scientific reports

Check for updates

OPEN

Effects of tianeptine on mTORC1-mediated neuronal autophagy in primary rat hippocampal neurons under nutrient deprivation

Mi Kyoung Seo[1,2], Hyewon Kim[3], Ah Jeong Choi[1], Dae-Hyun Seog[2,4,5], Weon-Gyu Kho[1,6], Sung Woo Park[1,2]✉ & Jung Goo Lee[1,3]✉

The aim of this study was to investigate the effects of the antidepressant tianeptine on the mechanistic target of rapamycin complex 1(mTORC1)-mediated autophagy pathway in primary hippocampal neurons exposed to B27-deprived conditions. When primary hippocampal neurons were treated with tianeptine at doses of 1, 10, 50, and 100 μM for 3 days under B27-deprived conditions, we observed that it activated autophagy and increased the formation of autophagosomes through the upregulation of autophagic proteins, including autophagy-activating kinase 1 (ULK1), Beclin 1, LC3B-II/I, and p62. And at a concentration of 100 μM tianeptine, the decrease in mTORC1 phosphorylation induced by B27 deprivation was significantly reversed. Changes in the expression of autophagic proteins induced by B27 deprivation were reversed by tianeptine treatment in a concentration-dependent manner, and tianeptine significantly reduced the increase in LC3B membrane number induced by B27 deprivation, an effect that was blocked by pretreatment with rapamycin. In conclusion, tianeptine attenuated the activity of mTORC1-mediated autophagy in primary rat hippocampal neurons under B27-deprived conditions. These results may suggest a novel mechanism by which tianeptine may affect autophagy in neurons.

Keywords Tianeptine, Autophagy, mTORC1, Hippocampal neurons, B27 deprivation

Depression is a mental illness characterized by symptoms such as low mood, loss of interest, and decreased activity[1]. Depression is known to be chronic and not respond well to treatment. According to the World Health Organization, approximately 280 million people worldwide currently suffer from depression, and more than 700,000 people die by suicide each year[2]. While a variety of treatments for depression exist, pharmacotherapy with antidepressants, including selective serotonin reuptake inhibitors (SSRIs), is commonly and widely used[3–5]. Despite their widespread use, the exact mechanism of action of antidepressants remains unclear. The monoamine hypothesis posits that SSRIs work by inhibiting the reuptake of monoamine neurotransmitters, including serotonin, dopamine, and noradrenaline[5,6]. However, pharmacotherapy with these antidepressants has significant drawbacks: approximately 50–60% of patients do not respond adequately to initial treatment, and it can take weeks to months for treatment effects to appear and it can take weeks to months to observe treatment effects[7,8]. This delayed treatment response is not fully explained by the monoamine hypothesis, which means that our understanding of the pathophysiology of depression is still incomplete.

Therefore, recent research has focused on changes in signaling and neuroplasticity after neurotransmitters bind to neuronal receptors[9]. In particular, recent studies have focused on changes in neuroplasticity caused by activation of the mechanistic target of rapamycin complex 1 (mTORC1) and its antidepressant effects[10]. mTORC1 is a protein complex that is important in cell signaling and regulation and is involved in cell growth, proliferation, metabolism, and autophagy[11,12]. The activity of mTORC1 is regulated by a variety of signals, including growth factors, nutrient availability, cellular energy levels, and stress signals[13–15]. When conditions

[1]Paik Institute for Clinical Research, Inje University, Busan 47392, Republic of Korea. [2]Department of Convergence Biomedical Science, College of Medicine, Inje University, Busan 47392, Republic of Korea. [3]Department of Psychiatry, College of Medicine, Haeundae Paik Hospital, Inje University, Busan 48108, Republic of Korea. [4]Department of Biochemistry, College of Medicine, Inje University, Busan 47392, Republic of Korea. [5]Dementia and Neurodegenerative Disease Research Center, College of Medicine, Inje University, Busan 47392, Republic of Korea. [6]Department of Parasitology, College of Medicine, Inje University, Busan 47392, Republic of Korea. ✉email: swpark@inje.ac.kr; iybihwc@naver.com

are favorable, mTORC1 phosphorylates its key targets to promote cell growth and protein synthesis, resulting in increased protein synthesis and cell proliferation[16,17].

In particular, ketamine, an NMDA antagonist, has been shown in clinical studies to have antidepressant effects, which are thought to be due to changes in neuroplasticity caused by increased synaptogenesis through mTORC1 activation[18,19]. Preclinical studies have also shown that ketamine reduces depressive behavior and increases mTORC1 signaling and synaptogenesis in animal models of depression[20,21].

In addition to its role in neuroplasticity, mTORC1 signaling plays an important role in autophagy, a cellular process that maintains cellular health by breaking down and recycling damaged proteins and organelles[22–24]. Furthermore, the autophagy and mTORC1 pathways have been found to be interconnected, acting as a regulatory network that influences a variety of cellular processes, including cell growth, metabolism, and autophagy itself[22–24]. Notably, dysregulation of autophagy may contribute to the pathogenesis of neurodegenerative diseases[25]. mTORC1 serves as a central regulator of cell growth and metabolism in response to nutrient availability and growth factor signaling[12]. When nutrients and growth factors are abundant, mTORC1 is active, promoting cell growth and protein synthesis while inhibiting autophagy[26]. This inhibition occurs through the phosphorylation of key autophagy regulators such as ULK1 (Unc-51-like autophagy activating kinase 1) and ATG13, which suppress autophagy initiation[11]. Conversely, under conditions of nutrient deprivation or other stressors, mTORC1 activity is inhibited, leading to the activation of autophagy[27]. This inhibition of mTORC1 releases inhibitory phosphorylation on ULK1 and ATG13, allowing the formation of the ULK1-ATG13-FIP200 complex, which initiates autophagy[28]. This allows cells to recycle cellular components to generate energy and maintain cellular homeostasis[26]. Consequently, the relationship between autophagy and mTORC1 is a dynamic and finely tuned one that enables cells to adapt to changing nutritional conditions, maintain cellular homeostasis, and influence neuroplasticity[29].

Tianeptine is a drug used primarily as an antidepressant and anxiolytic[30,31]. The mechanism of action for tianeptine's antidepressant and anxiolytic effects is thought to be different from other currently used antidepressants, including SSRIs, and the exact mechanism of action is not clearly understood[32,33]. However, studies have suggested that tianeptine may exert its antidepressant and anxiolytic effects by increasing BDNF expression and affecting neuroplasticity, similar to traditional antidepressants[34–36]. The mechanism of tianeptine's effects on neuroplasticity is also unclear. Recent studies suggest that tianeptine may exert its antidepressant effects by affecting neuronal autophagy[37].

The limitations of current antidepressants, including delayed therapeutic effects and incomplete understanding of mechanisms, now emphasize the need for new approaches. Given the critical role of mTORC1 in neuroplasticity and autophagy and the recent findings on ketamine, it is important to investigate how other antidepressants, such as tianeptine, affect these pathways. Therefore, this study aims to investigate the effects of tianeptine on the mTORC1-mediated autophagy pathway in primary hippocampal neurons under B27-deprived conditions, potentially revealing a novel mechanism of its antidepressant effects.

Results

Effects of nutrient deprivation on apoptosis and autophagy in cultured primary hippocampal neurons

Growth medium B27, which contains a variety of growth factors, proteins, and lipid-rich compounds, enhances the growth of primary neurons while inhibiting the growth of glia[38]. To investigate the effects of B27 deprivation on autophagy and neurotoxicity of hippocampal cells, cells were maintained without B27 supplementation for 3 days. B27 deprivation resulted in a significant reduction in cell viability by approximately 18% (82.4% of control, $t = 4.070$, $p < 0.001$, Fig. 1A). Furthermore, treatment with 0.5 μM rapamycin and 10 μM chloroquine, used as a positive control for autophagy detection, decreased cell viability to approximately 35.4% (64.6% of control, $t = 12.200$, $p < 0.001$, Fig. 1A).

The levels of ULK1 phosphorylation, Beclin 1, LC3B-II/I ratio, and p62 were quantified by Western blot analysis. The level of phosphorylated ULK1 was found to be decreased by B27 deprivation (64.6% of control, $t = 5.500$, $p < 0.001$, Fig. 1B). The levels of Beclin 1 and LC3B-II/I were increased by approximately 49% (148.5% of control, $t = 4.330$, $p < 0.001$, Fig. 1C) and 52% (151.6% of control, $t = 2.960$, $p = 0.010$, Fig. 1D), respectively. In B27 deprivation conditions, the level of p62 expression was reduced to a level similar to that observed in the positive control (60.3% of control, $t = 8.840$, $p < 0.001$, Fig. 1E).

