has shown that PL neurons are significantly activated in sleep-deprived mice (Murack et al., 2021). Taken together, the PL is an ideal region for investigating emotion-related memory impairment caused by sleep deprivation.

Transient receptor potential vanilloid (TRPV4) channels are widely expressed in the brain and function as gated, non-selective cation channels, regulating numerous physiological processes (Grace et al., 2017). Previous studies suggest that TRPV4 expression is regulated by clock genes, which are linked to circadian rhythms (Ihara et al., 2017, 2018b). Disturbance of circadian rhythms significantly alters TRPV4 protein expression. Upregulation of TRPV4 expression, induced by exogenous and endogenous factors, enhances Ca^{2+} influx, leading to intracellular Ca^{2+} overload (Baratchi et al., 2019; Veteto et al., 2020). Consequently, changes in TRPV4 expression are involved in cognitive processes, such as amyloid-β (Aβ)-induced hippocampal cell impairment (Bai & Lipski, 2014), and neuronal apoptosis (Deng et al., 2022). The TRPV channel family has been identified as a risk factor for synaptic plasticity impairment (Shi et al., 2013) and neurobiological diseases, including Alzheimer's disease (Bai & Lipski, 2014), brain edema (Jie, Tian, et al., 2015; Lu et al., 2017), cerebral ischemic reperfusion injury (Jie et al., 2015a, 2016). However, it remains unclear whether TRPV4 plays a critical role in learning and memory impairment induced by sleep deprivation or how its expression and function are altered following sleep deprivation.

The postsynaptic density (PSD) is a dense protein complex localized in excitatory synapses, including various receptors, signal molecules, and structural proteins essential for synaptic plasticity. PSD95, one of the most abundant proteins in the PSD, plays a critical role in dendritic spine development, excitatory neurotransmitter transmission, and synaptic plasticity (Coley & Gao, 2018) Notably, knockout of the transient receptor potential cation channel 6 (TRPC6) enhances PSD95 expression and alleviates learning and memory dysfunction by reducing Ca^{2+} influx (Kong et al., 2023).

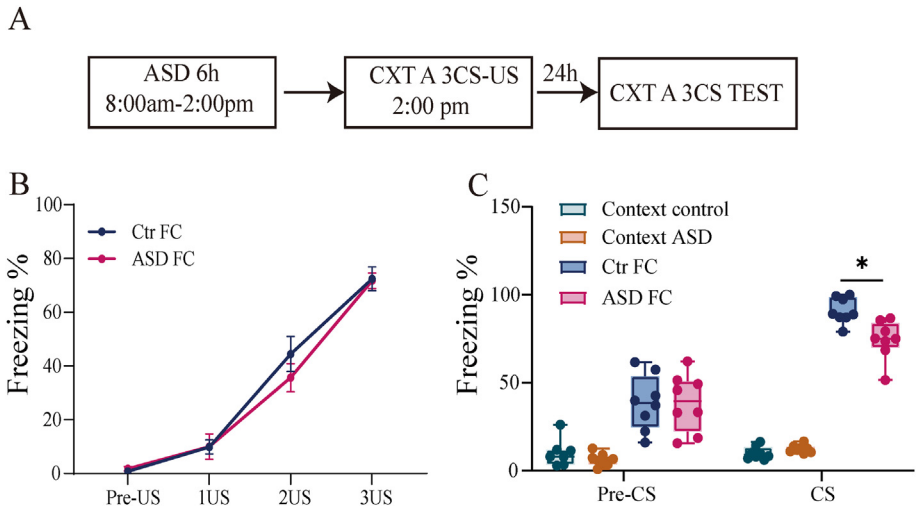

Fig. 1. Acute sleep deprivation impaired memory formation. (A) Timeline of acute sleep deprivation and behavior testing. (B) No significant difference was observed between the control and sleep-deprived groups during memory acquisition. (C) Acute sleep deprivation did not affect the percentage of freezing in the context control and context ASD groups, However, sleep-deprived mice exhibited a reduced percentage of freezing (n = 8 per group). FC: fear conditioned; ASD: acute sleep deprivation; Mean ±SEM. *$p < 0.05$.

Based on this evidence, we employed an acute sleep deprivation (ASD) mouse model combined with fear conditioning to investigate how sleep deprivation affects memory formation, focusing on the underlying molecular mechanisms in the prelimbic cortex (PL). We hypothesized that TRPV4 contributes to sleep deprivation-induced memory impairment by reducing PSD95 expression and disrupting synaptic plasticity. This study aims to elucidate the role of TRPV4 in synaptic plasticity and its association with learning and memory impairment induced by sleep deprivation.

2. Results

2.1. Acute sleep deprivation impairs the formation of memory

To evaluate the effects of acute sleep deprivation (ASD) on learning and memory, mice were subjected to 6 h of sleep deprivation followed by three conditioned stimulus-unconditioned stimulus (CS-US) pairing trials for fear conditioning training on the first day. Fear memory expression was assessed 24 h later using a fear conditioning test across different groups (Fig. 1(A)). Context control groups were included to assess the impact of ASD on memory acquisition, which showed no significant effect (Fig. 1(B)). No significant differences were observed between the context control group and the ASD context control group (Fig. 1(C)), indicating that ASD minimally affected the mice's response to the contextual environment. However, ASD-treated mice exhibited significant impairment in memory formation (Two-way ANOVA with Tukey's post hoc test: Ctr FC vs. ASD FC in CS, $p = 0.018$).

2.2. mRNA sequence reveals gene expression alterations in memory impaired mice induced by sleep deprivation

Following fear conditioning training, mPFC tissues from sleep-deprived mice were collected for RNA sequencing (Fig. 2(A)). Transcriptional profiles were compared between sleep-deprived mice and mice allowed to sleep ad libitum. Fig. 2(B)–(C) illustrates the differentially expressed genes (DEGs) in the ASD and control (Ctr) groups. Compared to the Ctr group, the ASD group exhibited 99 downregulated and 115 upregulated genes. Gene Ontology (GO) analysis identified enriched biological processes and molecular functions in the two groups (Fig. 2(D)). Upregulated DEGs in sleep-deprived mice were associated with “negative regulation of apoptotic process”, “apical plasma membrane”, and “calmodulin binding”, while downregulated DEGs were enriched for “activation of adenylate cyclase activity” and “mitotic spindle”.

To validate the reliability of the mRNA sequencing data, RT-qPCR was performed on selected genes (Fig. 2(E)–(F), $n = 6$, unpaired t-test). In the ASD FC group, the downregulation of genes (Adnp, Dbp, and Henmt1) identified through DEG analysis was confirmed by RT-qPCR, as was the upregulation of genes (Trpv4, Pim1, and Htr5b), demonstrating the reliability and stability of the DEG analysis. Compared to the Ctr group, the mRNA expression of Trpv4, Pim1, and Htr5b was significantly increased in the ASD group (all $p < 0.01$, Fig. 2(E)). Conversely, the mRNA expression of Adnp ($p = 0.013$), Dbp ($p < 0.01$), and Henmt1 ($p < 0.01$) was significantly downregulated in the ASD group (Fig. 2(F)).

