Brain-derived neurotropic factor (BDNF) has been shown to play critical roles in neural development, plasticity, and neurodegenerative diseases. The main function of BDNF in the brain is widely accepted to be synaptic regulation. However, how BDNF modulates synaptic transmission, especially the underlying signaling cascades between presynaptic and postsynaptic neurons, remains controversial. In the present study, we investigated the actions of BDNF at rat calyx-type synapses of either sex by measuring the excitatory postsynaptic current (EPSC) and presynaptic calcium current and capacitance changes. We found that BDNF inhibits the EPSC, presynaptic calcium influx, and exocytosis/endocytosis via activation of the presynaptic cannabinoid Type 1 receptors (CB1Rs). Inhibition of the CB1Rs abolished the BDNF-induced presynaptic inhibition, whereas CB1R agonist mimicked the effect of BDNF. Exploring the underlying signaling cascade, we found that BDNF specifically activates the postsynaptic TrkB receptors, inducing the release of endocannabinoids via the PLC$\gamma$DGL pathway and retrogradely activating presynaptic CB1Rs. We also reported the involvement of AC/PKA in modulating vesicle endocytosis, which may account for the BDNF-induced calcium-dependent and -independent regulation of endocytosis. Thus, our study provides new insights into the BDNF/endocannabinoid-associated modulation of neurotransmission in physiological and pathologic processes.

Key words: AC/PKA; BDNF; endocannabinoid; endocytosis; exocytosis; retrograde signaling

Introduction

Precise and efficient neurotransmission is the basis of neuronal function and plasticity in the CNS (Saheki and De Camilli, 2012; L. G. Wu et al., 2014). Among all neurotrophins, BDNF has attracted much interest for its high expression and potent effects in neural development, functional neural circuit formation, and neurologic diseases (de Jong and Verhage, 2009; Park and Poo, 2013; Choo et al., 2017). BDNF is generally accepted to mainly regulate synaptic function on both excitatory and inhibitory synapses, and one key aspect of the diverse effects of BDNF stems from its complex signaling cascade (Lu et al., 2014). Although many studies have reported BDNF as a potent modulator of synaptic transmission via activation of the tropomyosin receptor kinase B (TrkB) receptor, the underlying signaling cascade is still
controversial (Reichardt, 2006; Guo et al., 2018; Lin et al., 2018). A recent study reported that BDNF inhibits synaptic transmission by slowing presynaptic calcium current (ICa) activation, and impairs subsequent exocytosis/endocytosis via activation of the TrkB receptors at a giant calyx-type synapse located in the brainstem (Baydyuk et al., 2015). However, at calyces, the TrkB receptors are expressed not only in the presynaptic nerve terminal, but also in the postsynaptic principal neuron. Whether presynaptic and/or postsynaptic TrkB receptors are involved in the BDNF-induced inhibitory effect is unclear. In addition, the signaling cascade downstream of TrkB activation remains unknown. Interestingly, a previous study reported that BDNF-TrkB signaling in the postsynaptic dendrite leads to a decrease in the probability of presynaptic GABA release on layer 2/3 neocortical inhibitory synapses by rapid mobilization of endocannabinoids (eCBs) into the synaptic cleft (Lemtiri-Chlieh and Levine, 2010). Activation of presynaptic cannabinoid Type 1 receptors (CB1Rs) at the inhibitory synapse makes it necessary to investigate whether BDNF-TrkB signaling at the glutamatergic postsynaptic neuron can also activate the release of eCBs to retrogradely inhibit presynaptic function.

Many studies have demonstrated that eCBs are key activity-dependent signals that can modulate synaptic transmission by activating presynaptic CB1Rs (Castillo et al., 2012). For example, strong depolarization of the postsynaptic neuron can lead to reduced synaptic transmission via the release of eCBs (Wilson and Nicoll, 2001). In addition, excessive glutamate release could activate metabotropic glutamate receptors (mGluRs), promoting the synthesis of eCBs to retrogradely regulate neurotransmission (Kushnir et al., 2004). Evidence also supports mutual interactions between BDNF and eCB signaling. CB1R antagonist has been shown to block BDNF-induced LTP and LTD (Maglio et al., 2018; Pan et al., 2019). Furthermore, CB1R activation upregulates BDNF expression via the PI3K/Akt/mTORC1 pathway (Blázquez et al., 2015), whereas BDNF can induce the release of eCBs at neocortical inhibitory synapses (Lemtiri-Chlieh and Levine, 2010; Zhao and Levine, 2014) and dopamine neurons in the mouse midbrain (Zhong et al., 2015). A recent study showed that the BDNF-induced increase in the mEPSC frequency can be unmasked by blocking eCB signaling at cortical excitatory synapses, suggesting opposing roles of BDNF (Yeh et al., 2017). Whether such opposing roles of BDNF also exist at the glutamatergic calyx-type synapse is not yet known.

CB1Rs are GPCRs and have a well-documented inhibitory effect on adenyl cyclase (AC) and protein kinase A (PKA) (Childers and Deadwyler, 1996; Castillo et al., 2012). A previous study demonstrated that the AC/PKA signaling pathway can modulate vesicle endocytosis in an activity-dependent manner (Yao and Sakaba, 2012). These findings urged us to investigate whether AC/PKA signaling is involved in BDNF-induced inhibition of synaptic transmission at calyx of Held synapses.

In the present study, we used time-resolved capacitance measurements at a giant glutamatergic synapse, the calyx of Held, to investigate the signaling cascade underlying BDNF-induced inhibition of synaptic transmission (Barnes-Davies and Forsythe, 1995; Borst et al., 1995). We found that BDNF selectively activates postsynaptic TrkB receptors, which induces the release of eCBs in a calcium-dependent manner and retrogradely activates presynaptic CB1Rs to induce presynaptic inhibition. These results suggest a different interpretation of the previous study (Baydyuk et al., 2015), and the trans-synaptic signaling cascade of BDNF-TrkB-eCB coupling may provide a comprehensive understanding of neurotrophin-regulated neurotransmission in the CNS.
Table 1. Statistical analysis per figure

