Activity- and BDNF-Induced Plasticity of Miniature Synaptic Currents in ES Cell-Derived Neurons Integrated in a Neocortical Network

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Copi, Andrea, Kay Jungling, and Kurt Gottmann. Activity- and BDNF-induced plasticity of miniature synaptic currents in ES cell-derived neurons integrated in a neocortical network. J Neurophysiol 94: 4538–4543, 2005; doi:10.1152/jn.00155.2005. In vitro differentiated embryonic stem (ES) cells have been proposed as potential donor cells for cell replacement therapies of neurodegenerative diseases. The functional synaptic integration of such cells appears conceivable because ES cell-derived neurons are well known to establish excitatory and inhibitory synapses. However, long-term synaptic plasticity, a prerequisite of memory formation, has not yet been demonstrated at these synapses. After in vitro differentiation and purification by immunolocalization, we co-cultured ES cell-derived neurons with neocortical explants, which strongly innervated the ES cell-derived target neurons. ES cell-derived neurons exhibited action potential firing similar to primary cultured neocortical neurons. The formation of glutamatergic synapses was indicated by AMPA receptor-mediated miniature excitatory postsynaptic currents (AMPa mEPSCs). In addition, a N-methyl-D-aspartate receptor-mediated, n-2-amino-5-phosphophenopentanoic acid-sensitive mEPSC component was observed. We first studied activity-dependent homeostatic plasticity (synaptic scaling) of mEPSCs at glutamatergic synapses. Chronic blockade of action potential activity by TTX resulted in an increase in the amplitudes of AMPA mEPSCs. This indicates that ES cell-derived neurons are capable of a homeostatic regulation of postsynaptic AMPA receptors. In addition, we investigated neurotrophin-induced synaptic plasticity of mEPSCs at glutamatergic synapses. Chronic addition of brain-derived neurotrophic factor (BDNF; 100 ng/ml) to the culture medium resulted in an increase in both the frequency and amplitudes of AMPA mEPSCs. These results suggest that BDNF induces the formation and/or the functional maturation of presynaptic release sites in parallel with an upregulation of postsynaptic AMPA receptors. Thus BDNF represents a potential co-factor that could improve functional synaptic integration of ES cell-derived neurons into neocortical networks.

INTRODUCTION

Pluripotent embryonic stem (ES) cells are well known to differentiate in vitro into mature neurons and thus represent a potential source of donor cells for cell replacement therapies of neurodegenerative diseases. Cultured ES cell-derived neurons have been demonstrated to develop neuronal morphology, to discharge action potentials on stimulation, and to form functional synapses (Bain et al. 1995; Finley et al. 1996; Jungling et al. 2003; Strübing et al. 1995). Moreover, after transplantation to the brain ES cell-derived neurons show organotypic morphology and become synaptically integrated into cortical neuronal networks (Benninger et al. 2003; Wernig et al. 2004). Both major types of synapses, excitatory synapses with AMPA- and N-methyl-D-aspartate (NMDA)-type glutamate receptors and inhibitory GABAergic synapses, have been described in synthetically connected ES cell-derived neurons (Finley et al. 1996; Jungling et al. 2003).

In addition to the basic properties of synapses, the correct functioning of transplanted ES cell-derived neurons within complex cortical networks requires long-term synaptic plasticity as an essential feature. Long-term synaptic plasticity at excitatory glutamatergic synapses is dependent on action potential activity patterns and is either input-specific (Malenka and Nicoll 1999; Malinow and Malenka 2002) or affects all synapses of a neuron (homeostatic plasticity) (Turrigiano et al. 1998; Turrigiano 1999; Turrigiano and Nelson 2004). Moreover, neurotrophic factors, e.g., brain-derived neurotrophic factor (BDNF), induce long-term synaptic plasticity at cortical glutamatergic synapses (Lessmann et al. 1994; Lessmann 1998; Lu 2003; Lu and Chow 1999). Both, an involvement in the input-specific potentiation of postsynaptic currents evoked by action potentials (Korte et al. 1995; Patterson et al. 1996) and in the cell-wide regulation of spontaneous miniature postsynaptic currents (Collin et al. 2001; McLean Bolton et al. 2000; Paul et al. 2001; Rutherford et al. 1998; Vicario-Abejon et al. 1998) have been described.