Figure 1F shows strong green fluorescence in the cytoplasm of positive control cells, indicating autophagy activation. In contrast, B27-deprived cells exhibit a slight reduction in fluorescence, while control cells display markedly diminished fluorescence. B27-deprived cells exhibited a significantly elevated number of LC3B puncta (155.1% of control, $t = 3.290$, $p = 0.001$, Fig. 1F).

The effects of tianeptine on autophagy in B27-deprived cells

The toxicity of tianeptine in B27-deprived hippocampal cells was assessed to further experiments. Tianeptine was treated at various doses (1-200 μM) to B27-deprived cells. No significant changes in viability were observed in cells treated with 1-100 μM tianeptine. In contrast, treatment with 200 μM tianeptine resulted in a significant decrease in cell viability ($F_{[8, 63]} = 40.900$, $p < 0.001$; B27 deprivation only versus B27 deprivation + tianeptine 200 μM, $p < 0.001$, Fig. 2A). Consequently, 200 μM tianeptine was excluded from the subsequent experiments. B27 deprivation resulted in a significant decrease in the phosphorylated level of mTORC1 ($F_{[5,30]} = 3.370$, $p = 0.016$; control versus B27 deprivation only, $p = 0.016$, Fig. 2B). This effect was significantly prevented by tianeptine 100 μM (B27 deprivation only versus B27 deprivation + tianeptine 100 μM, $p = 0.040$).

Figure 3 illustrates the effects of tianeptine (50 and 100 μM) on the expression of autophagic proteins and autophagosome formation in B27-deprived cells. The phosphorylated level of ULK1 was significantly reduced by B27 deprivation ($F_{[3, 28]} = 6.690$, $p = 0.002$; control versus B27 deprivation only, $p = 0.002$), but this reduction

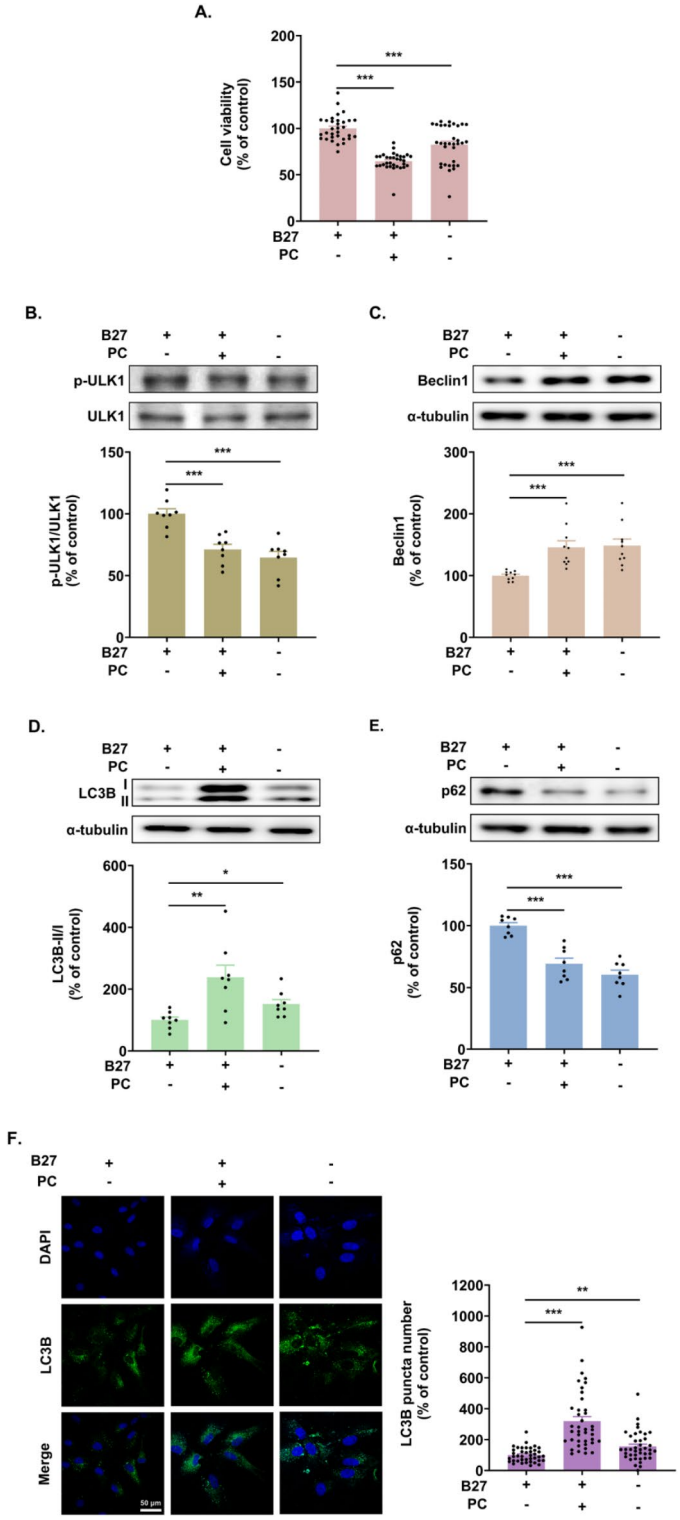

Fig. 1. The effect of B27 deprivation on apoptosis and autophagy of hippocampal cells. The cells were maintained with (B27 +) or without (B27 −) growth medium B27 for 3 days. Cells treated with 0.5 μM rapamycin and 10 μM chloroquine were employed as a positive control (PC). An MTT assay was conducted ($n = 32–33$) (**A**). Western blots were employed to assess the levels of ULK phosphorylation ($n = 8$) (**B**), Beclin 1 ($n = 10$) (**C**), the LC3B-II/I ratio ($n = 8$) (**D**), and p62 ($n = 8$) (**E**). Quantitative analyses were normalized to the total ULK or the α-tubulin band. Full-length uncropped gels for all Western blots are available in the Supplementary Information. The cells were stained with the Cyto-ID Green autophagy dye and analyzed by confocal microscopy ($n = 39–40$ fields) (**F**: left, representative image; right, Quantitative analyses). The values are presented as the mean ± standard error of the mean (SEM) expressed as a percentage of the control values (B27 + /PC −). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ versus control.