Based on DEG analysis, TRPV4 expression was significantly enhanced in the ASD FC group ($p < 0.0001$, Fig. 3(A)). GO analysis revealed that TRPV4 is involved in several biological processes and molecular functions enriched in the ASD group, such as “apical plasma membrane” and “calmodulin binding”. Furthermore, previous studies have demonstrated a close relationship between TRPV4 and learning and memory. These findings preliminarily identify TRPV4 as a critical target in learning and memory impairment induced by acute sleep deprivation.

2.3. Enhanced expression of TRPV4 in sleep-deprived mice

To investigate factors influencing TRPV4 expression, TRPV4 mRNA levels were measured in the context control, context ASD, control FC, and ASD FC groups. The results revealed that acute sleep deprivation significantly increased TRPV4 expression, whereas fear conditioning training did not affect TRPV4 levels. As shown in Fig. 3(A), TRPV4 mRNA levels were significantly elevated in both the context ASD and ASD FC groups compared to their respective controls (Two-way ANOVA with Bonferroni's post hoc test: context Ctr vs. context ASD, $p < 0.0001$; Ctr FC vs. ASD FC, $p < 0.0001$). Additionally, TRPV4 protein expression was assessed in the Ctr FC and ASD FC groups. A significant increase in TRPV4 protein levels was observed in the PL of the ASD FC group (unpaired t-test, $p < 0.001$, Fig. 3(B)).

2.4. Memory deficits are associated with enhanced TRPV4 expression induced by acute sleep deprivation

We designed and validated TRPV4-shRNA based on established protocols (Li et al., 2019), and packaged it into a lentiviral vector. The lentivirus was bilaterally infused into the PL 7 days before ASD and fear conditioning training (Fig. 4(A)–(C)). The knockdown efficiency of TRPV4-shRNA was approximately 50 % (Fig. 4(B)). TRPV4 knockdown did not affect memory acquisition (Fig. 4(D)). However, the percentage of freezing was significantly higher in mice infused with TRPV4-shRNA compared to the control virus group after sleep deprivation, indicating that TRPV4 knockdown rescued memory deficits in sleep-deprived mice (Fig. 4(E), $p = 0.032$).

To further investigate the temporal role of TRPV4 in memory formation, an additional experimental timeline was established (Fig. 4(F)). Lentivirus-TRPV4-shRNA or control virus was infused after fear conditioning training and behavioral tests were performed 7 days later. As shown in Fig. 4(G), sleep deprivation did not affect memory acquisition. However, the freezing level was significantly increased in animals infused with TRPV4-shRNA compared to the control virus group (Fig. 4(H), Two-way ANOVA with Tukey's post hoc test: $p = 0.013$), without altering anxiety-like behavior or spontaneous locomotion (Fig. S1). These findings demonstrate that TRPV4 inhibition, either before or after ASD, rescues memory deficits induced by ASD. Given that TRPV4 is a cation channel involved in calcium signaling, we further investigated whether acute sleep deprivation affects Ca^{2+} concentrations in the PL.

2.5. Ca^{2+} overload and synaptic plasticity impairment in mice with ASD-induced memory deficits

To investigate how sleep deprivation affects memory formation, we examined Ca^{2+} concentrations and synaptic plasticity by assessing PSD95 expression and dendritic spine density. The tissue collection protocol is illustrated in Fig. 5(A). ASD significantly increased Ca^{2+} concentrations in the PL (Fig. 5(B), unpaired t-test, $p = 0.003$). We next evaluated PSD95 expression, a key regulator of synaptic plasticity associated with learning and memory, in mice with ASD-induced memory impairment. As shown in Fig. 5(C), PSD95 mRNA levels were significantly reduced (unpaired t-test, $p = 0.025$). Similarly, PSD95 protein expression was significantly decreased (Fig. 5(D)–(E), unpaired t-test, $p = 0.019$). Representative immunofluorescence images are shown in Fig. 5(F), with quantitative analysis confirming significant downregulation of PSD95 protein in the PL (Fig. 5(G), $p = 0.0014$, unpaired t-test). The timeline for dendritic spine analysis is depicted in Fig. 5(H). Dendritic segments of PL neurons directly traceable to the soma were selected for analysis (Fig. 5(I)). Representative images from the control and ASD groups are shown in Fig. 5(J). The ASD group exhibited significantly reduced spine density (unpaired t-test, Fig. 5(K), $p < 0.0001$).