Because BDNF readily induces long-term synaptic plasticity, this neurotrophin represents a potentially important co-factor in cell replacement therapy that might boost synaptic integration of transplanted ES cells-derived neurons. However, it has not yet been investigated whether BDNF can induce long-term synaptic changes also in ES cell-derived neurons. To address this, we established a co-culture system consisting of neocortical explants and ES cell-derived neurons and studied glutamatergic synapses electrophysiologically. We focused on BDNF effects on miniature EPSCs because a cell-wide form of plasticity appears more interesting than input-specific plasticity of a few synapses to improve the overall synaptic integration of transplanted cells. Here, we report that ES cell-derived neurons show activity-dependent synaptic scaling of all glutamatergic synapses on a given neuron. Most notably, we demonstrate that BDNF is able to induce a long-term enhancement of miniature EPSCs in ES cell-derived neurons.
METHODS

Proliferation and in vitro differentiation of mouse ES cells (line R1) was performed as described previously (Jüngling et al. 2003). In brief, ES cells were cultured on inactivated mouse feeder cells in the presence of LIF. Embryoid bodies (EBs) were formed in hanging drop culture in the presence of retinoic acid. After two further weeks in culture, EBs were dissociated and ES cell-derived neurons were purified by immunoisolation using antibodies against the cell adhesion molecule L1 (Jüngling et al. 2003).

To study the synaptic integration of ES cell-derived neurons into neocortical networks, we used a simple co-culture system consisting of presynaptic explants that innervated dissociated postsynaptic target neurons as described previously (Gottmann et al. 1997; Mohrmann et al. 1999). In brief, mouse neocortical explants were obtained from E18 mouse (C57/black6) fetuses and cultured for 3 days as free-floating explants in Neurobasal medium with addition of B27 supplement, glutamax, and penicillin/streptomycin (all from Invitrogen). Cultivation as free-floating explants led to the formation of “spheres,” which was critical to avoid outward migration of neurons from the explants during later cultivation. These explants were added to the purified ES cell-derived neurons that had been seeded at low density during later cultivation. These explants were added to the culture in the presence of retinoic acid. After two further weeks in culture in the presence of LIF. Embryoid bodies (EBs) were formed in hanging drop culture. EBs were dissociated and ES cell-derived neurons were cultured in the presence of retinoic acid. At 11–14 DIV, AMPA receptor-mediated mEPSCs were recorded in the presence of TTX (1 μM), picrotoxin (100 μM), and Mg2+ (1 mM) at a holding potential of −60 mV (Fig. 1C). Using an elevated extracellular K+ concentration (30 mM), mEPSCs occurred at a mean frequency of 5.9 ± 0.9 Hz (n = 18) and had a mean amplitude of 13.6 ± 1.3 pA. Their mean rise time was 0.86 ± 0.06 ms (average mEPSCs), and their mean decay time constant was 5.6 ± 0.8 ms. AMPA receptor-mediated mEPSCs were completely blocked by the addition of 6,7-dinitroquinoxalin-2,3-dione (20 μM, n = 6; Fig. 1D). To further study the presence of a NMDA receptor-mediated component, mEPSCs were recorded in Mg2+-free extracellular solution at a holding potential of −60 mV. For analysis, mEPSCs of an individual cell were averaged to obtain a mean mEPSC. Averaged mEPSCs showed a clear NMDA receptor-mediated slow component that was selectively blocked by the addition of d-AP5 (50 μM, n = 5; Fig. 1E). We further compared AMPA mEPSCs from co-cultures containing ES cell-derived neurons as postsynaptic targets with AMPA mEPSCs from control co-cultures consisting of neocortical explants and dissociated neocortical target neurons. The mean frequency (10.3 ± 1.5 Hz, n = 21) and the mean amplitude (12.7 ± 0.6 pA) of AMPA mEPSCs in neocortical neurons were not significantly different (Fig. 1F). Thus glutamatergic mEPSCs in ES cell-derived neurons had properties very similar to those of glutamatergic mEPSCs in primary cultured neocortical neurons in the same type of co-culture system (Mohrmann et al. 1999). Functional glutamatergic synapses between ES cell-derived neurons or glutamatergic autapses were observed only very rarely, thus demonstrating that synaptic input to ES cell-derived neurons in our co-culture system is strongly dominated by the explant fibers. In whole cell recordings from pairs of ES cell-derived neurons action potential-evoked AMPA EPSCs were detectable in only 1 of 14 potential connections.
tested. Similarly, autaptic AMPA EPSCs were detected in only 2 of 14 cells studied. In both cases, the amplitudes of evoked AMPA EPSCs were very small (<5 pA) in the range of AMPA mEPSCs, suggesting the presence of only a single release site.

We next wanted to demonstrate that glutamatergic synapses in ES cell-derived neurons show synaptic scaling, a homeostatic form of activity-dependent long-term plasticity (Turrigian et al. 1998). Synaptic scaling has been described in dissociated neocortical neurons (empty bars; Co/Co).