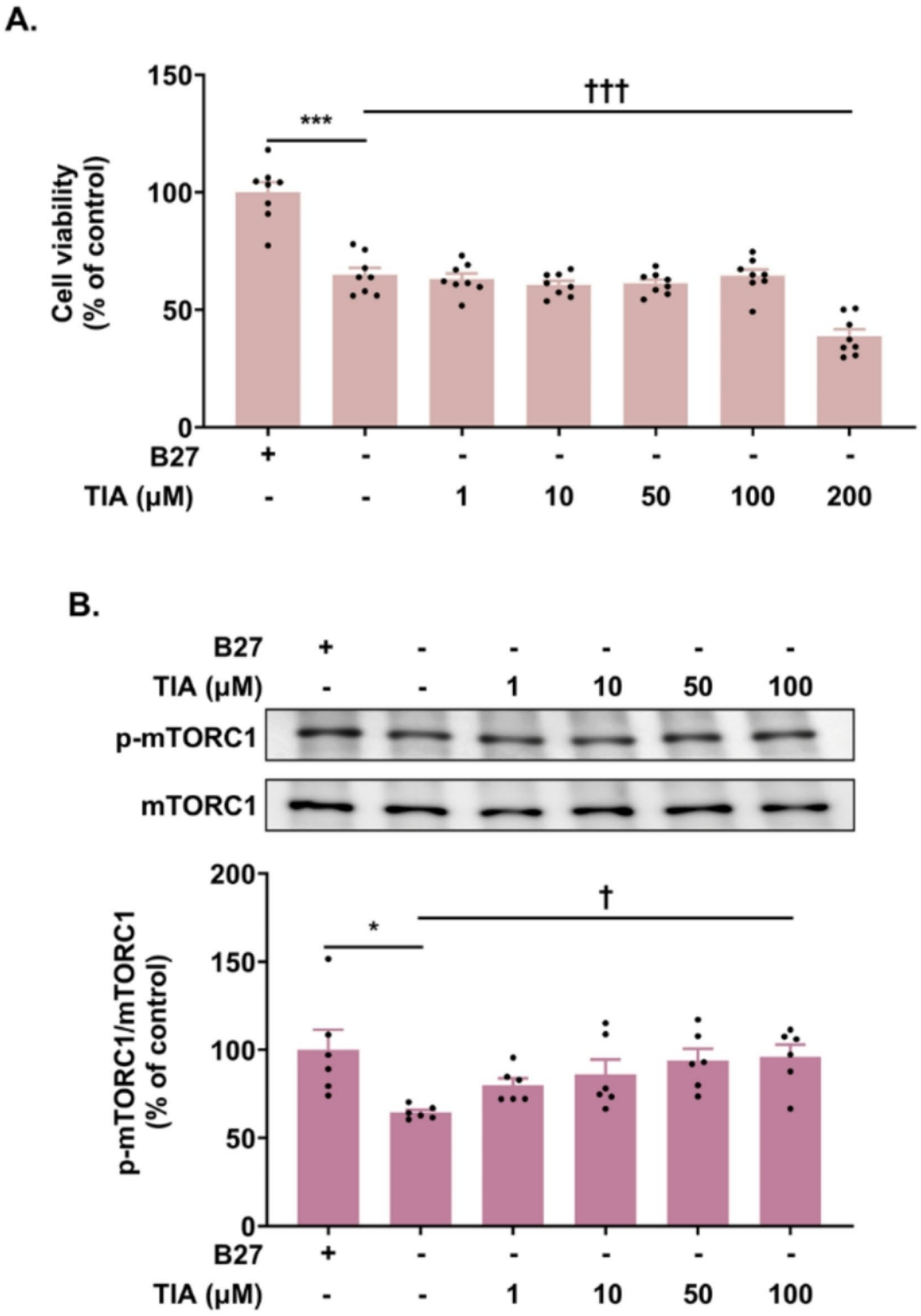

Fig. 2. The effect of tianeptine on the viability of B27-deprived hippocampal cells. The cells were treated with tianeptine (TIA; 1–200 μM) in the absence of growth medium B27 (B27 −) for 3 days. An MTT assay was conducted ($n = 8$) (**A**). Western blots were employed to ascertain the levels of mTORC1 phosphorylation ($n = 6$) (**B**). The quantitative analyses were normalized to the total mTORC1 band. Full-length uncropped gels for all Western blots are available in the Supplementary Information. The values are presented as the mean ± standard error of the mean (SEM) expressed as a percentage of the control (B27+/TIA−) values. $^{*}p < 0.05$ and $^{***}p < 0.001$ versus control; $^{\dagger}p < 0.05$ and $^{\dagger\dagger\dagger}p < 0.001$ versus B27 deprivation only (B27−/TIA−).

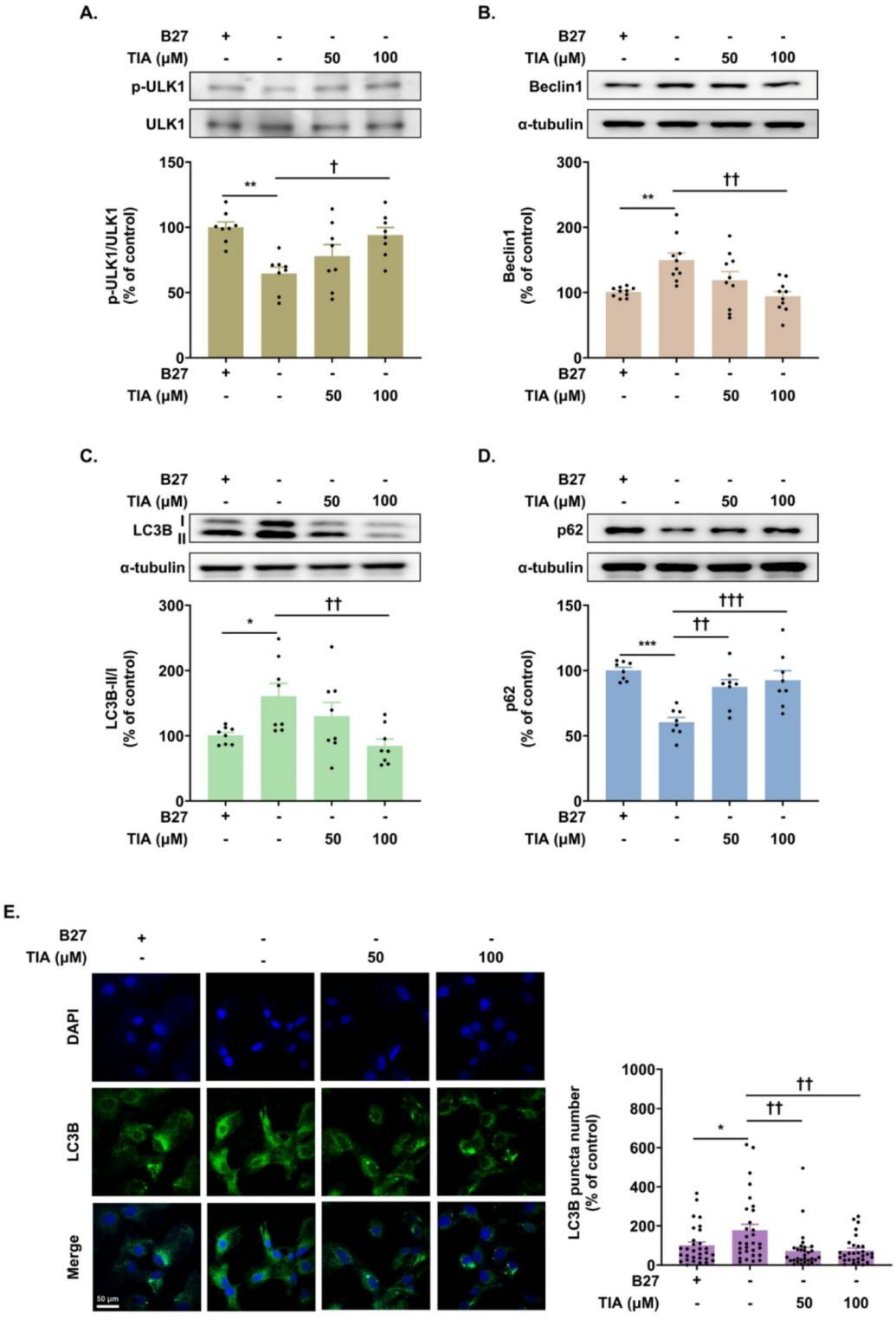

Fig. 3. The effects of tianeptine on the expression of autophagic proteins and autophagosome formation in B27-deprived hippocampal cells. Cells were treated with tianeptine (TIA; 50 and 100 μM) in the absence of growth medium B27 (B27 −) for 3 days. Western blots were employed to assess the levels of ULK phosphorylation ($n = 8$) (**A**), Beclin 1 ($n = 10$) (**B**), the LC3B-II/I ratio ($n = 8$) (**C**), and p62 ($n = 8$) (**D**). Quantitative analyses were normalized to the total ULK or the α-tubulin band. Full-length uncropped gels for all Western blots are available in the Supplementary Information. The cells were stained with the Cyto-ID Green autophagy dye and analyzed by confocal microscopy ($n = 30$ fields) (**E**). The values are expressed as a percentage of the control (B27 +/TIA −) and presented as the mean ± standard error of the mean (SEM). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ versus control; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ and $^{\dagger\dagger\dagger}p < 0.001$ versus B27 deprivation only (B27−/TIA−).

was significantly reversed by tianeptine 100 μM (B27 deprivation only vs. B27 deprivation + tianeptine 100 μM, $p = 0.012$, Fig. 3A). B27 deprivation was found to significantly elevate Beclin 1 and LC3B-II/I levels (Beclin 1: $F_{[3,36]} = 6.810$, $p < 0.001$; control versus B27 deprivation only, $p = 0.005$; LC3B-II/I ratio: $F_{[3,28]} = 4.680$, $p = 0.009$; control versus B27 deprivation only, $p = 0.049$), whereas 100 μM tianeptine significantly reversed these effects (Beclin 1: B27 deprivation only versus B27 deprivation + tianeptine 100 μM, $p = 0.001$, Fig. 3B; LC3B-II/I ratio: B27 deprivation only versus B27 deprivation + tianeptine 100 μM, $p = 0.009$, Fig. 3C). Treatment with tianeptine 50 or 100 μM reversed the decrease in p62 expression induced by B27 deprivation ($F_{[3,28]} = 11.500$, $p < 0.001$; control versus B27 deprivation only, $p < 0.001$; B27 deprivation only versus B27 deprivation + tianeptine 50 μM, $p = 0.004$; B27 deprivation only versus B27 deprivation + tianeptine 100 μM, $p < 0.001$, Fig. 3D).