| Figure | Test | Post hoc comparison |
|--------|------|---------------------|
| 1B     | Repeated-measures one-way ANOVA | Bonferroni’s multiple comparisons test |
|        | EPSC: F(2,37) = 12.90, p = 0.0021 | EPSC: Ctrl vs BDNF, p = 0.0020; BDNF vs Washout, p = 0.0218 |
|        | PPR: F(2, 35) = 38.98, p < 0.0001 | PPR: Ctrl vs BDNF, p = 0.0006; BDNF vs Washout, p = 0.0007 |
| 1C     | Unpaired Student’s t test (two-tailed) | |
|        | t = 7.851, df = 13, p < 0.0001; Rise time: t = 0.5952, df = 13, p = 0.5619 | |
| 1D     | Unpaired Student’s t test (two-tailed) | |
|        | −10 mV: t = 3.233, df = 9, p = 0.0103; 0 mV: t = 2.750, df = 9, p = 0.0225; 10 mV: t = 2.809, df = 9, p = 0.0204; 20 mV: t = 2.798, df = 9, p = 0.0208; 30 mV: t = 2.627, df = 9, p = 0.0275; 40 mV: t = 2.594, df = 9, p = 0.0290 | |
| 1E     | Kolmogorov-Smirnov test | |
|        | p = 0.9819 | |
| 1G     | Unpaired Student’s t test (two-tailed) | |
|        | ΔCm(ta): t = 3.256, df = 9, p = 0.0099; ΔCm(ta): t = 2.483, df = 9, p = 0.0348; ΔCm(ta): t = 4.400, df = 9, p = 0.0015; ΔCm(ta): t = 4.181, df = 9, p = 0.0024; ΔCm(ta): t = 3.958, df = 9, p = 0.0033; ΔCm(ta): t = 1.600, df = 9, p = 0.1375; ΔCm(ta): t = 0.02962, df = 9, p = 0.9770 | Probability: t = 3.999, df = 9, p = 0.0031 |
| 2D     | One-way ANOVA | |
|        | ΔCm: F(7,50) = 7.459, p < 0.0001 | |
|        | ICA: F(7,50) = 8.813, p < 0.0001 | |
|        | Rate(ta): F(2, 50) = 6.820, p < 0.0001 | |
|        | ΔCm(ta)%: F(7,50) = 6.459, p < 0.0001 | |
| 2E     | One-way ANOVA | |
|        | ICA: F(2,10) = 10.80, p = 0.0011 | |
| 2F     | One-way ANOVA | |
|        | ΔCm: F(2,10) = 12.78, p = 0.0005 | |
|        | Rate(ta): F(2,10) = 13.64, p = 0.0003 | |
|        | ΔCm(ta)%: F(2,10) = 5.362, p = 0.0165 | |
| 3B     | One-way ANOVA | |
|        | ΔCm: F(1,62) = 7.346, p < 0.0001 | |
|        | QICA: F(7,62) = 8.623, p < 0.0001 | |
|        | Rate(ta): F(7,62) = 17.11, p < 0.0001 | |
|        | ΔCm(ta)%: F(7,62) = 6.363, p < 0.0001 | |
| 3C     | One-way ANOVA | |
|        | QICA: F(2,10) = 10.01, p = 0.0011 | |
| 3D     | One-way ANOVA | |
|        | ΔCm: F(2,10) = 4.853, p = 0.0198 | |
|        | Rate(ta): F(2,10) = 14.97, p < 0.0001 | |
|        | ΔCm(ta)%: F(2,10) = 23.39, p < 0.0001 | |
| 4      | Unpaired Student’s t test (two-tailed) | |
|        | t = 9.838, df = 24, p < 0.0001 | |
| 5B     | Paired Student’s t test (two-tailed) | |
|        | EPSC: t = 1.761, df = 4, p = 0.8688; PPR: t = 1.430, df = 4, p = 0.2259 | |
| 5C     | One-way ANOVA | |
|        | ICA: F(2,10) = 13.26, p = 0.0006 | |
| 5D     | One-way ANOVA | |
|        | ΔCm: F(2,10) = 6.408, p = 0.0106 | |
|        | Rate(ta): F(2,10) = 8.801, p = 0.0033 | |

(Table continues.)
| Figure | Test | Post hoc comparison |
|--------|------|---------------------|
| 5E     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{QICa: } F_{(2,14)} = 23.81, \ p < 0.0001 \) | Bonferroni’s multiple comparisons test |
| 5F     | One-way ANOVA | \( \Delta Cm: \) Vehicle vs Vehicle + BDNF, \( p < 0.0001; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0002 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 6B     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 6D     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 7A     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 7B     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 7C     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 7D     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 7E     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 7F     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |
| 8B     | One-way ANOVA | \( \Delta Cm_{15}\%: \) Vehicle vs Vehicle + BDNF, \( p = 0.0010; \) Vehicle + BDNF vs BAPTA + BDNF, \( p = 0.0021 \) |
|        | \( \text{Unpaired Student t test (two-tailed) } \) | Bonferroni’s multiple comparisons test |

(Table continues.)
as secondary antibodies. ImageJ software (National Institutes of Health) was used to quantify the TrkB activation. The relative fluorescence was calculated as the fluorescence ratio between p-TrkB and MAP2.

Data collection and measurements. As described previously (Xue et al., 2012b; Sun et al., 2016), capacitance measurements were made within 10 min after break-in to avoid rundown, and capacitance jumps were measured 250 ms after depolarization to avoid artifacts. The time constant (τ) was obtained from monoequexponential or biequexponential fitting of the capacitance decay. The initial rate of endocytosis (Rateendo) was measured 1–2 s after depolarization. The percentage of residual capacitance 15 (ΔCm30s%) or 30 s (ΔCm60s%) after depolarization was measured to represent capacitance recovery.

For typical drug bath application experiments, BDNF was applied to the recording chamber by a peristaltic pump at least 30 min before recording and continuously present throughout the experiment. For intracellular drug application experiments, drugs were added to the presynaptic or postsynaptic pipette solution before recording. To specifically block the postsynaptic TrkB receptors, 200 nM K252a was included in the postsynaptic pipette solution via a whole-cell configuration before application of BDNF. After bath application of BDNF for 20 min, we obtained paired recordings by applying another presynaptic pipette at the nerve terminal of the same synapse. The same methods were applied to postsynaptic delivery of BAPTA (20 mM).

Experimental design and statistical analyses. For experiments recording the ICa, capacitance changes, and postsynaptic responses, each group of data were collected from 5 to 13 calyces, which were from 3 to 7 rats of either sex. All data are presented as mean ± SEM. We used the Kolmogorov-Smirnov test to assess differences in calcium inactivation and paired or unpaired Student’s t test to test differences between two groups. One-way ANOVA with post hoc Bonferroni test was applied for multiple group comparisons. A p value < 0.05 was considered significant. All statistical analyses were performed using MATLAB (2019b, The MathWorks). Details of all statistical analysis are provided in Table 1.