2.6. TRPV4 knockdown attenuates Ca^{2+} overload and synaptic plasticity impairment in sleep-deprived mice

Based on these findings, we hypothesized that TRPV4 knockdown

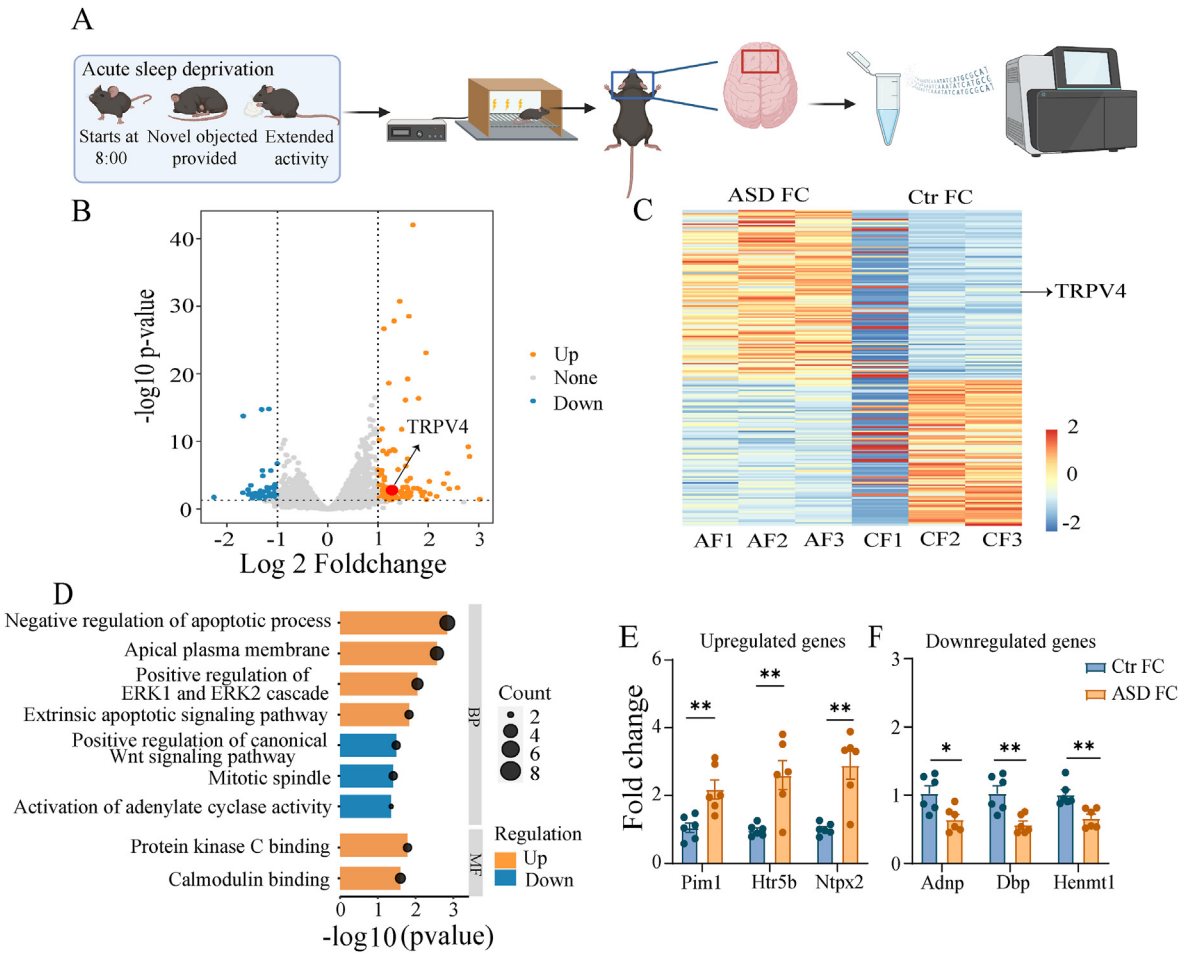

Fig. 2. Characterization of differentially expressed genes (DEGs) between the control and ASD groups. (A) Timeline of behavioral modeling and mRNA sequencing. (B) Volcano plots showing upregulated (yellow) and downregulated (blue) DEGs in ASD vs. Control (Ctr) in the PL. (C) Heatmap of DEG based on mRNA sequencing data from the two groups ($p < 0.05$). (D). Gene Ontology (GO) term analysis of ASD FC vs. Ctr FC, $n = 3$ per group, p value < 0.05. (E)-(F) Verification of upregulated genes (Trpv4, Pim1, and Htr5b) and downregulated genes (Adnp, Dbp, and Henmt1) using RT-qPCR in two groups. FC: fear conditioned; ASD: acute sleep deprivation; Mean ±SEM. *$p < 0.05$, **$p < 0.01$.

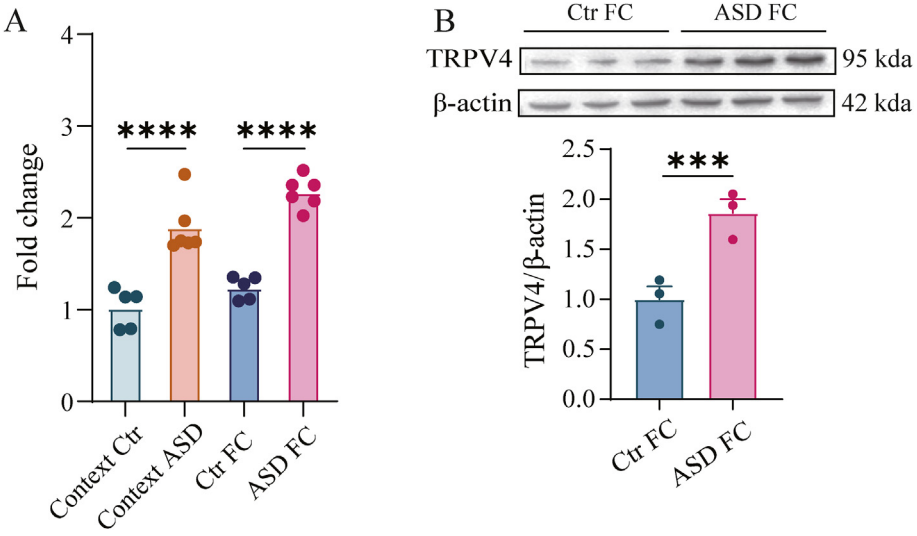

Fig. 3. TRPV4 expression is increased in the PL following acute sleep deprivation but is not influenced by fear conditioning. (A) Acute sleep deprivation significantly increased TRPV4 mRNA expression in both the context control and fear-conditioned groups ($n = 5$–6 per group). (B) TRPV4 protein expression was significantly higher in the ASD FC group compared to the Ctr FC group ($n = 3$ per group). FC: fear conditioned; ASD: acute sleep deprivation; Mean ±SEM; ***$p < 0.001$, ****$p < 0.0001$.