B27 deprivation significantly affected the number of LC3B puncta ($F_{[3,116]} = 5.705$, $p = 0.001$, control versus B27 deprivation only, $p = 0.044$, Fig. 3E). Tianeptine 50 or 100 μM significantly reduced the number of puncta in B27-deprived cells (B27 deprivation only versus B27 deprivation + tianeptine 50 μM, $p = 0.003$; B27 deprivation + tianeptine 100 μM, $p = 0.003$).

The effects of mTORC1 inhibition on tianeptine-induced autophagy in B27-deprived cells

To investigate the role of mTORC1 signaling in the inhibition of tianeptine-induced autophagy, cells were pretreated with rapamycin under B27 deprivation conditions. The levels of mTORC1 phosphorylation, the LC3B-II/I ratio, and p62 were quantified by Western blotting (Fig. 4).

Two-way ANOVA revealed significant differences for tianeptine ($F_{[1,20]} = 9.681$, $p = 0.006$), rapamycin ($F[_{1,20]} = 4.726$, $p = 0.042$), and the tianeptine × rapamycin interaction ($F_{[1,20]} = 10.010$, $p = 0.005$) for the phosphorylated levels of mTORC1. *Post hoc* analyses showed that tianeptine significantly increased mTORC1 phosphorylation ($p = 0.002$) and this increase was blocked by rapamycin ($p = 0.007$, Fig. 4A). Significant differences in LC3B-II/I levels were also observed for tianeptine ($F_{[1,20]} = 13.800$, $p = 0.001$), rapamycin ($F_{[1,20]} = 4.990$, $p = 0.037$), and the interaction between tianeptine and rapamycin ($F_{[1,20]} = 4.770$, $p = 0.041$). The tianeptine-induced decrease in LC3B-II/I levels was completely blocked by rapamycin ($p = 0.032$, Fig. 4B). A two-way ANOVA of p62 levels revealed a significant effect of tianeptine ($F_{[1,20]} = 14.900$, $p < 0.001$) and rapamycin ($F_{[1,20]} = 4.490$, $p = 0.047$), as well as a trend for an interaction between tianeptine and rapamycin ($F_{[1,20]} = 3.830$, $p = 0.064$). In a post hoc comparison, p62 level was increased by tianeptine ($p = 0.003$), and this effect was blocked by rapamycin ($p = 0.044$, Fig. 4C).

Discussion

In this study, tianeptine reversed the decrease in mTORC1 phosphorylation and changes in autophagy protein expression induced by B27 deprivation. It also reduced the ratio of Beclin 1 to LC3B-II/I and the number of LC3B puncta, suggesting an inhibitory effect on autophagy. These effects were also blocked by rapamycin, confirming that the effect of tianeptine on autophagy is mediated by the mTORC1 pathway.

Autophagy is a cellular metabolic process that degrades and removes unneeded organelles or proteins via lysosomes[27]. Autophagy has been implicated in the pathophysiological mechanisms of several psychiatric disorders, including depression. Recent studies have shown that autophagy-mediated cell death impairs neuroplasticity, leading to depression, and that antidepressants can modulate autophagy[20,27]. However, the exact role of autophagy in the pathophysiology of depression is not fully understood[39].

Autophagy markers are biological measurements that play an important role as indicators of autophagy during normal physiological processes, pathogenic processes, or pharmacological responses to drugs[40] and there are different types of autophagy markers, including macroautophagy, selective autophagy, chaperone-mediated autophagy, and microautophagy. These autophagy markers can be used to detect autophagy levels in cells or tissues dynamically, in real time, and quantitatively, but each has its own significance and limitations[41]. In this study, we measured changes in the macroautophagy markers Beclin 1, LC3B, ULK1, and p62, as well as changes in LC3B puncta, in neurons under B27 deprivation conditions.

Beclin 1 is a protein that plays an important role in the regulation of autophagy in cells and is an essential component of the phosphatidylinositol 3-kinase class III (PI3K-III) complex, which is involved in the formation and maturation of autophagosomes[42,43]. Cancer and neurodegenerative diseases have been shown to occur when Beclin 1 does not function properly[44]. LC3B also plays an important role in the autophagy process. L3CB identifies autophagy targets, triggers the production of autophagosomes, and can also be used to measure autophagosome levels. The formed autophagosomes are used to engulf cytoplasmic elements, including cytoplasmic proteins and organelles, and to engulf organelles[41,45]. In addition, LC3B-I is converted to LC3B-II, which forms an autophagic membrane[41]. ULK1 plays an important role in the early stages of autophagy[46]. In the absence of nutrients, dephosphorylation of ULK1 leads to dissociation of a ULK1-containing complex (ULK1-Atg13-Atg101-FIP200 complex) from the mTORC1 complex[47,48]. This leads to increased activation of the Beclin1/Vps34 complex (Beclin1-Atg14L-Vps15-Vps34 complex), which induces the production of phosphatidylinositol 3-phosphate (PI3P) and subsequent nucleation of vesicles with a bilayer structure by aggregated DFCP1 and WIPI proteins[49]. This is followed by vesicle elongation by proteins containing two ubiquitin-like conjugation systems [Atg12-Atg5-Atg16 and LC3-phosphatidylethanolamine (PE)], leading to autophagosome formation[45,50]. p62, also known as sequestosome-1 (SQSTM1), is a multifunctional protein that acts as an autophagy receptor. It interacts with ubiquitinated proteins and delivers them to autophagosomes for degradation. During this process, p62 degrades itself and a corresponding decrease in p62 levels is observed when autophagy is induced. Similarly, when autophagy is severely inhibited, p62 is observed to accumulate in the cell[51,52]. Therefore, p62 can be a useful metric for measuring autophagy activity. The level of p62 indicates the degree of autophagy, and a decrease in p62 is associated with an increase in autophagy[53].

In this study, we observed changes in autophagy in primary hippocampal neurons from mice exposed to a B27-deprived environment. In cell culture, a B27-deprived environment refers to the omission or removal of