Results

BDNF inhibits apparent EPSC, presynaptic ICa, and release probability

First, we investigated how BDNF modulates synaptic transmission by examining the EPSCs of the postsynaptic principal neurons at rat calyces in a whole-cell configuration with 0.5 mM EGTA in the pipette solution. We applied a pair of stimuli with an interval of 20 ms every 10 s near the midline of the MNTB in p8–p10 rats, which can induce two consecutive EPSCs. The PPR was used to evaluate the change in release probability as described above. Recording a baseline for ~10 min, the averaged EPSC amplitude was 3.2 ± 0.5 nA (n = 8 calyces). After bath application of BDNF (100 ng/ml), the EPSC gradually decreased and reached a plateau in ~15–20 min (Fig. 1, A, top). The EPSC amplitude decreased ~50% (1.6 ± 0.3 nA, n = 8; Fig. 1B) and was significantly smaller than the baseline (p = 0.0020, repeated-measures one-way ANOVA with Bonferroni post hoc test). The reduced EPSCs recovered to a level similar to baseline after an ~15–30 min washout (99 ± 7%; p > 0.9999, repeated-measures one-way ANOVA with Bonferroni post hoc test) after BDNF application (p = 0.0006, repeated-measures one-way ANOVA with Bonferroni post hoc test; n = 8; Fig. 1A, bottom, B), suggesting a presynaptic mechanism for BDNF-inhibited synaptic transmission (Liu et al., 2019).

A recent study reported that BDNF slows presynaptic ICa activation to inhibit EPSCs at the rat calyx synapse (Baydyuk et al., 2015). Therefore, we applied a similar 20 ms depolarization pulse from ~80 to 10 mV (depolarization) and recorded the ICa at the presynaptic nerve terminal of rat calyces (Fig. 1C). We observed an obvious reduction in ICa amplitude after bath application of BDNF (100 ng/ml; Ctrl: 2.1 ± 0.1 nA, n = 7; BDNF: 1.4 ± 0.1 nA, n = 8; p < 0.0001, unpaired t test; Fig. 1C). However, we did not find a slowing of ICa activation. The 20%–80% rise time for ICa after depolarization in control rats was 0.60 ± 0.05 ms (n = 7), which is similar to the previous report (Baydyuk et al., 2015). After incubation with BDNF (100 ng/ml) in the extracellular solution for 30 min, the 20%–80% rise time was not significantly changed (0.64 ± 0.06 ms, n = 8; p = 0.5619, unpaired t test; Fig. 1C). Next, we plotted the current–voltage curve induced by a 200 ms depolarization pulse from ~80 mV to ~70, ~60, . . . 80 mV with an interval of 30 s (Fig. 1D, left), the ICa in the presence of BDNF was smaller than control at every voltage step, but the peak amplitude did not shift (Ctrl: n = 5; BDNF: n = 6; Fig. 1D, right), confirming that BDNF inhibits ICa amplitude but does not affect its activation. We also examined...
Figure 1.  BDNF inhibits EPSC, presynaptic ICa, and release probability. A, Top, Sampled paired EPSC recordings in response to 0.1 Hz fiber stimulation at the midline of the trapezoid body; 100 ng/ml BDNF was added to the extracellular solution after obtaining a baseline, and BDNF was washed out after the reduced EPSC amplitudes were stable. Bottom, The corresponding PPR calculated from the paired EPSC amplitudes. B, Top, Sampled EPSC pairs at time points a (baseline, black), b (BDNF treatment, red), and c (washout, blue) from A were overlapped, showing the EPSC changes in response to BDNF application. Bottom, Statistics for EPSC amplitude and PPR (n = 8) from A using a repeated-measures one-way ANOVA with Bonferroni’s multiple comparisons test. **p < 0.01. C, Top, Averaged traces of ICa induced by depol20ms in the control group (n = 7; black) and in the presence of BDNF (100 ng/ml, n = 8; red). ICa of the BDNF-treated group is scaled for comparison (BDNF-scaled, blue). Dashed lines indicate the 20% and 80% rise time. Bottom, Statistics for ICa amplitude and the 20%-80% rise time induced by depol20ms using an unpaired Student’s t test. **p < 0.01. D, Left, Sampled ICa traces in response to 200 ms depolarization pulses from −80 to −40 mV (black), −10 (green), 0 (purple), 10 (red), and 40 mV (blue) in the control and BDNF-treated groups. Right, Plot of the current–voltage relationship in control (n = 5 for each data point; black) and BDNF-treated calyces (n = 6 for each data point; red). p values were calculated using an unpaired Student’s t test. *p < 0.05. E, Plot of ICa inactivation curves in the control (n = 5 for each data point; black) and BDNF-treated calyces (n = 5 for each data point; red). F, Sampled ICa (top) and capacitance changes (bottom) induced by 1 (black), 2 (red), 5 (blue), 10 (green), 20 (yellow), 30 (brown), and 50 ms (purple) depolarization pulses from −80 to 10 mV in the control and BDNF-treated groups. G, Left, The relationship between ΔCm and the duration of depolarization pulses in the control (n = 6 for each data point; black) and BDNF-treated (n = 5 for each data point; red) groups. Right, Statistics of the release probability measured by the percentage of RRP release induced by a 1 ms depolarization pulse from −80 to 10 mV in the control (n = 6; black) and BDNF-treated groups (n = 5; red) using an unpaired Student’s t test. Data were from left. **p < 0.01. n.s., not significant. Detailed statistical information is provided in Table 1.
the inactivation curve of the ICa and found no significant difference between the control and BDNF-treated groups \((p = 0.9819, \text{Kolmogorov-Smirnov test}; n = 5; \text{Fig. 1F})\). These results suggest that inhibition of the presynaptic ICa amplitude, not a slowdown of ICa activation, is involved in the BDNF-induced inhibition of EPSC amplitude.