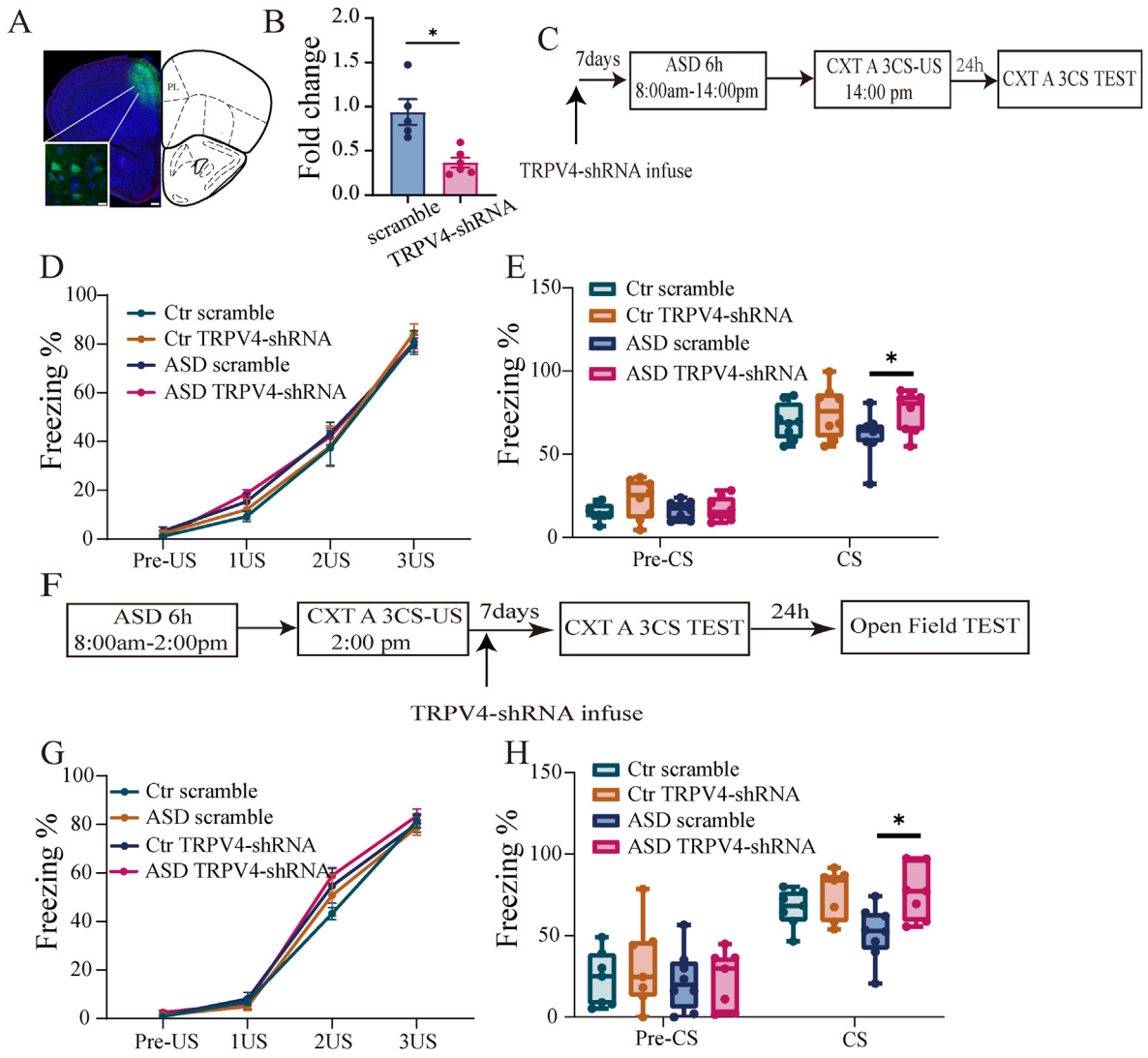

Fig. 4. Knockdown of TRPV4 in the PL reversed memory impairment induced by sleep deprivation. (A) Fluorescent images showing the location of the TRPV4-shRNA lentivirus infusion. Scale bars: 100 μm (white) for the main image and 10 μm (red) for the inset. (B) The knockdown efficiency of TRPV4-shRNA was approximately 50 %. (C) Timeline of lentivirus infusion, sleep deprivation, and behavioral testing. (D) TRPV4-shRNA lentivirus did not affect memory acquisition. (E) The percentage of freezing was significantly higher in animals infused with TRPV4-shRNA compared to those infused with the control virus after sleep deprivation. (F) Timeline of sleep deprivation, behavioral training, and lentivirus infusion. (G) Sleep deprivation did not affect memory acquisition. (H) Freezing levels were significantly higher in ASD animals infused with TRPV4-shRNA compared to the control virus. FC: fear conditioned; ASD: acute sleep deprivation; Mean ±SEM. *$p < 0.05$.

alleviates Ca^{2+} overload and improves synaptic plasticity, thereby rescuing memory deficits. As shown in Fig. 6(A), lentivirus-TRPV4-shRNA was bilaterally infused into the PL 7 days before the experimental day. Mice underwent 6 h of ASD followed by fear conditioning training. Immediately after training, brain tissues were collected for analysis.

TRPV4 knockdown significantly reduced Ca^{2+} concentrations in the PL (Fig. 6(B), unpaired *t*-test, $p = 0.012$). To assess the effects of TRPV4 on synaptic plasticity, we measured PSD95 expression and dendritic spine density. Both PSD95 mRNA (Fig. 6(C), unpaired *t*-test, $p = 0.019$) and protein levels (Fig. 6(D)–(E), unpaired *t*-test, $p = 0.005$) were significantly increased. Representative immunofluorescence images of PSD95 in the PL are shown in Fig. 6(F), with quantitative analysis confirming increased PSD95 expression following TRPV4 knockdown (Fig. 6(G), unpaired *t*-test, $p = 0.005$).

The timeline for dendritic spine analysis is depicted in Fig. 6(H). Dendritic segments directly traceable to the soma were selected (Fig. 6(I)). Representative images from the ASD scramble and ASD TRPV4-shRNA groups are shown in Fig. 6(J). TRPV4 knockdown reversed the ASD-induced reduction in spine density (unpaired *t*-test, Fig. 6(K), $p < 0.001$).

3. Discussion

In this study, we investigated the effects of sleep deprivation on memory formation. Fear conditioning provides a robust model for understanding how memories, particularly those related to fear or trauma, are encoded, consolidated, and retrieved in the brain. PL, a key region associated with fear-related behaviors, served as the focus of our investigation. We employed an ASD mouse model combined with fear conditioning to explore how sleep deprivation affects memory formation and its underlying molecular mechanisms in the PL.

Through mRNA sequencing analysis, we identified TRPV4 as a potential mediator of sleep deprivation-induced memory impairment. To further elucidate the role of TRPV4, we packaged a lentivirus to specifically knockdown TRPV4 expression in the PL. TRPV4 knockdown