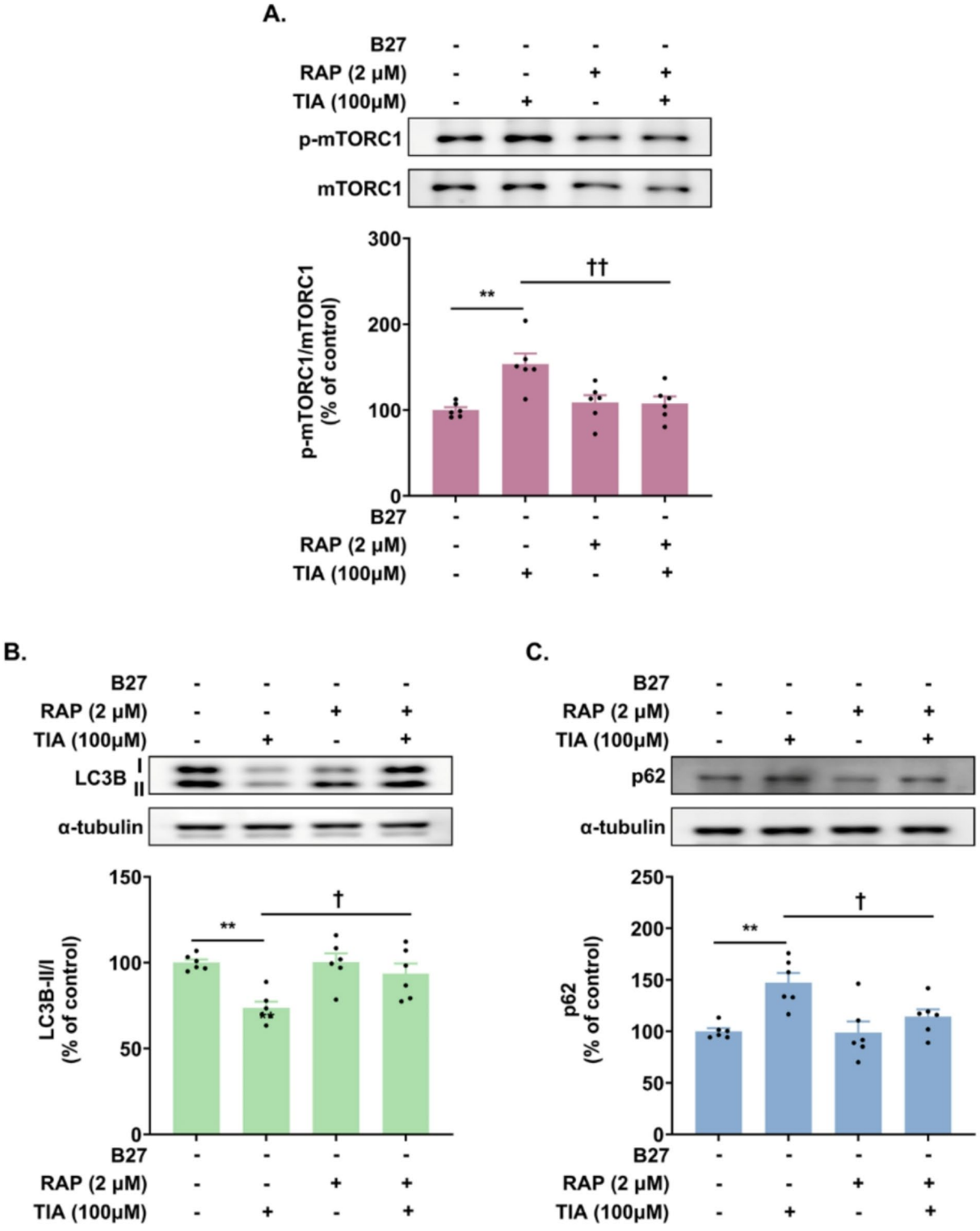

Fig. 4. The effects of the mTORC1 inhibitor rapamycin on the regulation of tianeptine-induced autophagic proteins expression in B27-deprived hippocampal cells. B27-deprived cells were incubated with rapamycin (RAP, 2 μM) for 30 min prior to treatment with tianeptine (TIA; 100 μM). Following a three-day incubation period, Western blotting was conducted to assess the levels of mTORC1 phosphorylation ($n = 6$) (**A**), the LC3B-II/I ratio ($n = 6$) (**B**), and p62 ($n = 6$) (**C**). Quantitative analyses were normalized to the total mTORC1 or the α-tubulin band. Full-length uncropped gels for all Western blots are available in the Supplementary Information. The values are expressed as the mean ± standard error of the mean (SEM) and represent the percentage of the control (B27−, no drug) values. $^{**}p < 0.01$ versus control; $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ versus tianeptine-only-treated cells.

B27 supplementation from the growth medium used to grow neurons or other cell types in vitro. Depriving cells of B27 can be used to study a variety of cellular processes, including cell death, survival mechanisms, and the effects of nutritional stress on cell health and function[54]. For example, studies using neuronal cell cultures have shown that the absence of B27 can simulate conditions that may cause cellular stress or damage, which may be useful for studying neurodegenerative diseases or the effects of certain treatments aimed at improving cell survival and resilience. In the present study, a B27-deprived environment decreased phosphorylated ULK1 levels and increased Beclin 1 and LC3BII/I ratios. It also decreased p62 levels compared to the chloroquine-treated group. Immunofluorescence studies also showed an increase in the number of LC3B membranes in B27-deprived hippocampal neurons. Taken together, these results indicate that a B27-deprived environment increases autophagy in primary rat hippocampal neurons. This is consistent with previous findings by Young et al. who observed that B27 deprivation increased autophagy in primary cortical neurons[55], and another study confirming that starvation induces autophagy[56]. The phosphorylation levels of ULK1, which were reduced by B27 deprivation, were significantly reversed by tianeptine 100 μM treatment. In addition, the levels of Beclin 1 and LC3B-II/I, which were increased by B27 deprivation, were significantly reversed by tianeptine 100 μM treatment. Tianeptine 50 or 100 μM also reversed the Beclin 1 and LC3B-II/I ratios decreased by B27 deprivation. B27 deprivation also significantly affected the number of mucosal LC3B on immunofluorescence. Tianeptine 50 or 100 μM significantly reduced the number of membranes in B27-deprived cells. In this study, tianeptine treatment reduced the activation of autophagy induced by B27 deprivation in primary rat hippocampal neurons. And the reversal of autophagy markers by tianeptine suggests a reduction in autophagy activity, indicating a protective mechanism against excessive autophagy which could lead to cellular damage[57,58]. Previous studies have shown that antidepressants used to treat depression and anxiety can have a variety of effects on neuronal autophagy[59]. However, these effects are highly complex and may vary depending on the specific antidepressant and the context in which it is used[56,59]. Several studies have shown that antidepressants affect neuronal autophagy; however, these findings are inconsistent and offer several possible explanations.

Serotonin (5-hydroxytryptamine, 5-HT) plays a crucial role in mood regulation, neuroplasticity, and metabolic homeostasis[60]. Emerging evidence suggests that serotonin signaling may influence autophagy, potentially through its interaction with mTORC1, a key autophagy-regulating pathway[61,62]. However, direct evidence linking specific serotonin receptor subtypes to autophagy regulation remains limited. Some studies indicate that serotonin receptors might affect cellular survival pathways that intersect with autophagy-related signaling cascades. For example, 5-HT2A receptor activation has been associated with the ERK1/2 signaling pathway, which regulates neuronal survival and apoptosis and may have implications for autophagy regulation[63]. Similarly, 5-HT1A receptor activation modulates the cAMP-PKA pathway, which influences neuronal survival and metabolic homeostasis[64]. While these signaling mechanisms are known to interact with autophagy, their precise role in serotonin receptor-mediated autophagy modulation requires further elucidation. Given the established role of serotonin receptors in synaptic plasticity and cellular metabolism, investigating their potential effects on autophagy may provide valuable insights into the pathophysiology of neurodegenerative disorders and mood regulation. Clarifying these mechanisms could help determine whether targeting serotonin signaling pathways represents a viable approach for modulating autophagy in therapeutic applications.

Furthermore, emerging evidence suggests that SSRIs, including fluoxetine and citalopram, can enhance autophagy in neurons and astrocytes[37]. This suggests that serotonergic modulation may influence autophagic flux, although the precise molecular mechanisms remain unclear. Additionally, autophagy itself has been shown to regulate serotonin receptor turnover and synaptic function, indicating a potential bidirectional interaction between serotonin signaling and autophagy[39]. Tianeptine is known to modulate serotonergic neurotransmission; however, its exact mechanism remains under investigation. While early studies suggested that tianeptine may enhance serotonin reuptake[65,66], more recent findings indicate that its primary effects involve modulation of serotonin receptor signaling and synaptic plasticity rather than direct serotonin transporter regulation[32,67]. Given the role of serotonin receptors in autophagy regulation, further research is needed to determine whether tianeptine indirectly influences autophagy through serotonin receptor activity. Although this study did not directly measure serotonin levels, the observed changes in autophagy following tianeptine treatment suggest a potential link between serotonin receptor activity and mTORC1-mediated autophagy regulation. Future studies should investigate this relationship using pharmacological and genetic approaches, such as receptor knockdown models or selective agonist/antagonist treatments in hippocampal neurons. Specifically, the use of 5-HT1A and 5-HT2A receptor antagonists in neuronal cultures, as well as conditional knockout mouse models, could provide insights into whether serotonin receptor activity is necessary for tianeptine-mediated autophagy modulation.

We were the first to report that tianeptine increases neuroplasticity by activating mTORC1 signaling[68], and in this study we observed that autophagy was activated by B27 deprivation and that treatment with tianeptine reduced autophagy. We also found that rapamycin treatment blocked tianeptine-induced increases in mTORC1 phosphorylation and decreases in LC3B I/II ratio and p62, suggesting that tianeptine-induced changes in autophagy are associated with activation of mTORC1 signaling. Therefore, although the changes in autophagic proteins and autophagosomes observed in this study may not be sufficient to determine whether autophagy is promoted or blocked, we have shown that tianeptine can activate mTORC1 in hippocampal neurons and affect autophagy. These findings suggest that modulation of the autophagic process may be a promising therapeutic target for the treatment of depression.

To our knowledge, this study provides initial evidence that tianeptine modulates autophagy under nutrient deprivation conditions, emphasizing the involvement of mTORC1 signaling in this process. By elucidating the role of mTORC1 signaling, our findings contribute to a better understanding of the molecular mechanisms underlying the neuroprotective and antidepressant effects of tianeptine. However, despite these insights, certain limitations must be acknowledged. First, the primary hippocampal neuronal culture model lacks the complexity of the in vivo brain environment, including interactions with glial cells, blood-brain barrier permeability, and

systemic factors that may influence autophagy[69]. While this model provides a controlled setting to examine cellular mechanisms, future studies should incorporate co-culture systems or organoid models to better mimic physiological conditions and better characterize the role of tianeptine in autophagy regulation.