In co-cultures containing ES cell-derived neurons as target neurons (filled bars; Co/ndES) and in control co-cultures consisting of neocortical explants and neurons, we again performed paired recordings from ES cell-derived neurons into a neocortical network. We addressed whether BDNF is able to induce long-term plasticity at glutamatergic synapses in ES cell-derived neurons. We added BDNF (100 ng/ml) to our co-cultures at 8 DIV and recorded AMPA mEPSCs at 11–14 DIV as described in the preceding text. Strikingly, chronic BDNF application strongly increased both mEPSC frequency and mEPSC amplitude (Fig. 3). The mean frequency of AMPA mEPSCs significantly \((P < 0.01)\) increased from 6.0 \(\pm 1.3\) Hz \((n = 22)\) in parallel control cultures to 12.7 \(\pm 1.5\) Hz \((n = 16)\) in BDNF-treated cultures. In addition, the mean amplitude of AMPA mEPSCs significantly increased from 11.5 \(\pm 1.7\) to 17.9 \(\pm 1.3\) pA \((P < 0.01)\). No significant change in the mean rise time of average AMPA mEPSCs was observed (control: 0.9 \(\pm 0.04\) ms; BDNF-treated: 0.84 \(\pm 0.04\) ms; Fig. 2E) supporting that a postsynaptic mechanism. These results clearly indicate that ES cell-derived neurons integrated in neocortical networks are capable of cell-wide synaptic long-term plasticity involving postsynaptic AMPA receptor regulation.

Neurotrophic factors, e.g., BDNF are potentially important co-factors in cell replacement therapies of neurodegenerative diseases because they might enhance functional synaptic integration of ES cell-derived neurons. Therefore we further addressed whether BDNF is able to induce long-term plasticity at glutamatergic synapses in ES cell-derived neurons. We added BDNF (100 ng/ml) to our co-cultures at 8 DIV and recorded AMPA mEPSCs at 11–14 DIV as described in the preceding text. Strikingly, chronic BDNF application strongly increased both mEPSC frequency and mEPSC amplitude (Fig. 3). The mean frequency of AMPA mEPSCs significantly \((P < 0.01)\) increased from 6.0 \(\pm 1.3\) Hz \((n = 22)\) in parallel control cultures to 12.7 \(\pm 1.5\) Hz \((n = 16)\) in BDNF-treated cultures. In addition, the mean amplitude of AMPA mEPSCs significantly increased from 11.5 \(\pm 1.7\) to 17.9 \(\pm 1.3\) pA \((P < 0.01)\). No significant change in the mean rise time of average AMPA mEPSCs was observed (control: 0.9 \(\pm 0.04\) ms; BDNF-treated: 0.84 \(\pm 0.04\) ms; Fig. 2E), supporting that a postsynaptic mechanism underlies the increase in AMPA mEPSC amplitudes. To test whether BDNF led to the formation of glutamatergic autapses or synapses between the ES cell-derived neurons after chronic BDNF treatment. We could not detect any functional synapses \((n = 12\) potential connections tested) or autapses \((n = 12\) cells), thus demonstrating that the observed effects of BDNF occur at synaptic.

**FIG. 1.** Functional synaptic integration of mouse embryonic stem (ES) cell-derived neurons into a neocortical network. A: co-culture system (left photomicrograph) consisting of a neocortical explant innervating added ES cell-derived neurons (arrows) that were purified by immunosolation. Right photomicrograph: typical dendritic arborization of a strongly innervated ES cell-derived neuron at higher magnification; 12 day in vitro (DIV).

B: typical action potential responses of an ES cell-derived target neuron to depolarizing current injection. Current pulses used are shown below action potential responses. C: AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) recorded from an ES cell-derived target neuron using an elevated extracellular K\(^+\) concentration. Two individual events are shown at an enlarged time scale. Holding potential: \(-60\) mV. D: AMPA mEPSCs were completely blocked by addition of DNQX (20 \(\mu M\)) in the presence of Mg\(^2+\). E: mean mEPSCs obtained by averaging individual mEPSCs that were recorded in Mg\(^2+\)-free extracellular solution at \(-60\) mV for 100 ms. Two individual events are shown at an enlarged time scale. Holding potential: \(-60\) mV. F: quantitative comparison of the mean frequency and the mean amplitude of AMPA mEPSCs in co-cultures containing ES cell-derived neurons as target neurons (filled bars; Co/ndES) and in control co-cultures consisting of neocortical explants and dissociated neocortical neurons (empty bars; Co/Co).
contacts between explant fibers and ES cell-derived neurons as postsynaptic targets. In summary, our results strongly suggest that BDNF induces long-term plasticity of glutamatergic synapses in ES cell-derived neurons similar to primary cultured cortical neurons (Lessmann et al. 1994; Vicario-Abejon et al. 1998).