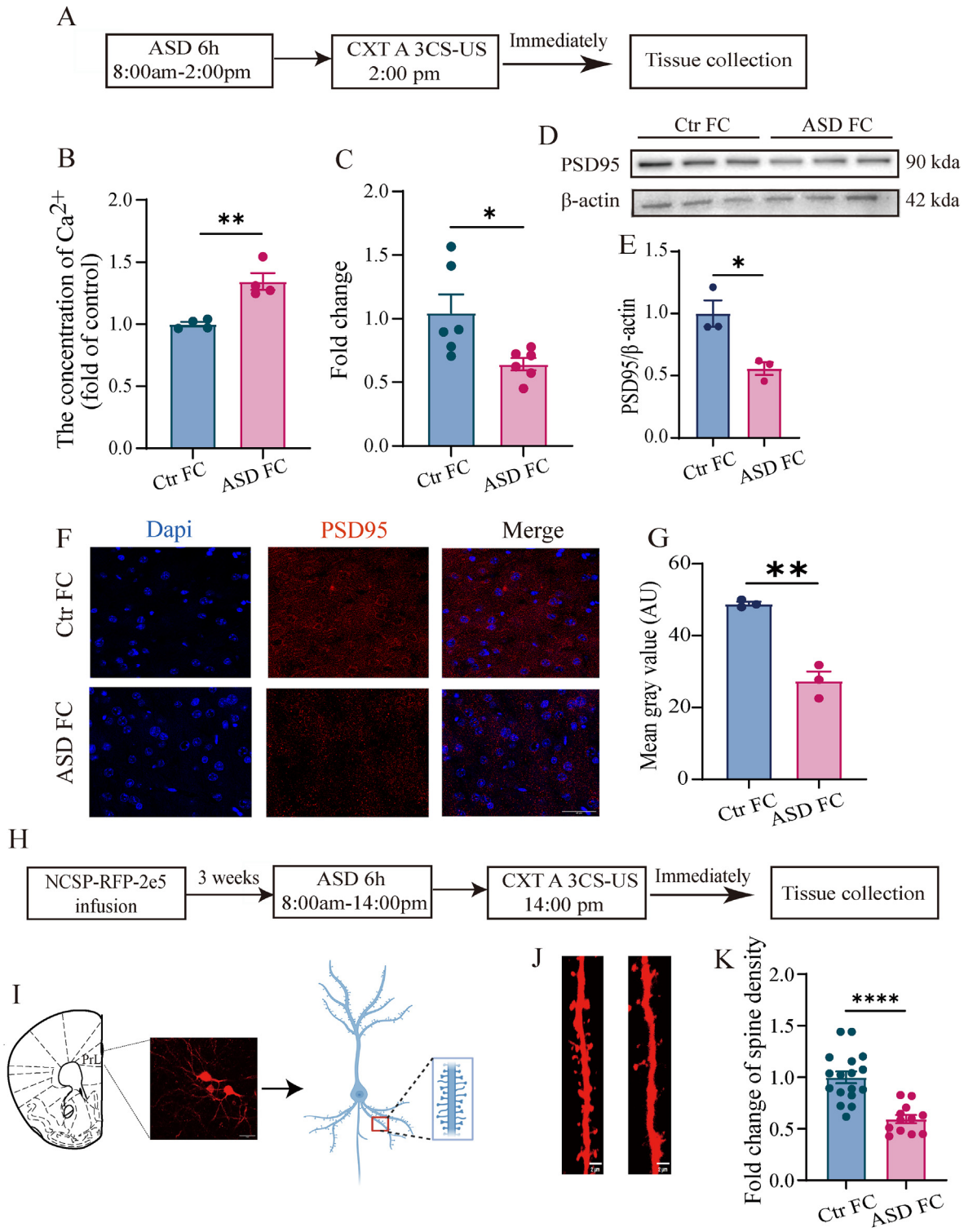

Fig. 5. Acute sleep deprivation caused Ca^{2+} overload and impaired synaptic plasticity in the PL of mice with memory impairment. (A) Timeline of tissue collection for measuring Ca^{2+} concentration and PSD95 expression. (B) Ca^{2+} concentration in the PL was significantly higher in the ASD FC group. (C) PSD95 mRNA level was significantly lower in the ASD FC group. (D) Representative immunoblot images of PSD95. (E) The expression of PSD95 proteins was significantly reduced in sleep deprived mice. (F) Representative immunofluorescence of PSD95 in two groups. (G) Quantitative analysis showed that PSD95 protein expression was significantly decreased. (H) Timeline for dendrite spine density analysis. (I) Schematic diagram showing the dendritic spines of PL neurons. Secondary basal spine dendrites were analyzed. Scale bar: 10 μm (J) Representative dendrites spines of PL neurons from control and sleep-deprived mice. Scale bar: 2 μm. (K) Dendritic spine densities of PL neurons in control and sleep-deprived mice were quantified. n = 12–15 dendrites from 3 mice per group. FC: fear conditioned; ASD: acute sleep deprivation; Mean ±SEM. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. Scale bar: 40 μm.

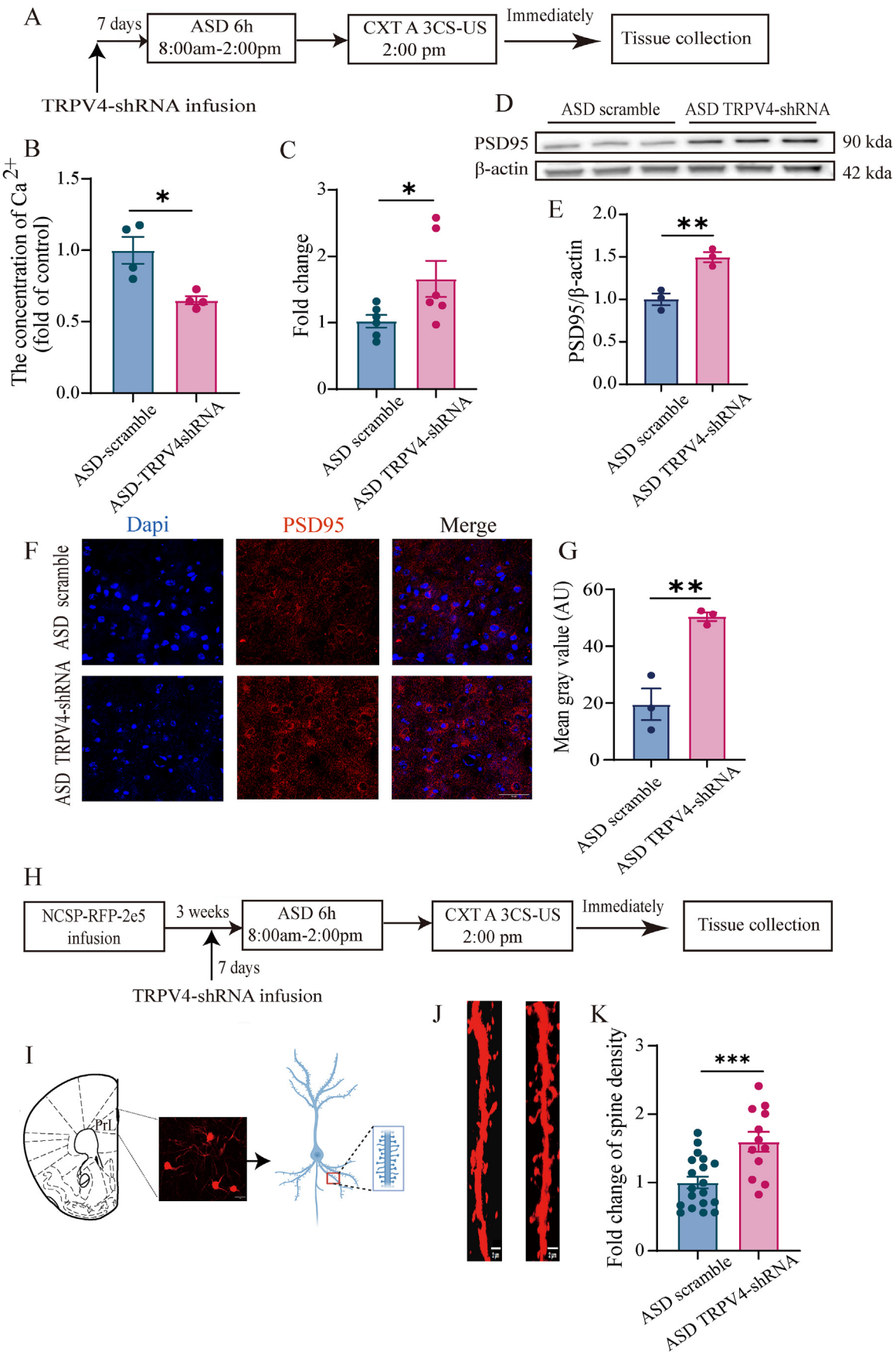

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Fig. 6. Ca^{2+} concentration was reduced, and impaired synaptic plasticity was reversed in the PL following TRPV4-shRNA lentivirus infusion in sleep-deprived mice. (A) Timeline of tissue collection for measuring Ca^{2+} concentration and PSD95 expression after TRPV4-shRNA knockdown. (B) TRPV4 knockdown significantly reduced the concentration of Ca^{2+} in the PL of sleep-deprived mice. (C) PSD95 mRNA level was significantly increased in the ASD TRPV4-shRNA group. (D) Representative immunoblot images of PSD95 in the two groups. (E) PSD95 protein expression was significantly increased after TRPV4-shRNA infusion compared to scramble virus infusion. (F) Representative immunofluorescence of PSD95 in the two groups. (G) Quantitative analysis showed that PSD95 protein expression was significantly enhanced. (H) Timeline for dendrite spine density analysis in different groups. (I) Schematic diagram showing dendritic spines of PL neurons. Secondary basal spine dendrites were analyzed. Scale bar: 10 μm. (J) Representative dendrites spines of PL neurons from sleep-deprived mice after scramble or TRPV4-shRNA lentivirus infusion. Scale bar: 2 μm. (K) Dendritic spine densities of PL neurons in sleep-deprived mice were quantified after different virus infusions (n = 12–18 dendrites from 3 mice per group). FC: fear conditioned; ASD: acute sleep deprivation; Mean ±SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Scale bar: 40 μm.

effectively reversed memory deficits caused by sleep deprivation, concurrently mitigating dendritic spine loss and PSD95 protein reduction. These findings suggest that sleep deprivation-induced TRPV4 upregulation leads to dendritic spine damage and PSD95 reduction, ultimately impairing memory.