Second, whether this mechanism directly contributes to tianeptine's antidepressant effects in vivo remains unclear. To determine the translational relevance of these findings, further validation in animal models of depression is essential. Future studies should utilize rodent models such as chronic unpredictable stress and chronic social defeat stress to investigate whether tianeptine-induced autophagy modulation correlates with behavioral improvements[70,71]. Additionally, using conditional knockout models lacking mTORC1 activity in neurons could help clarify whether mTORC1 signaling is both necessary and sufficient for tianeptine's effects on autophagy and depressive behaviors[72].

Finally, our study primarily assessed autophagy through changes in autophagy-related proteins (LC3B, Beclin 1, p62) and immunofluorescence-based puncta analysis. While these approaches provide valuable insights into autophagic activity, they do not fully capture autophagic flux. Future research should incorporate lysosomal inhibitors (e.g., bafilomycin A1, chloroquine) and live-cell imaging techniques to distinguish between increased autophagosome formation and impaired degradation, allowing for a more comprehensive assessment of autophagy dynamics[27,53].

In conclusion, our findings demonstrate that tianeptine can modulate autophagy by regulating key autophagy-related signaling pathways, which may contribute to its neuroprotective and antidepressant effects. These insights may have implications for the development of therapeutic strategies targeting autophagy regulation in depression and other neurological disorders.

Methods

Primary hippocampal cell cultures

Primary cultures of hippocampal neurons were prepared from fetal brains of embryonic day 19 in Sprague-Dawley rats (Hana Biotec; Pyeongtaek, Gyeonggi-do, Korea) as previously described[38]. All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals for scientific purposes, following approved protocols from the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje Medical College (Approval No. 2018-019). Furthermore, all methods were performed in compliance with relevant institutional and national regulations, as well as the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The hippocampi were dissected in neurobasal medium. The cells were maintained in neurobasal medium with growth medium B27 supplement (components: Biotin, α-tocopherol acetate, α-tocopherol, vitamin A, bovine serum albumin, catalase, insulin, transferrin, superoxide dismutase, corticosterone, galactose, ethanolamine, glutathione, Carnitine, linoleic acid, linolenic acid, progesterone, putrescine, selenium, and triodo-L-thyronine, L-glutamine, and penicillin/streptomycin (all culture reagents from Invitrogen; Carlsbad, CA, USA). To stabilize the cells, they were grown for 7–10 days under the aforementioned conditions (normal conditions). Subsequently, the impact of nutrient deprivation on neural autophagy was investigated by culturing cells in the absence of B27 supplement (B27 deprivation conditions) for 3 days. To serve as positive controls for the induction of autophagy, 0.5 μM of rapamycin and 10 μM of chloroquine were added under normal conditions.

Drug treatment

One hundred mM of tianeptine (Tocris Bioscience; Ballwin, MO, USA) was completely dissolved in distilled water and diluted to various concentrations in neurobasal medium. To ascertain whether tianeptine affects autophagy induced by B27 deprivation, hippocampal cells were incubated with 1-200 μM of tianeptine (Tocris Bioscience; Ballwin, MO, USA) in B27 deprivation conditions for 3 days. To investigate the inhibitory effects, cells were treated with 2 μM rapamycin (Calbiochem, San Diego, CA, USA) 30 min prior to tianeptine administration. To select an appropriate rapamycin dose, rapamycin was tested at concentrations ranging from 0.5 μM to 2 μM in B27-deprived cells. These concentrations did not affect cell viability, mTORC1 phosphorylation, or the expression of autophagic proteins (data not shown). Consequently, the highest dose of rapamycin tested (2 μM) was selected for further investigation.

MTT assay

The viability of the cells was assessed using the MTT assay. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 3 days. Subsequently, the cells were incubated with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA) solution at 37 °C for 4 h to allow the formazan to dissolve. The absorbance was recorded at a wavelength of 570 nm using an ELISA reader (Spectramax M2e; Molecular Devices, San Jose, CA, USA). The MTT assay was conducted in two to three independent cultures.

Western blot analysis

Primary hippocampal cells were plated on 6-well dishes at a density of 2×10^5 cells per well and harvested in ice-cold lysis buffer (20 mM Tris-HCl, 137 mM NaCl, 10% glycerol, 1% Nonidet™ P-40, 0.1% SDS, 0.5% sodium deoxycholate, and 2 mM EDTA) containing one complete protease inhibitor tablet (Roche; Laval, Quebec, Canada). The lysates were then centrifuged, and the supernatants were collected. Twenty μg of protein were separated by SDS-PAGE and then transferred to polyvinylidene fluoride membranes (PVDF; Millipore, Bedford, MA, USA). The membranes were incubated in Tris-buffered saline and Tween 20 (TBST) containing 5% bovine serum albumin for 1 h at room temperature, followed by overnight incubation at 4 °C with primary antibodies. The primary antibodies utilized in this study were as follows: anti-phospho-mTORC1 (Ser2448, #2971), anti-mTORC1 (#2972), anti-phospho-Unc-51-like autophagy-activating kinase-1 (ULK1) (Ser757, #14202), anti-

ULK1 (#8054), anti-Beclin 1 (#3738), and anti-LC3B (#2775) from Cell Signaling Technology (1:1000 Beverly, MA, USA); anti-p62 (ab56416) from Abcam (1:1000, Cambridge, UK); anti-α-tubulin (T9026) from Sigma (1:2000). The secondary antibody was anti-α-tubulin (T9026) from Sigma (1:2000). Following a washing step, the membranes were incubated for 1 h at room temperature in TBST containing horseradish peroxidase-conjugated secondary antibodies (mouse anti-rabbit IgG for anti-phospho-mTORC1, anti-mTORC1, phospho-ULK1, anti-ULK1, anti-Beclin 1, and anti-LC3B [1:2000]; anti-mouse IgG for anti-p62 [1:2000] and anti-α-tubulin [1:10000]). The membranes were subsequently washed and the signals were visualized using a Clarity Western enhanced chemiluminescence (ECL) solution (Bio-rad, Hercules, CA, USA). Chemiluminescence was detected using an Amersham imager 600 (AI600; GE Healthcare Life Sciences, Fairfield, CT, USA). The densitometric values of protein bands were quantified using the Multi-Gauge Software (Fuji Photo Film Co., Ltd., Tokyo, Japan). Western blot analysis was conducted in duplicate on each of three to five independent cultures.

Immunofluorescence for autophagy detection

Hippocampal cells were plated on 12-well dishes with glass bottoms at a density of 2×10^4 cells per well. After 3 days of incubation, cells were stained for autophagic vacuoles using the Cyto-ID® Autophagy Detection Kit (#ENZ-51031, Enzo, Farmingdale, NY, USA) according to the manufacturer's protocol. In brief, the cells were washed once with 1×assay buffer and then incubated in a solution of the Cyto-ID green dye and the nuclear stain Hoechst 33,342 at 37 °C for 30 min. For fixation, cells were treated with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Images were obtained using a confocal laser scanning microscope (Nikon, Japan). ImageJ (version 1.25a) was used to quantify the number of puncta per cell applying a puncta size range (5-Infinity), a pixel count threshold (auto) and a circularity range (0.00–1.00) to exclude nonspecific signals. The number of LC3B puncta was quantified using the ImageJ 1.52a software (National Institutes of Health, Bethesda, MD, USA). A minimum of 150 to 200 cells were counted in 30 fields for a minimum of nine sets of slides from three independent cultures.

Statistical analysis

All statistical analyses were conducted using GraphPad Software version 10.1.2 (La Jolla, CA, USA). A comparison of control and B27-deprived cells was conducted using an unpaired *Student's t-test*. The dose-dependent effect of tianeptine was analyzed using one-way analysis of variance (ANOVA). In order to evaluate the effects of rapamycin blockade, a two-way ANOVA was employed to assess the main effect of tianeptine or rapamycin, as well as the interaction between tianeptine and rapamycin. For *post hoc* comparison, *Tukey's* multiple-comparison test was employed. In all analyses, a *p*-value of less than 0.05 was considered statistically significant.

Data availability

Data is available on request from the corresponding authors.