The increased PPR recorded for the EPSC after bath application of BDNF suggests a reduction in release probability (Fig. 1A,B). To address this issue at the presynaptic site, we applied stimulation pulses of various lengths \((1, 2, 5, 10, 20, 30, \text{and} 50 \text{ms})\) from \(-80 \text{ to } 10 \text{ mV}\) to induce vesicle release and determined the changes in the readily releasable pool (RRP) size and release probability in the presence of BDNF as described previously \((\text{Xue et al., 2012b})\). In control rats, depol20ms induced a capacitance jump of \(493 \pm 35 \text{fF}\) \((n = 6)\), which represents the RRP size \((\text{Sun and Wu, 2001})\). Depolarization pulses of 1, 2, 5, and 10 ms induced 6 \pm 1\%, 23 \pm 4\%, 65 \pm 4\%, and 87 \pm 5\%, respectively, of the capacitance jump induced by depol20ms measured at the same synapses \((n = 6\) for each depolarization step; Fig. 1F, left, G, black). No further capacitance increase was observed in the control group when the step duration was increased after 30 ms, which is consistent with previous studies showing that a 10–20 ms depolarization pulse from \(-80 \text{ to } 10 \text{ mV}\) can deplete the RRP \((\text{Sun and Wu, 2001; Xue et al., 2012a})\). After application of BDNF \((100 \text{ng/ml})\) to the extracellular solution, depol20ms induced a capacitance increase of \(378 \pm 13 \text{fF}\) \((n = 5)\), which is smaller than the capacitance jump measured in controls \((p = 0.0033, \text{unpaired} t \text{test})\). When longer stimulation pulses \((30–50 \text{ ms})\) were applied, the capacitance jump increased further, until reaching a similar level as controls \((528 \pm 23 \text{fF} \text{after} 50 \text{ ms depolarization pulse}, n = 5; p = 0.9770, \text{unpaired} t \text{test}; \text{Fig. 1G, red})\), suggesting that the RRP size is not affected. However, depolarization pulses of 1, 2, 5, and 10 ms induced 4 \pm 1\%, 13 \pm 2\%, 39 \pm 4\%, and 56 \pm 3\%, respectively, of the capacitance jump induced by depol1ms after incubation with BDNF \((n = 5\) for each depolarization step, Fig. 1F, right, G, red), which is much smaller than the capacitance jump in controls. These results clearly indicate a reduction in the vesicle release probability, which was calculated the percentage of RRP release \((\Delta \text{Cm}_{15s}/\text{RRP})\) induced by an action potential-like stimulation \((\text{depol1ms:} 1 \text{ ms depolarization pulse from } -80 \text{ to } 10 \text{ mV})\), the data for depol1ms were obtained from the same experiments; Fig. 1G) \((\text{Sun and Wu, 2001})\).

**BDNF induces presynaptic inhibition via activation of postsynaptic TrkB receptor and presynaptic CB1R**

BDNF regulates synaptic functions via the activation of TrkB receptors, the major BDNF receptors in the CNS \((\text{Reichardt, 2006})\). We examined the location of TrkB receptors at the calyx of Held synapse by immunostaining. The antibody against Bassoon (BSN), a presynaptic cytomatrix protein selectively localized at the active zone of the nerve terminal \((\text{tom Dieck et al., 1998})\), overlapped partially with the staining of the antibody against TrkB receptors \((\text{Fig. 2A})\). Staining of TrkB receptors was also observed at the postsynaptic principal neurons, indicating that the TrkB receptors have both presynaptic and postsynaptic expression. We asked whether presynaptic and/or postsynaptic TrkB receptors are involved in the BDNF-induced inhibition of synaptic transmission. A previous study demonstrated that BDNF can act on postsynaptic TrkB receptors to activate the release of eCBs, which would then diffuse retrogradely to reduce the presynaptic release probability of neurotransmitters at cortical inhibitory synapses \((\text{Lemtiri-Chlieh and Levine, 2010})\). In the glutamatergic calyx-type synapse, eCBs are synthesized postsynaptically, and the CB1Rs are specifically localized at the synaptic nerve terminal \((\text{Kushmerick et al., 2004; Zou and Kumar, 2018})\) \((\text{Fig. 2B})\). Therefore, the postsynaptic expression of TrkB receptors, together with the presynaptic localization of CB1Rs, prompted reinvestigation of the previous study to examine the possibility of BDNF-TrkB-CB1R-induced inhibition of synaptic transmission at calyces \((\text{Baydyuk et al., 2015})\).

First, we investigated whether BDNF-induced reduction of postsynaptic ICa can inhibit presynaptic vesicle exocytosis and endocytosis at the calyx of Held synapse reported previously \((\text{Baydyuk et al., 2015})\). In control p8-p10 rats, depol20ms induced a calcium influx \((2.1 \pm 0.1 \text{nA}; n = 9; \text{Fig. 2C, top, black}, D)\) and a capacitance jump representing exocytosis \((\Delta \text{Cm}; 538 \pm 30 \text{fF}; n = 9; \text{Fig. 2C, bottom, black}, D)\). The immediately subsequent capacitance decay reflecting endocytosis could be fitted monoexponentially with a time constant of 16.9 \pm 1.6 s. The Rateendo measured 1–2 s after depol20ms was \(48 \pm 4 \text{fF/s} (n = 9; \text{Fig. 2C, bottom, black})\), which demonstrates slow endocytosis \((\text{Wu et al., 2005; Xue et al., 2012b})\). In the presence of BDNF \((100 \text{ng/ml})\) in the extracellular solution, depol20ms induced a ICa of \(1.5 \pm 0.1 \text{nA}\) and a ACm of \(387 \pm 18 \text{fF}\) \((n = 8)\), both of which were significantly lower than control \((\text{ICa:} p = 0.0037; \Delta \text{Cm:} p = 0.0017; \text{one-way ANOVA with Bonferroni post hoc test; Fig. 2C, red, D})\). The Rateendo was also reduced \((30 \pm 3 \text{fF/s}; n = 8; p = 0.0032, \text{one-way ANOVA with Bonferroni post hoc test; Fig. 2D})\), suggesting inhibition in slow endocytosis. The capacitance decay did not return to baseline within 30 s, which is difficult to fit monoexponentially. The remaining ACm 15 s after depol20ms was \(49 \pm 3\% (\Delta \text{Cm}_{15s}: n = 8; \text{Fig. 2D})\) of the maximum ACm after depol20ms which is still remarkably higher than control \((30 \pm 3\%; n = 9; p = 0.0002, \text{one-way ANOVA with Bonferroni post hoc test})\), further confirming a slowdown in slow endocytosis.

Second, we investigated whether activation of TrkB receptors was involved in BDNF-induced presynaptic inhibition. We applied ANA-12 \((500 \text{ nm})\), a potent and selective TrkB receptor antagonist \((\text{Montalbano et al., 2013})\), with BDNF \((100 \text{ng/ml})\) in the extracellular solution. After incubating for 30 min, the BDNF-induced inhibition of calcium influx, exocytosis, and endocytosis after depol20ms was abolished, confirming that the effect of BDNF was mediated by the activation of TrkB receptors \((\text{Fig. 2D})\). Incubation with only ANA-12 did not affect the presynaptic ICa and exocytosis/endocytosis \((\text{Fig. 2D})\).