Collectively, our results highlight the critical role of TRPV4 in sleep deprivation-induced memory impairment and its impact on synaptic plasticity. This study underscores the therapeutic potential of targeting TRPV4 to alleviate the cognitive deficits associated with sleep deprivation.

Sleep plays a crucial role in memory consolidation. Given the importance of neuronal activity in synaptic plasticity, sleep regulates synaptic connections essential for memory learning and formation (Lichtman & Colman, 2000; G. Yang et al., 2009). Therefore, understanding how sleep deprivation affects memory formation is critical. This study demonstrates that acute sleep deprivation impairs both synaptic morphological and functional plasticity, leading to learning and memory deficits. Previous studies have shown that 8 h of sleep deprivation significantly reduces spine formation and impairs learning and memory, with additional training failing to reverse these effects (Guang Yang et al., 2014). Interestingly, increased dendritic spine density and spinogenesis of pyramidal neurons were observed 24 h after sleep deprivation (Wu et al., 2024). These findings suggest that acute sleep deprivation may initially reduce spine density and impair learning and memory, but subsequent sleep and rest could potentially rescue the impaired synaptic plasticity.

Neural activities in the brain are highly complex and sensitive to changes in sleep conditions. Sleep deprivation can disrupt normal neural transmission, leading to deficits in attention and memory. PL, a subregion of the medial prefrontal cortex (mPFC), not only regulates the sleep-wake cycle but also plays a critical role in cognitive processes. Previous studies have shown that sleep disturbances and fragmentation often precede cognitive deficits in alcohol-dependent patients with abnormal mPFC function (J. Liu et al., 2019). Furthermore, chronic sleep deprivation suppresses neural networks associated with the PL and impairs synaptic plasticity (Zhu et al., 2023), indicating that sleep deprivation can induce cognitive dysfunction through abnormal neural activity in the PL.

Additionally, the PL is a key region for memory retrieval (DeNardo et al., 2019). Activation of PL neurons has been linked to increased freezing behavior during memory formation (Vidal-Gonzalez et al., 2006), whereas inactivation of these neurons suppresses learning and memory (Corcoran & Quirk, 2007; Sierra-Mercado et al., 2011). Given these findings, it is essential to elucidate the regulatory mechanisms within the prelimbic cortex that contribute to memory impairment caused by acute sleep deprivation.

In this study, we found that 6 h of ASD significantly enhanced TRPV4 expression. TRPV4 appears to be particularly sensitive to sleep loss, with its expression pattern closely linked to circadian rhythms regulated by clock genes (Ihara et al., 2017, 2018a). TRPV4 levels are elevated during wakefulness and decrease during sleep stages (Ihara et al., 2017). Interestingly, previous studies have shown that members of the TRPV family play a role in regulating sleep homeostasis (Deng et al., 2022). For example, mechanosensory neurons expressing TRPV channels can indirectly influence sleep centers in the brain and modulate circadian rhythms (Lone et al., 2021). These findings highlight the critical role of TRPV4 in sleep regulation (Kumar et al., 2022).

TRPV4 channels are nonselective plasma membrane Ca^{2+} channels widely distributed in the brain (Liu, Yan, et al., 2020). Given their importance in neuronal transmission, dysregulation of TRPV4 channels may contribute to various neurobiological diseases, including Parkinson's disease (Liu et al., 2022), Alzheimer's disease (Liu, Yan, et al., 2020), and brain edema (Faropoulos et al., 2021). Our findings that TRPV4 knockdown rescues memory deficits in acute sleep deprivation align with its reported roles in some neurological disorders. In Alzheimer's mice, astrocytic TRPV4 channels exacerbate calcium dyshomeostasis and Aβ-induced hippocampal neuronal death (Bai & Lipski, 2014) — a mechanism strikingly analogous to the calcium dysregulation we observed in ASD-impaired mice. In PD models, TRPV4-driven ER stress and neuroinflammation mediate dopaminergic neuron loss in the substantia nigra (Liu et al., 2022; Liu, Liu, et al., 2020), mirroring our finding that TRPV4 inhibition attenuates synaptic plasticity damage through calcium homeostasis restoration. Clinically, TRPV4 inhibitor HC067047 alleviates cognitive deficits in scopolamine-induced amnesia models, further supporting its broad neuroprotective potential. (Deng et al., 2022). These collective findings underscore TRPV4 as a convergent molecular node governing calcium-dependent neuronal dysfunction, highlighting its potential as a versatile therapeutic target for neurological disorders.

TRPV4 activation triggers Ca^{2+} influx from the extracellular matrix and intracellular Ca^{2+} release, leading to elevated overall Ca^{2+} concentrations (Caterina et al., 1997; Senning & Gordon, 2015). Ca^{2+} acts as a critical secondary messenger, regulating various cellular activities, and imbalances in Ca^{2+} homeostasis are closely linked to neurological diseases. In this study, TRPV4 expression was significantly enhanced in mice with ASD-induced memory impairment. Notably, TRPV4 knockdown in the PL reversed memory deficits caused by sleep deprivation and increased dendritic spine density. These findings suggest that TRPV4 hyperactivation due to sleep deprivation may lead to Ca^{2+} overload, impair synaptic plasticity, and ultimately result in memory deficits. Similarly, a previous study demonstrated that knockout of TRPC6, another Ca^{2+}-permeable channel, improved Ca^{2+} homeostasis, alleviated neuronal damage, and mitigated cognitive deficits (Kong et al., 2023). We found that TRPV4 knockdown contributes to the recovery of PSD95 expression in sleep-deprived mice. In sleep-deprived mice, TRPV4 knockdown normalizes intracellular Ca^{2+} levels, preventing prolonged calmodulin (CaM) activation and its downstream proteolytic/transcriptional cascades. Balanced Ca^{2+}/CaM signaling enables transient activation of CaMKII, a kinase critical for synaptic plasticity (Colomer & Means, 2007; Nicoll & Schulman, 2023; Yasuda et al., 2022), which promotes PSD95 expression (Cai et al., 2021; Mi et al., 2022). This cascade aligns with reports that NR2B-CaMKII-PSD95 coupling rescues neuronal injury in LPS-induced neuroinflammation models (Song et al., 2019), suggesting evolutionary conservation of calcium-dependent synaptic remodeling mechanisms.