Received: 7 December 2024; Accepted: 4 March 2025
Published online: 25 April 2025

References

1. Richards, D. Prevalence and clinical course of depression: A review of the literature on the prevalence and clinical course of depression. *Clin. Psychol. Rev.* **31**, 1117–1125. https://doi.org/10.1016/j.cpr.2011.07.004 (2011).
2. Moradi, Y., Dowran, B. & Sepandi, M. The global prevalence of depression, suicide ideation, and attempts in the military forces: A systematic review and meta-analysis of cross-sectional studies. *BMC Psychiatry* **21**, 510. https://doi.org/10.1186/s12888-021-03526-2 (2021).
3. Pereira, V. S. & Hiroaki-Sato, V. A. A brief history of antidepressant drug development will be presented, with a focus on the evolution from tricyclic antidepressants to more recent advances. *Acta Neuropsychiatry* **30**, 307–322. https://doi.org/10.1017/neu.2017.39 (2018).
4. Hillhouse, T. M. & Porter, J. H. A brief history of the development of antidepressant drugs from monoamines to glutamate. *Exp. Clin. Psychopharmacol.* **23**, 1–21. https://doi.org/10.1037/a0038550 (2015).
5. López-Muñoz, F. & Alamo, C. Monoaminergic neurotransmission: A historical account of the discovery of antidepressants from the 1950s to the present. *Curr. Pharm. Des.* **15**, 1563–1586. https://doi.org/10.2174/138161209788168001 (2009).
6. Delgado, P. L. Depression: The case for a monoamine deficiency. *J. Clin. Psychiatry* **61**, 7–11 (2000).
7. Frazer, A. & Benmansour, S. Delayed pharmacological effects of antidepressants. *Mol. Psychiatry* **7**, S23–S28. https://doi.org/10.1038/sj.mp.4001015 (2002).
8. Otte, C. et al. Major depressive disorder. *Nat. Rev. Dis. Primers* **2**, 16065. https://doi.org/10.1038/nrdp.2016.65 (2016).
9. Harmer, C. J., Duman, R. S. & Cowen, P. J. How do antidepressants work? New perspectives for the refinement of future treatment approaches. *Lancet Psychiatry* **4**, 409–418. https://doi.org/10.1016/S2215-0366(17)30015-9 (2017).
10. Duman, R. S. The pathophysiology of depression and innovative treatments: Remodeling glutamatergic synaptic connections. *Dialogues Clin. Neurosci.* **16**, 11–27. https://doi.org/10.31887/DCNS.2014.16.1/rduman (2014).
11. Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. *Cell* **149**, 274–293. https://doi.org/10.1016/j.cell.2012.03.017 (2012).
12. Condon, K. J. & Sabatini, D. M. Nutrient regulation of mTORC1 at a glance. *J. Cell. Sci.* **132**, jcs222570. https://doi.org/10.1242/jcs.222570 (2019).
13. Battaglioni, S., Benjamin, D., Wälchli, M., Maier, T. & Hall, M. N. mTOR substrate phosphorylation in growth control. *Cell* **185**, 1814–1836. https://doi.org/10.1016/j.cell.2022.04.013 (2022). .
14. Zhang, S. et al. Regulation of mTORC1 by amino acids in mammalian cells: A general picture of recent advances. *Anim. Nutr.* **7**, 1009–1023. https://doi.org/10.1016/j.aninu.2021.05.003 (2021).
15. Takahara, T., Amemiya, Y., Sugiyama, R., Maki, M. & Shibata, H. Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *J. Biomed. Sci.* **27**, 87. https://doi.org/10.1186/s12929-020-00679-2 (2020).
16. Ben-Sahra, I. & Manning, B. D. mTORC1 signaling and the metabolic control of cell growth. *Curr. Opin. Cell. Biol.* **45**, 72–82. https://doi.org/10.1016/j.ceb.2017.02.012 (2017).
17. Szwed, A., Kim, E. & Jacinto, E. Regulation and metabolic functions of mTORC1 and mTORC2. *Physiol. Rev.* **101**, 1371–1426. https://doi.org/10.1152/physrev.00026.2020 (2021).

18. Zarate, C. A. et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* **63**, 856–864. https://doi.org/10.1001/archpsyc.63.8.856 (2006).
19. Dwyer, J. M., Lepack, A. E. & Duman, R. S. mTOR activation is required for the antidepressant effects of mGluR2/3 Blockade. *Int. J. Neuropsychopharmacol.* **15**, 429–434. https://doi.org/10.1017/S1461145711001702 (2012).
20. Kato, T. Role of mTOR1 signaling in the antidepressant effects of ketamine and the potential of mTORC1 activators as novel antidepressants. *Neuropharmacology* **223**, 109325. https://doi.org/10.1016/j.neuropharm.2022.109325 (2023).
21. Li, N. et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329**, 959–964. https://doi.org/10.1126/science.1190287 (2010).
22. Rabanal-Ruiz, Y., Otten, E. G. & Korolchuk, V. I. mTORC1 as the main gateway to autophagy. *Essays Biochem.* **61**, 565–584. https://doi.org/10.1042/EBC20170027 (2017).
23. Fleming, A. & Rubinsztein, D. C. Autophagy in neuronal development and plasticity. *Trends Neurosci.* **43**, 767–779. https://doi.org/10.1016/j.tins.2020.07.003 (2020).
24. Maday, S. & Holzbaur, E. L. F. Autophagosome biogenesis in primary neurons follows an ordered and spatially regulated pathway. *Dev. Cell.* **30**, 71–85. https://doi.org/10.1016/j.devcel.2014.06.001 (2014).
25. Nixon, R. A. The role of autophagy in neurodegenerative disease. *Nat. Med.* **19**, 983–997. https://doi.org/10.1038/nm.3232 (2013).
26. Kim, J. & Guan, K. L. mTOR as a central hub of nutrient signalling and cell growth. *Nat. Cell. Biol.* **21**, 63–71. https://doi.org/10.1038/s41556-018-0205-1 (2019).
27. Deleyto-Seldas, N. & Efeyan, A. The mTOR–autophagy axis and the control of metabolism. *Front. Cell. Dev. Biol.* **9**, 655731. https://doi.org/10.3389/fcell.2021.655731 (2021).
28. Hosokawa, N. et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell* **20**, 1981–1991. https://doi.org/10.1091/mbc.e08-12-1248 (2009).
29. Henry, F. E. et al. A unique homeostatic signaling pathway links synaptic inactivity to postsynaptic mTORC1. *J. Neurosci.* **38**, 2207–2225. https://doi.org/10.1523/JNEUROSCI.1843-17.2017 (2018).
30. Defrance, R., Marey, C. & Kamoun, A. Antidepressant and anxiolytic activities of tianeptine: An overview of clinical trials. *Clin. Neuropharmacol.* **11**, S74–S82 (1998).
31. Kasper, S. & McEwen, B. S. Neurobiological and clinical effects of the antidepressant tianeptine. *CNS Drugs* **22**, 15–26. https://doi.org/10.2165/00023210-200822010-00002 (2008).
32. McEwen, B. S. et al. The Neurobiological properties of tianeptine (Stablon): From monoamine hypothesis to glutamatergic modulation. *Mol. Psychiatry* **15**, 237–249. https://doi.org/10.1038/mp.2009.80 (2010).
33. Della, F. P. et al. Tianeptine treatment induces antidepressive-like effects and alters BDNF and energy metabolism in the brain of rats. *Behav. Brain Res.* **233**, 526–535. https://doi.org/10.1016/j.bbr.2012.05.039 (2012).
34. Nowacka-Chmielewska, M. M. et al. The effects of desipramine, fluoxetine, or tianeptine on changes in bulbar BDNF levels induced by chronic social instability stress and inflammation. *Pharmacol. Rep.* **69**, 520–525. https://doi.org/10.1016/j.pharep.2017.01.004 (2017).
35. Reagan, L. P. et al. Tianeptine increases brain-derived neurotrophic factor expression in the rat amygdala. *Eur. J. Pharmacol.* **565**, 68–75. https://doi.org/10.1016/j.ejphar.2007.02.023 (2007).
36. Mutlu, O. et al. Effects of fluoxetine, tianeptine, and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. *Life Sci.* **91**, 1252–1262. https://doi.org/10.1016/j.lfs.2012.09.023 (2012).
37. Gulbins, A. et al. Antidepressants act by inducing autophagy controlled by sphingomyelin-ceramide. *Mol. Psychiatry* **23**, 2324–2346. https://doi.org/10.1038/s41380-018-0090-9 (2018).
38. Kaech, S. & Banker, G. Culturing hippocampal neurons. *Nat. Protoc.* **1**, 2406–2415. https://doi.org/10.1038/nprot.2006.356 (2006).
39. Pierone, B. C., Pereira, C. A., Garcez, M. L. & Kaster, M. P. Stress and signaling pathways regulating autophagy: From behavioral models to psychiatric disorders. *Exp. Neurol.* **334**, 113485. https://doi.org/10.1016/j.expneurol.2020.113485 (2020).
40. Yim, W. W. & Mizushima, N. Lysosome biology in autophagy. *Cell. Discov.* **6**, 6. https://doi.org/10.1038/s41421-020-0141-7 (2020).
41. Yoshii, S. R. & Mizushima, N. Monitoring and measuring autophagy. *Int. J. Mol. Sci.* **18**, 1865. https://doi.org/10.3390/ijms18091865 (2017).
42. Mukhopadhyay, S., Panda, P. K., Sinha, N., Das, D. N. & Bhutia, S. K. Autophagy and apoptosis: Where do they meet? *Apoptosis* **19**, 555–566 (2014). https://doi.org/10.1007/s10495-014-0967-2
43. Kang, R., Zeh, H. J., Lotze, M. T. & Tang, D. The Beclin 1 network regulates autophagy and apoptosis. *Cell. Death Differ.* **18**, 571–580. https://doi.org/10.1038/cdd.2010.191 (2011).
44. Klionsky, D. J. et al. Autophagy in major human diseases. *EMBO J.* **40**, e108863. https://doi.org/10.15252/embj.2021108863 (2021).
45. Tanida, I., Ueno, T. & Kominami, E. LC3 and autophagy. *Methods Mol. Biol.* **445**, 77–88. https://doi.org/10.1007/978-1-59745-157-4_4 (2008).
46. Wang, C. et al. Phosphorylation of ULK1 affects autophagosome fusion and links chaperone-mediated autophagy to macroautophagy. *Nat. Commun.* **9**, 3492. https://doi.org/10.1038/s41467-018-05449-1 (2018).
47. Kim, J., Kundu, M., Viollet, B. & Guan, K. L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell. Biol.* **13**, 132–141. https://doi.org/10.1038/ncb2152 (2011).
48. Poole, L. P., Bock-Hughes, A., Berardi, D. E. & Macleod, K. F. ULK1 facilitates mitophagy via phosphorylation and stabilization of BNIP3. *Sci. Rep.* **11**, 20526. https://doi.org/10.1038/s41598-021-00170-4 (2021).
49. Ohashi, Y. Activation mechanisms of the VPS34 complexes. *Cells* **10**, 3124. https://doi.org/10.3390/cells10113124 (2021).
50. Dooley, H. C. et al. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. *Mol. Cell.* **55**, 238–252. https://doi.org/10.1016/j.molcel.2014.05.021 (2014).
51. Bjørkøy, G. et al. Monitoring the autophagic degradation of p62/SQSTM1. *Methods Enzymol.* **452**, 181–197. https://doi.org/10.1016/S0076-6879(08)03612-4 (2009).
52. Kageyama, S. et al. The p62/SQSTM1-droplet serves as a platform for autophagosome formation and the anti-oxidative stress response. *Nat. Commun.* **12**, 16. https://doi.org/10.1038/s41467-020-20185-1 (2021).
53. J Klionsky, D. et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). *Autophagy* **17**, 1–382. https://doi.org/10.1080/15548627.2020.1797280 (2021).
54. Sünwoldt, J., Bosche, B., Meisel, A. & Mergenthaler, P. Neuronal culture microenvironments determine preferences in bioenergetic pathway use. *Front. Mol. Neurosci.* **10**, 305. https://doi.org/10.3389/fnmol.2017.00305 (2017).
55. Young, J. E., Martinez, R. A. & Spada, A. R. L. Nutrient deprivation induces neuronal autophagy and implicates reduced insulin signaling in neuroprotective autophagy activation. *J. Biol. Chem.* **284**, 2363–2373. https://doi.org/10.1074/jbc.M806088200 (2009).
56. Vargas, J. N. S., Hamasaki, M., Kawabata, T., Youle, R. J. & Yoshimori, T. The mechanisms and roles of selective autophagy in mammals. *Nat. Rev. Mol. Cell. Biol.* **24**, 167–185. https://doi.org/10.1038/s41580-022-00542-2 (2023).
57. Zhu, J. H. et al. Regulation of autophagy by extracellular signal-regulated protein kinases during 1-methyl-4-phenylpyridinium-induced cell death. *Am. J. Pathol.* **170**, 75–86. https://doi.org/10.2353/ajpath.2007.060524 (2007).
58. Chu, C. T. Autophagic stress in neuronal injury and disease. *J. Neuropathol. Exp. Neurol.* **65**, 423–432. https://doi.org/10.1097/01.jnen.0000229233.75253.be (2006).
59. Gassen, N. C. & Rein, T. Is there a role of autophagy in depression and antidepressant action? *Front. Psychiatry* **10**, 337. https://doi.org/10.3389/fpsyt.2019.00337 (2019).
60. Berger, M., Gray, J. A. & Roth, B. L. The expanded biology of serotonin. *Annu. Rev. Med.* **60**, 355–366. https://doi.org/10.1146/annurev.med.60.042307.110802 (2009).