Next, we examined whether BDNF-TrkB signaling can retrogradely activate presynaptic CB1Rs to inhibit the presynaptic ICa and exocytosis/endocytosis. We applied 1 \text{mM} of AM251, the CB1R antagonist \((\text{Lemtiri-Chlieh and Levine, 2010})\), in the extracellular solution in the presence of BDNF \((100 \text{ng/ml})\). We found that, after incubation for 30 min, BDNF modulation of the ICa and exocytosis/endocytosis was no longer apparent after depol20ms \((\text{ICa:} 2.1 \pm 0.1 \text{nA}; p > 0.9999; \Delta \text{Cm:} 509 \pm 27 \text{fF}; p > 0.9999; \text{Rateendo:} 45 \pm 3 \text{fF/s}; p > 0.9999; \Delta \text{Cm}_{15s}: 34 \pm 3\% ; p > 0.9999; \text{one-way ANOVA with Bonferroni post hoc test for all four groups:} n = 7; \text{Fig. 2C, blue, D})\), suggesting that CB1R was the downstream effector in BDNF-inhibited synaptic transmission. Furthermore, adding only AM251 to the extracellular solution did not result in any difference from control \((\text{Fig. 2D})\).

To confirm the activation of CB1Rs in BDNF-induced presynaptic inhibition, we included 2 \text{mM} WIN55212-2, the exogenous cannabinoid agonist \((\text{Lemtiri-Chlieh and Levine, 2010})\), in the extracellular solution to investigate whether it can mimic the
Figure 2. BDNF inhibits presynaptic ICa and exocytosis/endocytosis via activation of postsynaptic TrkB receptors and presynaptic CB1Rs. A, Immunostaining of presynaptic cytomatrix (Bassoon, green) and TrkB receptors (red) at calyces. Scale bar, 10 µm. B, Immunostaining of presynaptic cytomatrix (Bassoon, green) and CB1Rs (red) at calyces. Scale bar, 10 µm. C, Averaged presynaptic ICa (top) and Cm (bottom) induced by depol20ms in the control (Ctrl, black), BDNF treatment (BDNF, red), BDNF treatment in the presence of AM251 (AM251+BDNF, blue), and WIN55212-2 treatment (WIN, green) groups. D, Statistics for ΔCm, ICa, Rateendopl, and ΔCm15s% from different treatments in extracellular solution (Ctrl, n = 9; BDNF, n = 8; ANA-12+BDNF, n = 7; ANA-12, n = 8; AM251+BDNF, n = 7; AM251, n = 6; WIN, n = 8; AM251+WIN, n = 5). *p values were calculated using a one-way ANOVA with Bonferroni's multiple comparisons test. *p < 0.05. **p < 0.01. E, Left, Averaged presynaptic ICa induced by depol20ms in the control (K252apost, 200 nM K252a in the postsynaptic pipette solution, n = 5; black), BDNF treatment (BDNF, without K252a in the postsynaptic pipette solution in the presence of BDNF, n = 7; red), and BDNF treatment with K252a (K252apost+BDNF, 200 nM K252a in the postsynaptic pipette solution in the presence of BDNF, n = 7; blue) groups. All recordings are made in the paired-recording mode. Right, Statistics for ICa in all three groups using a one-way ANOVA with Bonferroni's multiple comparisons test. *p < 0.05. **p < 0.01. n.s., not significant. Detailed statistical information is provided in Table 1.
showing that the BDNF-induced inhibition of rapid endocytosis was mediated by activation of TrkB receptors. We further examined the involvement of CB1Rs in the BDNF-induced inhibition of rapid endocytosis. After incubation with AM251 (1 µm) in the presence of BDNF (100 ng/ml) in the extracellular solution for 30 min, the calcium influx, exocytosis, and rapid/slow endocytosis recovered after depol20max10 (QICa: 307 ± 20 pC, p > 0.9999; ΔCm: 1513 ± 74 fF, p > 0.9999; Rateendo: 221 ± 17 fF/s, p > 0.9999; ΔCm30s%: 14 ± 3%, p > 0.9999; one-way ANOVA with Bonferroni post hoc test for all four groups; n = 8; Fig. 2C, green, D). An additional 1 µM AM251 fully abolished the WIN-induced inhibition of ICA and vesicle exocytosis/endocytosis (Fig. 2D). These results confirm that BDNF inhibits synaptic transmission via activation of postsynaptic CB1Rs.

We further investigated the involvement of presynaptic and/or postsynaptic TrkB receptors by specifically blocking the postsynaptic TrkB receptors. We applied K252a (200 nM), another TrkB receptor inhibitor, to the postsynaptic pipette solution in a whole-cell configuration, and then recorded the ICA and vesicle exocytosis/endocytosis at the presynaptic nerve terminal of the same synapse after bath application of BDNF (100 ng/ml) for 20 min. Inhibition of postsynaptic TrkB receptors in the presence of 100 ng/ml BDNF abolished the depol20max-induced inhibition of ICA and ΔCm (ICA: 2.1 ± 0.1 nA, p > 0.9999; ΔCm: 503 ± 36 fF, p > 0.9999; one-way ANOVA with Bonferroni post hoc test), and subsequent endocytosis recovered (Rateendo: 49 ± 4 fF/s, p > 0.9999; ΔCm30s%: 29 ± 4%, p > 0.9999; one-way ANOVA with Bonferroni post hoc test; n = 7; Fig. 2E,F, blue). Administration of K252a in the postsynaptic pipette solution did not affect the presynaptic ICA and vesicle exocytosis/endocytosis (Fig. 2E,F, black). Therefore, we concluded that BDNF induces presynaptic inhibition via activation of postsynaptic TrkB receptors at calyces.

**BDNF inhibits rapid endocytosis via the eCB signaling pathway**

After exocytosis, the fused presynaptic membrane is retrieved via endocytosis to maintain efficient synaptic transmission and normal morphology of the presynaptic nerve terminal (L. G. Wu et al., 2014). Two forms of endocytosis are commonly observed at calyces: clathrin- and dynamin-dependent slow endocytosis, and clathrin-independent dynamin-dependent rapid endocytosis (W. Wu et al., 2005; X. S. Wu et al., 2009). We examined whether BDNF also affects rapid endocytosis.

We applied a stronger stimulation of 10 depol20ms at 10 Hz (depol20max10) to induce rapid endocytosis at calyces. In control p8-p10 rats, depol20max10 induced a much larger calcium influx (QICa; 308 ± 10 pC) and a higher ΔCm (1527 ± 94 fF, n = 10; Fig. 3A, top, black), representing more vesicle release. The capacitance decayed biexponentially with a rapid and slow τ of 1.9 ± 0.1 s and 16.4 ± 1.6 s, respectively (n = 10; Fig. 3A, bottom, black). In the presence of BDNF (100 ng/ml) in the extracellular solution, depol20max10 evoked a reduced calcium influx (224 ± 12 pC, p = 0.0002; one-way ANOVA with Bonferroni post hoc test) and smaller ΔCm (1166 ± 49 fF, p = 0.0021; one-way ANOVA with Bonferroni post hoc test; n = 13; Fig. 3A, red). Both rapid and slow endocytosis on depol20max10 was greatly inhibited (control: Rateendo 222 ± 19 fF/s; ΔCm30s%, 11 ± 4%; n = 10; BDNF: Rateendo 111 ± 7 fF/s, p < 0.0001; ΔCm30s%, 30 ± 4%, p = 0.0025; one-way ANOVA with Bonferroni post hoc test; n = 13; Fig. 3A, bottom), confirming that BDNF can also inhibit clathrin-independent rapid endocytosis.