TRPV4 channels are widely expressed in neurons, microglia, astrocytes, smooth muscle cells of cerebral vasculature, as well as vascular endothelial cells in the brain. Importantly, existing literature strongly supports that TRPV4 in either cell type can regulate neuronal function through calcium signaling (Bai & Lipski, 2014; Cibelli et al., 2024; Lee & Choe, 2014; Shibasaki et al., 2014, 2015). For example, astrocytic TRPV4 affects Aβ-induced hippocampal neuronal function via calcium dyshomeostasis (Bai & Lipski, 2014), and neuronal TRPV4 channel is regarded

as a potential target for cognitive and aging-related neurological disorders (Kanju & Liedtke, 2016). In the current study, we found that TRPV4 knockdown in the IL rescues the memory deficits via upregulating PSD95 expression and improving neuronal functions. We fully acknowledge that this approach cannot distinguish cell type-specific contributions, and future studies need to explore this issue.

In summary, ASD induces memory impairment, accompanied by increased TRPV4 expression and Ca^{2+} overload. Furthermore, TRPV4 knockdown alleviated synaptic plasticity damage and reversed memory deficits in sleep-deprived mice. These findings suggest that TRPV4 may serve as a promising therapeutic target for mitigating cognitive impairments associated with sleep deprivation and underscore the importance of sleep management.

4. Materials and methods

Mice: Nine-week-old male C57BL/6J mice were given free access to food and water under a 12-h light/dark cycle. All behavioral testing was conducted in red-light rooms. The protocols for this study were approved by the Animal Ethics Committee of Zhongnan Hospital of Wuhan University (Ethics approval number: ZN2023121).

Acute sleep deprivation and fear conditioning test protocol: Before the experiment, the mice were allowed to acclimate to the environment and establish a circadian cycle (with the light phase from 8:00 a.m. to 8:00 p.m.). On the experimental day, the mice were kept awake by gentle stimulation from 8:00 a.m. to 2:00 p.m. (Guo et al., 2022). During this period, animals were allowed to explore freely without disturbance until a sleep attempt was captured. At this point, they were kept awake using mild auditory and tactile stimulation with a pencil-sized paintbrush.

The experimenter was prohibited from touching the mice at any time to minimize stress. After sleep deprivation, the mice were placed in the operant chambers (Xinyi Instruments, Shanghai, China). The conditioning chambers featured two walls made of transparent material and two walls constructed from stainless steel, with a metal grid floor (3.2 mm in diameter and spaced 8 mm apart at the center). The context was scented with a lemon fragrance. Following a previous study's protocol, the training included three trials pairing the tone with the shock (80 dB tone as the context stimulus (CS) for 120 s and a 0.6 mA foot shock as the unconditioned stimulus (US) lasting 1 s) (Li et al., 2019). Each trial was separated by 120-s intervals. The mice's locomotion was recorded by cameras in the chambers, and the data were processed and analyzed using FreezeFrame 4 software to determine the percentage of freezing, defined as at least 1s of immobility. The context control mice were exposed to the same context for 14 min (matching the duration of the training protocol) without any tone or shock. On the second day after training, the mice were returned to the lemon-scented context for recall tests. They were allowed to explore for 120 s, followed by three trials consisting of 120-s CS presentations alternating with 120-s intervals. The percentage of freezing time during the recall tests was assessed to evaluate memory retention.

Open field test The open field test was performed to measure anxiety-related behaviors and spontaneous movement. The testing apparatus consisted of a white plastic chamber (30 cm × 30 cm × 30 cm). Animals from each group were placed individually at the center of the chamber. Their activities were recorded for 20 min using a ceiling-mounted camera. Video data were analyzed with Any-Maze tracking software (Xinruan Technology, Shanghai, China) to quantify three parameters: (1) total distance traveled, (2) time spent in the central area, and (3) number of entries into the central zone. All tests were conducted under consistent lighting conditions.

4.1. RNA sequence and data analysis

mRNA from mPFC tissue was extracted, and rRNA was removed using a kit from Vazyme (Nanjing, China). The Dynabeads purification kit (Invitrogen, USA) was used to purify the mRNA. The mRNA was broken into fragments, and cDNA libraries were constructed. Subsequently, the cDNA library was sequenced on the Illumina HiSeq 4000 platform. The final data were mapped to the mouse genome (GRCm39), and read counts were analyzed to determine gene expression across different groups (fold change >1 and p value < 0.05). Clustering and Gene Ontology (GO) enrichment analysis were performed using R packages.

RNA extraction and RNA reverse transcription: Trizol reagent (Vazyme, Nanjing, China) was added in the Eppendorf tubes containing the tissues, which were then homogenized using electric grinding rods. Subsequent steps for RNA isolation were performed according to the manufacturer's instructions. RNA concentration was measured using a Nanondrop spectrophotometer (N50, IMPLEN, Germany). RNA reverse transcription was performed using a kit from Vazyme (Cat: R423-01, Nanjing, China) according to the provided protocol.

Quantitative PCR: After preparing, the cDNA was diluted 1:5 with nuclease-free water. Quantitative PCR (qPCR) was performed according to the manufacturer's protocol (Cat: Q711-03, Vazyme, Nanjing, China) in a 10 μL reaction volume (5ul SYBR Green Master Mix, 2uL cDNA, 1uL primer (5 μM, Sangon Biotech), and 2 μL nuclease-free water per reaction). The cDNA concentration was adjusted based on the abundance of the target gene. All reactions were performed in duplicate using a Rotor-Gene Q PCR cycler. Data were normalized to the internal control Pgk1 (phosphoglycerate kinase) and analyzed using the δδCT method. qPCR primers of each gene were listed below: Trpv4 F: AAACCTGCGTATGAAGTTCCAG; R: CCGTAGTCGAACAAGGAATCCA; Pim1 F:TGTCCAAGATCAACTCCCTGG; R:CCACCTGGTACTGCGACTC; Htr5b F: CTGGTGAGCGAGTTGTCCG; R: GCGTGATAGTCCAGTAGCGA; Adnp F: GACTCCCACCACGAATCAGC; R:CCCGTTGAATTTAAGTTGGGCT; Dbp F: GGAAACAGCAAGCCCAAAGAA; R:CAGCGGCGCAAAAAGACTC; Henmt1 F: AGATTTAGTGGATCGCCATGAAC; R: CGATGCCCATTTGAATGCAATTT; Pgk1 F: TGCACGCTTCAAAAGCGCACG; R: AAGTCCACCCTCATCACGACCC.