61. Kim, Y. C. & Guan, K. L. mTOR: A pharmacologic target for autophagy regulation. *J. Clin. Invest.* **125**, 25–32. https://doi.org/10.1172/JCI73939 (2015).
62. Zschocke, J. et al. Antidepressant drugs diversely affect autophagy pathways in astrocytes and neurons-dissociation from cholesterol homeostasis. *Neuropsychopharmacology* **36**, 1754–1768. https://doi.org/10.1038/npp.2011.57 (2011).
63. Zhang, G. & Stackman, R. W. Jr. The role of serotonin 5-HT2A receptors in memory and cognition. *Front. Pharmacol.* **6**, 225. https://doi.org/10.3389/fphar.2015.00225 (2015).
64. Albert, P. R., Benkelfat, C. & Descarries, L. The neurobiology of depression-revisiting the serotonin hypothesis. I. Cellular and molecular mechanisms. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 2378–2381. https://doi.org/10.1098/rstb.2012.0190 (2012).
65. Mennini, T., Mocaer, E. & Garattini, S. Tianeptine, a selective enhancer of serotonin uptake in rat brain. *Naunyn Schmiedebergs Arch. Pharmacol.* **336**, 478–482. https://doi.org/10.1007/BF00169302 (1987).
66. Fattaccini, C. M., Bolaños-Jimenez, F., Gozlan, H. & Hamon, M. Tianeptine stimulates uptake of 5-hydroxytryptamine in vivo in the rat brain. *Neuropharmacology* **29**, 1–8. https://doi.org/10.1016/0028-3908(90)90076-4 (1990).
67. Uzbay, T. I. Tianeptine: Potential influences on neuroplasticity and novel pharmacological effects. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **32**, 915–924. https://doi.org/10.1016/j.pnpbp.2007.08.007 (2008).
68. Seo, M. K. et al. Tianeptine induces mTORC1 activation in rat hippocampal neurons under toxic conditions. *Psychopharmacol. (Berl)* **233**, 2617–2627. https://doi.org/10.1007/s00213-016-4309-7 (2016).
69. Zhang, J. et al. Recent progresses in novel in vitro models of primary neurons: A biomaterial perspective. *Front. Bioeng. Biotechnol.* **10**, 953031. https://doi.org/10.3389/fbioe.2022.953031 (2022).
70. Duman, R. S. Neurobiology of stress, depression, and rapid acting antidepressants: Remodeling synaptic connections. *Depress. Anxiety*. **31**, 291–296. https://doi.org/10.1002/da.22227 (2014).
71. Krishnan, V. et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* **131**, 391–404. https://doi.org/10.1016/j.cell.2007.09 (2007).
72. Cloëtta, D. et al. Inactivation of mTORC1 in the developing brain causes microcephaly and affects gliogenesis. *J. Neurosci.* **33**, 7799–7810. https://doi.org/10.1523/JNEUROSCI.3294-12.2013 (2013).

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government Ministry of Science and ICT (MSIT) (RS-2023-NR077070 to S.W. Park and RS-2024-00347714 to J.G. Lee).

Author contributions

Conceptualization: JGL, SWP, and MKS; Formal analysis: MKS, HYK, and AJC; Funding acquisition: JGL and SWP; Investigation: JGL, SWP, MKS, and HYK; Methodology: MKS, HYK, AJC, DHS, WGK, SWP, and JGL; Resources: MKS, HYK, AJC, DHS, and WGK; Supervision: JGL, SWP, and MKS; Writing-original draft: JGL and SWP; Editing: MKS, DHS, and WGK. All authors have read and agreed to the published version of the paper.

Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

The animal study was reviewed and approved by Inje Medical College Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board (Approval No. 2018-019).

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-92988-5.

Correspondence and requests for materials should be addressed to S.W.P. or J.G.L.