Similarly, when we applied ANA-12 (500 nM) with BDNF (100 ng/ml) in the extracellular solution for 30 min, the calcium influx, exocytosis, and endocytosis evoked by depol20max10 were not obviously different from those in the control group (Fig. 3B),

**Postsynaptic release of eCBs is required for the BDNF-induced presynaptic inhibition**

Having shown that BDNF acts on the postsynaptic TrkB receptors and retrogradely induces presynaptic inhibition of the ICA and exocytosis/endocytosis via activation of the presynaptic CB1Rs, we investigated the involvement of postsynaptic eCB synthesis. The eCBs have been shown to be released from postsynaptic neurons via Ca2+-dependent mechanisms (Kushmerick et al., 2004). We recorded the EPSCs with 20 mM BAPTA in the postsynaptic pipette solution to examine whether the BDNF-induced inhibition of synaptic transmission could be abolished. After 30-60 min bath application of BDNF (100 ng/ml), the EPSC amplitude was not changed (baseline: 5.1 ± 0.8 nA, BDNF: 5.1 ± 0.8 nA; p = 0.8688, paired t test; n = 5; Fig. 5A,B), confirming that the release of eCBs is required for BDNF-induced inhibitory effect.

To examine the presynaptic effect when calcium is chelated, we included 0 (vehicle) or 20 mM BAPTA in the postsynaptic pipette solution via a whole-cell configuration, and then applied BDNF (100 ng/ml) in the extracellular solution. After 20 min, we
performed presynaptic recordings at the nerve terminal of the same synapse to examine the calcium influx and exocytosis/endocytosis. We found that 20 mM BAPTA can completely abolish the BDNF-induced inhibition of presynaptic calcium influx and exocytosis/endocytosis after either depol20ms (ICa: 2.0 ± 0.1 nA, p > 0.9999; ΔCm: 530 ± 46 fF, p > 0.9999; Rateendo: 50 ± 4 ff/s, p > 0.9999; ΔCm30s: 30 ± 2%, p > 0.9999; one-way ANOVA with Bonferroni post hoc test for all four groups; n = 6) or depol20msx10 (QICa: 302 ± 12 pC, p > 0.9999; ΔCm: 1522 ± 69 fF, p > 0.9999; Rateendo: 224 ± 13 ff/s, p > 0.9999; ΔCm30s: 9 ± 2%, p > 0.9999; one-way ANOVA with Bonferroni post hoc test for all four groups; n = 6; Fig. 5C–F).

Next, we disrupted the synthesis of 2-arachidonoylgllycerol, the principal eCB for activity-dependent retrograde signaling, by blocking phospholipase C (PLC) or diacylglycerol lipase (DGL), both of which are key postsynaptic enzymes in generating 2-arachidonoylgllycerol (Ohno-Shosaku et al., 2012; Anderson et al., 2015). After application of U73122 (5 μM), an inhibitor of PLC (Rinaldi and Hansel, 2013), with BDNF (100 ng/ml) for 30 min, we found that the BDNF-inhibited ICa and exocytosis/endocytosis induced by depol20ms were fully abolished (ICa: 2.0 ± 0.1 nA, p > 0.9999; ΔCm: 522 ± 24 fF, p > 0.9999; Rateendo: 47 ± 4 ff/s, p > 0.9999; ΔCm30s: 29 ± 2%, p > 0.9999; one-way ANOVA with Bonferroni post hoc test for all four groups; n = 7; Fig. 6A, blue, B). When we induced rapid endocytosis with depol20msx10, the calcium influx, exocytosis, and subsequent rapid endocytosis were also similar to control (QICa: 286 ± 18 pC, p > 0.9999; ΔCm: 1447 ± 73 fF, p > 0.9999;
BDNF inhibits endocytosis in calcium-dependent and -independent ways

As BDNF can inhibit presynaptic calcium influx and subsequent exocytosis/endocytosis, which is consistent with our previous finding that calcium triggers exocytosis and initiates all forms of endocytosis (X. S. Wu et al., 2009; Xue et al., 2012b), we further investigated whether BDNF can directly modulate presynaptic vesicle endocytosis in a calcium-independent manner. To address this issue, we increased the extracellular calcium concentration to counterbalance the BDNF-induced reduction in the ICa. In the presence of BDNF (100 ng/ml) in the extracellular solution with 3.5 mM calcium, the ICa and exocytosis induced by depol20ms were similar to control (Ctrl3.5Ca: ICa, 2.2 ± 0.2 nA, R = 0.6351; ICa, 531 ± 29 F; p = 0.4757; unpaired t test; n = 7; Fig. 7A,B). The Rateendo and ΔCm15% were slightly reduced but still not significantly different from control (Ctrl3.5Ca: Rateendo, 48 ± 5 F/s; ΔCm15%, 31 ± 2%; n = 8; BDNF3.5Ca: Rateendo, 46 ± 4 F/s, p = 0.6343; ΔCm15%, 35 ± 4%, p = 0.2413; unpaired t test; n = 7; Fig. 7B). However, endocytosis was still inhibited (Ctrl3.5Ca: Rateendo, 238 ± 11 F/s; ΔCm30%, 8 ± 2%; n = 8; BDNF3.5Ca: Rateendo, 155 ± 20 F/s, p = 0.0029; ΔCm30%, 31 ± 5%, p = 0.0008; unpaired t test; n = 8; Fig. 7D) when we applied depol20msmax10 to induce rapid endocytosis, although calcium influx and exocytosis were similar to control in the presence of BDNF (100 ng/ml) with 3.5 mM extracellular calcium (Ctrl3.5Ca: QICa, 309 ± 14 pC; ΔCm, 1510 ± 57 F; n = 8; BDNF3.5Ca: QICa, 310 ± 26 pC, p = 0.7844; ΔCm, 1457 ± 85 F, p = 0.6111; unpaired t test; n = 8; Fig. 7C,D). These results confirm that, in addition to calcium-dependent regulation of slow and rapid endocytosis, BDNF can also directly modulate the rapid form of endocytosis in a calcium-independent manner. We also performed similar experiments with 1.3 mM calcium in the extracellular solution of the control group to mimic the BDNF-reduced calcium influx. Although calcium influx and exocytosis were reduced to a level similar to the BDNF-treated group, endocytosis was still significantly inhibited in the presence of BDNF (100 ng/ml) after depol30max10 (Ctrl3.5Ca: Rateendo, 147 ± 7 F/s; ΔCm30%, 15 ± 3%; n = 7; BDNF3.5Ca: Rateendo, 108 ± 6 F/s, p = 0.0018; ΔCm30%, 33 ± 2%, p = 0.0004; unpaired t test; n = 7; Fig. 7E,F). Therefore, we concluded that BDNF can modulate presynaptic vesicle endocytosis in both a calcium-dependent and -independent manner.