Western blot: PL tissue from mice was collected for protein extraction. The tissue was lysed using RIPA buffer (Servicebio, Wuhan, China) and denatured at 65 °C for 30 min to ensure complete protein denaturation. The protein samples were diluted to a concentration of 5 μg/μL using PBS and loading buffer. Proteins were separated by gel electrophoresis for 45 min and then transferred to a PVDF membrane (0000287894, Immobilon-P, Germany). The membrane was blocked with quick blocking buffer (HYC00811, HYCEZMBIO, Wuhan, China) for 30 min at room temperature. After blocking, the membrane was washed three times with Tris-buffered saline containing 1 % Triton-X-100 (TBST) for 7 min. The membrane was then incubated with 10 mL of rabbit antibody (TRPV4 1:1000, A5660, RRID: AB_2766420; PSD 95 1:1000, A7889; abclonal, Wuhan, China) in antibody diluent buffer at 4 °C for 18 h, followed by three washes with TBST. Subsequently, the membrane was incubated with HRP-conjugated anti-rabbit secondary antibody (1:10000, bioswamp, SAB48169, Wuhan, China) in antibody diluent buffer for 1 h and washed three times with TBST for 7 min each. Due to a non-specific band at 43 kDa in the PSD95 Western blot (Fig. 6D), we stripped the membrane with antibody elution buffer, then re-incubated it with the loading control antibody and re-detected. The optical density and quantitative analysis of the blots were performed using an LI-COR analysis system.

Immunofluorescence: The expression of PSD95 protein was measured in fear-conditioned mice after sleep deprivation. The sleep-deprived mice underwent fear conditioning in operant chambers. Immediately afterward, the animals were anesthetized with isoflurane (RWD Life Science, Shenzhen, China) and transcardially perfused with PBS, followed by 4 % paraformaldehyde. The brain tissues were collected, embedded in optimal cutting temperature (OCT) compound, and sectioned into 40 μm slices using a freezing microtome.

The brain slices were washed four times with PBST and incubated with blocking solution for 2 h at 37 °C. Next, the brain slices were incubated overnight at 4 °C with anti-PSD95 primary antibody (1:1000, Proteintech, 20665-1-AP, RRID: AB_2687961, Wuhan, China). After

washing, the slices were incubated for 2 h at 25 °C with goat anti-rabbit IgG conjugated to Alexa Fluor 594 (1:400, Jackson, RRID: AB_2728112, USA). Following a final series of four washes, the slices were mounted on glass slides. Images were captured using a confocal microscope (Leica, Germany) and analyzed using ImageJ software (RRID: SCR_003070).

Ca^{2+} concentration measurement: The Ca^{2+} concentration in IL tissue was measured using an assay kit from Beyotime Biotechnology (Cat: S1063S). According to the manufacturer's protocol, the tissue was thoroughly homogenized and lysed. Subsequently, 75 μL of color reagent and 75 μL of calcium buffer were mixed with 50 μL of lysate and incubated for 10 min. Absorbance was measured at 575 nm using a microplate reader (Thermo Fisher Scientific, USA). Calcium levels in each group were determined using a standard curve.

Lentivirus packaging: Lentivirus was packaged following a previously described method (Lin et al., 2011). HEK293T cells (RRID: CVCL_0063) were used for lentivirus production. When the cells reached approximately 80 % confluency in monolayer flasks, they were transfected with three helper plasmids (pMDLg, pMDG, and pRSV) and a transfer vector (constructed by cloning TRPV4 shRNA into the FG12 plasmid) using Lipofectamine 2000 transfection reagent, according to the manufacturer's instructions. After 48 h of culture, the supernatant was collected, filtrated, and concentrated by ultracentrifugation. The concentrated virus, including a control lentivirus and a TRPV4-targeting lentivirus, were stored at −80 °C.

The H1 promotor sequence used in the lentiviral packaging is listed below.

5'-3':TATATACGACTCACTATAGGGAGATCCAAGCTTATCGATACCG TCGACCTCGAGGG.

GGGGCCCGGTACCAAGCTTTTTTT.

The sequences for the shRNA were as follows:

TRPV4 shRNA: CCCTGGCAAGAGTGAAATCTA;

Universal control shRNA: GCGCGATAGCGCTAATAAT.

The titers of each lentivirus were at least 1 x 10^8 IU/mL.

Stereotaxic injection surgery: Mice were anesthetized with 1 % pentobarbital (i.p., 50 mg/kg) and placed in a stereotaxic frame (RWD Life Science, Shenzhen, China). Throughout the procedure, a heating pad was used to maintain the animals' body temperature. Control lentivirus and TRPV4 shRNA lentivirus (approximately 1 μL at a rate of 80 nL/min) were injected bilaterally into the PL (2.34 mm anterior to the bregma, 0.20 mm lateral to the midline, and 1.90 mm ventral to the dura) using a glass pipette connected to a microinjection pump (RWD Life Science, Shenzhen, China). After injection, the pipette was left in place for 10 min to prevent viral diffusion. The scalp incision was then sutured. Once the mice regained consciousness, they were returned to their home cages. According to our previous study (Li et al., 2019), the mice were allowed at least one week to recover and ensure stable virus expression, which effectively reduced the synthesis of the target protein, thereby achieving a decrease in protein levels.

Virus injection and dendritic spine analysis: AAV (NCSP-RFP-2e5) was infused bilaterally into the PL to reconstruct single neurons. After 21 days, sleep-deprived and sleep ad libitum mice were subjected to fear conditioning in operant chambers. Immediately afterward, the mice were anesthetized with isoflurane (RWD Life Science, Shenzhen, China) and transcardially perfused with PBS, followed by 4 % paraformaldehyde (PFA). The brain tissues were collected, fixed in 4 % PFA for 24–48 h, and sliced into 35 μm slices. The brain sections were mounted on adhesive slides and sealed with an anti-fluorescent quencher (Servicebio, Wuhan, China). Images were acquired using a Zeiss 980 microscope with Airscan mode. A 63× oil immersion objective was used to capture detailed images of dendritic spines. Secondary basal dendrites located 50–110 μm from the soma were selected, as described in a previous study (Zhang et al., 2021), using the z-stack mode. The Zeiss 980 microscope was used to analyze spine density across different groups. The experimenters were blinded to the group assignments and treatments throughout the study.

4.2. Statistics

The experimenter was blinded to the group assignments throughout the experiment. Data were analyzed using GraphPad Prism 9.0 (GraphPad, RRID: SCR_002798, New York, USA) and Image J (RRID: SCR_003070, USA). The Shapiro-Wilk test was used to assess the data normality, and an unpaired *t*-test was used for comparisons between two groups. A two-way analysis of variance (ANOVA) was used to compare differences among four groups, followed by Tukey's multiple comparison test for pairwise comparisons. Data are presented as the mean ± the standard error of the mean (SEM), and a p-value <0.05 was considered statistically significant.

CRediT authorship contribution statement

Meimei Guo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Feiyang Zhang:** Writing – original draft, Methodology, Conceptualization. **Sha Liu:** Methodology, Conceptualization. **Yi Zhang:** Investigation. **Lesheng Wang:** Investigation. **Jian Song:** Supervision, Resources. **Wei Wei:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Xiang Li:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Conflict of interest

All authors declare no financial or nonfinancial conflicts of interest.

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Appendix A. Supplementary data

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