The AC/PKA signaling pathway is involved in CB1R-induced presynaptic inhibition

Many studies have suggested that activation of CB1R modulates synaptic transmission by reducing neurotransmitter release at both excitatory and inhibitory synapses (Heifets and Castillo, 2009; Kano et al., 2009; Ohno-Shosaku et al., 2012). However, how CB1R activation leads to the inhibition of synaptic transmission remains elusive (de Jong and Verhage, 2009). A previous study demonstrated that the AC/PKA signaling pathway modulates endocytosis in response to strong stimulation (Yao and Sakaba, 2012). Therefore, we investigated whether AC/PKA are also involved in BDNF-TrkB-CB1R-induced presynaptic inhibition.

We applied specific AC and PKA antagonists MDL (10 μM) and KT5720 (2 μM), respectively (Yao and Sakaba, 2012), to the presynaptic pipette solution. Presynaptic calcium influx, exocytosis, and endocytosis induced by depol30ms or depol20msmax10 were dramatically suppressed (Fig. 8A–D; Table 1). When we applied the exogenous cannabinoid agonist WIN55212-2 (2 μM) with MDL or KT5720, we did not detect further inhibition of calcium influx and exocytosis/endocytosis (Fig. 8A–D; Table 1), suggesting that AC/PKA signaling was involved in the BDNF-TrkB-CB1R-induced presynaptic inhibition at calyces. We also examined the effect of forskolin, a potent adenylate cyclase activator (Ho et al., 2015; Rey et al., 2020). In the presence of 50 μM forskolin in the presynaptic pipette solution, depol30ms or depol20msmax10 induced similar calcium influx and exocytosis/endocytosis to the control (DMSOpret, Fig. 8A,C). An additional 2 μM WIN55212-2 in the presence of forskolin did not induce any inhibitory effect (Fig. 8B,D; Table 1), confirming the
Figure 5. The BDNF-induced inhibitory effect is postsynaptic calcium-dependent. A. Left, Sampled paired EPSC recordings and corresponding PPR in response to 0.1 Hz fiber stimulation at the midline of the trapezoid body; 20 mM BAPTA was included in the postsynaptic pipette solution to chelate free calcium ions; 100 ng/ml BDNF was added to the extracellular solution after obtaining a stable baseline and EPSCs were recorded for another 30-60 min. Right, Sampled EPSCs at time points a (baseline, black) and b (BDNF treatment, red) from left. B. Statistics for EPSC amplitude and PPR (n = 5) from A using a paired Student’s t test. C. Left, Averaged presynaptic ICa induced by depol20ms in the control (Vehicle, without BAPTA in the postsynaptic pipette solution, n = 6; black), BDNF treatment (Vehicle + BDNF, without BAPTA in the postsynaptic pipette solution in the presence of BDNF, n = 5; red), and BDNF treatment with BAPTA (BAPTA + BDNF, 20 mM BAPTA in the postsynaptic pipette solution in the presence of BDNF, n = 6; blue) groups. All recordings were made in the paired-recording mode. Right, Statistics for ICa in all three groups using a one-way ANOVA with Bonferroni’s multiple comparisons test. **p < 0.01. D. Left, Averaged Cm induced by depol20msx10 (Vehicle, n = 5; Vehicle + BDNF, n = 6; BAPTA + BDNF, n = 6). **p < 0.01. n.s., not significant. Detailed statistical information is provided in Table 1.
involvement of the AC/PKA pathway. Interestingly, when we increased the extracellular calcium concentration to 3.5 mM, application of MDL (10 μM) or KT5720 (2 μM) still partially inhibited the endocytosis rate after depolarization (MDL3.5Ca: Rateendo, 129 ± 8 fl/s, p = 0.0002; KT3.5Ca: Rateendo, 139 ± 5 fl/s, p = 0.0456; n = 6; one-way ANOVA with Bonferroni post hoc test; Fig. 8E,F), which is consistent with calcium-dependent and -independent BDNF-induced modulation (Fig. 7).

Therefore, our results confirmed the involvement of the AC/PKA signaling pathway in the BDNF-TrkB-eCB-induced inhibition of synaptic transmission.

**Discussion**

BDNF and eCBs are widely expressed neuromodulators that play crucial roles in various neuronal functions, plasticity, and physiological processes. In the present study, we found that BDNF inhibits presynaptic calcium influx and vesicle exocytosis/endocytosis via the BDNF-TrkB-CB1R signaling pathway. BDNF selectively activates postsynaptic TrkB receptors to evoke the release of eCBs via the PLCγ/DGL pathway that retrogradely activates presynaptic CB1Rs, leading to the suppression of downstream AC/PKA signaling. Our study suggests a new mechanism of BDNF-induced inhibition of synaptic transmission at the calyx synapse (Fig. 9).
BDNF induces a retrograde eCB signaling pathway
How does activation of postsynaptic TrkB receptors lead to inhibition of presynaptic calcium influx and exocytosis/endocytosis? In the present study, we report the involvement of the eCB signaling pathway in BDNF-induced inhibition of synaptic transmission at calyces. Inhibition of the presynaptic CB1R or disruption of postsynaptic eCB synthesis abolishes the BDNF-induced presynaptic inhibition, and exogenous cannabinoid agonist WIN55212-2 can mimic the inhibitory effect of BDNF, demonstrating BDNF-induced retrograde presynaptic inhibition.

AC and PKA are widely reported to be involved in G-protein-activated presynaptic inhibition (Chevaleyre et al., 2007; Castillo et al., 2012). Here, we report that inhibition of AC/PKA leads to suppression of calcium influx and vesicle exocytosis/endocytosis at calyces. Activation of AC by 50 μM forskolin in the bath solution fully abolished the WIN-induced presynaptic

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**Figure 7.** BDNF inhibits endocytosis in a calcium-dependent and -independent manner. A. Left, Averaged presynaptic ICa induced by depol20ms in the control group (Ctrl2Ca, n = 8; black) and BDNF treatment with 3.5 mM extracellular calcium (BDNF3.5Ca, n = 7; red). Right, Statistics for ICa in two groups using an unpaired Student’s t test. B. Left, Averaged Cm induced by depol20ms from A. Right, Statistics for ΔCm, Rateendo, and ΔCm15s% in two groups using an unpaired Student’s t test. C. Similar to A, B, except the stimulation was depol20msx10 (Ctrl2Ca, n = 8; BDNF3.5Ca, n = 8). **p < 0.01. D. Left, Averaged presynaptic ICa induced by depol20msx10 in the control group with 1.3 mM extracellular calcium (Ctrl1.3Ca, n = 7; black) and BDNF treatment with 2 mM extracellular calcium (BDNF2Ca, n = 7; red). Right, Statistics for ICa in two groups using an unpaired Student’s t test. E. Left, Averaged Cm induced by depol20msx10 from D. Right, Statistics for ΔCm, Rateendo, and ΔCm30s% in two groups using an unpaired Student’s t test. **p < 0.01. n.s., not significant. Detailed statistical information is provided in Table 1.
Figure 8. The AC/PKA signaling pathway is involved in the inhibitory effects of BDNF. 

A. Averaged presynaptic ICₐ (top) and Cₘ (bottom) induced by depol₂₀ms in the control (DMSOₚₚ, 0.1% DMSO in the presynaptic pipette solution, black), WIN55212-2 treatment (WIN, red), WIN55212-2 treatment in the presence of MDL 12330A (MDL + WIN, blue), and WIN55212-2 treatment in the presence of KT 5720 (KT + WIN, green) groups. 

B. Statistics for ΔCₘ, ICₐ, Rateₑndo, and ΔCₘ₃₀s% from different treatments in extracellular solution (DMSOₚₚ, n = 6; WIN, n = 5; MDL, n = 8; KT, n = 7; MDL + WIN, n = 9; KT + WIN, n = 4; Forskolin + WIN, n = 5; Forskolin, n = 7). p values were calculated using a one-way ANOVA with Bonferroni’s multiple comparisons test. *p < 0.05. **p < 0.01.

C. Averaged ICₐ induced by depol₂₀msx₁₀ in the control (Ctrl₂₀Ca, identical to DMSOₚₚ in Fig. 8C, n = 6; black), MDL3.5Ca treatment with 3.5 mM extracellular calcium (MDL3.5Ca, n = 6; red), and KT3.5Ca treatment with 3.5 mM extracellular calcium (KT3.5Ca, n = 6; blue) groups. 

D. Statistics for QICₐ using a one-way ANOVA with Bonferroni’s multiple comparisons test.

E. Left, Averaged Cₘ induced by depol₂₀mxₐ₁₀ from E. Right, Statistics for ΔCₘ, Rateₑndo, and ΔCₘ₃₀s% using a one-way ANOVA with Bonferroni’s multiple comparisons test. *p < 0.05. **p < 0.01. n.s., not significant. Detailed statistical information is provided in Table 1.
BDNF induces endocytosis via calcium-dependent and -independent pathways

The previous study reported that BDNF inhibits slow and rapid endocytosis via a calcium-independent pathway because BDNF does not reduce the QICa on mild or intense stimulation (Baydyuk et al., 2015). However, in our study, we found that BDNF inhibits calcium influx and exocytosis/endocytosis on either depol20ms or depol20max10 (Figs. 2C,D, 3A,B). When we increased the extracellular calcium concentration in the BDNF-treated group or decreased the calcium concentration in the control group to induce similar amounts of calcium influx and vesicle exocytosis in the two groups, endocytosis was still partially inhibited because of direct modulation of endocytosis by the AC/PKA pathway. Therefore, our findings suggest that BDNF inhibits presynaptic endocytosis in both calcium-dependent and -independent ways.

Physiologic implication of the BDNF-TrkB-eCB signaling cascade

Many studies have shown that BDNF can facilitate the efficacy of excitatory synapses by altering either presynaptic neurotransmitter release (Carmignoto et al., 1997; Jovanovic et al., 2000) or the magnitude of postsynaptic responses (Alder et al., 2005) in brain slices or cultured neurons. However, several studies have shown that BDNF may play a different role in the brainstem (Balkowiec et al., 2000; Clark et al., 2011). For example, in the brainstem nucleus tractus solitarius slice, BDNF can reduce the amplitude of mEPSC, the evoked EPSC, and the action potential discharge, indicating reduced intrinsic neuronal excitability (Clark et al., 2011).

BDNF/eCB-induced inhibition of neurotransmission may serve as negative feedback to provide activity-dependent neuroprotection from excitotoxicity. Depolarization-induced suppression, a strong depolarization of postsynaptic neurons leading to reduced synaptic transmission via the release of eCBs, has been interpreted as an efficient means of neuronal protection (Wilson and Nicoll, 2001). Excessive glutamate release has also been shown to promote the synthesis of eCBs to avoid hyperexcitability (Kushmerick et al., 2004).

BDNF/eCB signaling may also exert neuroprotective effects on neurodegenerative diseases, such as Huntington’s disease. Delivery and overexpression of BDNF or activation of CB1R protect the striatal neurons from excitotoxicity, reduce motor disorders, and prevent the loss of medium spiny neurons (Kells et al., 2008; Blázquez et al., 2011; Connor et al., 2016; Ayerich et al., 2018).

A recent study reported that a reduction in BDNF expression impairs synaptic transmission at the calyx of Held (Jang et al., 2019). However, an increase in BDNF may modulate synaptic transmission via the activation of different signaling cascades, including the inhibition of synaptic transmission via eCB signaling shown here and in other studies (Lemtiri-Chlieh and Levine, 2010; Zhao and Levine, 2014; Zhong et al., 2015). Furthermore, increased BDNF expression has been observed in many physiological or pathologic conditions. For example, a protective mechanism of the CB1R-dependent increase in BDNF expression has been reported in mice with kainate-induced seizures (Marsicano et al., 2003). At calyces, the basal neuronal firing can be increased to >600–800 Hz on stimulation (Von Gersdorff and Borst, 2002; Hermann et al., 2007), which may increase the BDNF level in an activity-dependent manner (Y. J. Wu et al., 2004; Singer et al., 2014) to induce inhibitory neuroprotection from excitotoxicity. A recent study showed that, in the lower part of the auditory system in the brain, BDNF may improve the signal-to-noise ratio and sound sensitivity by increasing the inhibitory strength of neurons at hearing onset. A significant increase in central noise can be observed after auditory nerve injury (Chumak et al., 2016).

In conclusion, we examined the presynaptic mechanisms and signaling cascades of BDNF-induced inhibition of synaptic transmission at a glutamatergic central synapse. By uncovering the detailed mechanisms underlying how BDNF/TrkB couples with the eCB signaling pathway to modulate synaptic transmission, our study may provide a comprehensive understanding of how BDNF and eCBs associate in an overlapping set of neurologic diseases.
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