**DISCUSSION**

Pluripotent ES cells are a major donor source of neuronal precursor cells or neurons that might potentially be used for cell replacement therapies of neurodegenerative diseases. Mouse ES cells have been demonstrated to differentiate in vitro to neurons that show basic functional properties such as action potential firing (Bain et al. 1995; Strübng et al. 1995). In addition, cultured ES cell-derived neurons form both excitatory, glutamatergic synapses with postsynaptic AMPA and NMDA receptors and inhibitory, GABAergic synapses (Finley et al. 1996; Strübng et al. 1995). On transplantation of ES cell-derived neural precursor cells in slice cultures and in vivo, the ES cell-derived neurons exhibited functional properties similar to cortical neurons, i.e., action potential discharge and glutamatergic and GABAergic synaptic input (Benninger et al. 2003; Wernig et al. 2004). However, in addition to these basic properties, long-term synaptic plasticity is required for the proper functioning of central synapses within neuronal networks. Although the preceding studies have already well established a basic functional integration, the demonstration that ES cell-derived neurons are capable of long-term synaptic plasticity is still lacking.

In this paper, we describe a simple model system that allows to study the functional integration of ES cell-derived neurons under well-defined in vitro conditions. Our co-cultures consisted of mouse neocortical explants that innervated spatially

![FIG. 2. Activity-dependent synaptic scaling of AMPA mEPSC amplitudes in ES cell-derived neurons. A: AMPA mEPSCs recorded from co-cultures maintained in standard culture conditions (control). B: AMPA mEPSCs recorded from co-cultures after chronic addition of tetrodotoxin to the culture medium to block spontaneous action potentials (TTX). C: quantitative comparison of the mean frequencies (left) and the mean amplitudes (right) of mEPSCs recorded from control cultures (○) and from TTX-treated cultures (●). * significant difference. n is indicated above bars. D: plot of AMPA mEPSC frequency vs. mean AMPA mEPSC amplitude for each individual cell analyzed from control co-cultures (○) and from TTX-treated co-cultures (●). Note the increased amplitudes in TTX-treated co-cultures. E: time course of AMPA mEPSCs was not affected by TTX treatment. Mean mEPSCs from a control cell and a TTX-treated cell obtained by averaging individual mEPSCs are superimposed.](image)

![FIG. 3. Brain-derived neurotrophic factor (BDNF)-induced plasticity of AMPA mEPSC frequency and amplitudes in ES cell-derived neurons. A: AMPA mEPSCs recorded from co-cultures maintained in standard culture conditions (control). B: AMPA mEPSCs recorded from co-cultures after chronic addition of 100 ng/ml BDNF to the culture medium (BDNF). C: quantitative comparison of the mean frequencies (left) and the mean amplitudes (right) of mEPSCs recorded from control cultures (○) and from BDNF-treated cultures (●). * significant difference. n is indicated above bars. D: plot of AMPA mEPSC frequency vs. mean AMPA mEPSC amplitude for each individual cell analyzed from control co-cultures (○) and from BDNF-treated co-cultures (●). Note the increased frequencies and amplitudes in BDNF-treated co-cultures. E: time course of AMPA mEPSCs was not affected by BDNF treatment. Mean mEPSCs from a control cell and a BDNF-treated cell obtained by averaging individual mEPSCs are superimposed.](image)
separated mouse ES cell-derived neurons. This system allows for an efficient synaptic integration of ES cell-derived neurons in a neocortical network while preserving spatial separation. In particular, chronic application of pharmacological substances and neurotrophic factors at well-defined concentrations is easily possible in our co-culture system. Long-term synaptic plasticity of glutamatergic synapses is an essential feature of cortical neuronal networks and is thought to underlie developmental maturation of connectivity and memory formation. In addition to classical input-specific long-term potentiation/depression, a cell-wide, homeostatic form of plasticity, synaptic scaling, has been described (Turrigiano 1999; Turrigiano and Nelson 2004; Turrigiano et al. 1998). In neocortical neurons, chronic blockade of activity increased the amplitudes of AMPA receptor-mediated miniature EPSCs, while enhancing activity decreased miniature EPSC amplitudes. Both changes are caused by alterations in the number and postsynaptic accumulation of postsynaptic glutamate receptors (Desai et al. 2002; Turrigiano et al. 1998; Watt et al. 2000; Wierenga et al. 2005). Homeostatic plasticity, in particular the upscaling of mEPSCs, represents a cellular mechanism that could enhance the functional synaptic integration of ES cell-derived neurons into cortical networks. Here we demonstrated that a very similar scaling up of the amplitudes of AMPA receptor-mediated mEPSCs also occurs in neocortical explant-ES cell-derived neuron co-cultures during chronic activity blockade. This finding strongly suggests that glutamatergic synapses in ES cell-derived neurons are capable of homeostatic long-term plasticity.

The neurotrophin BDNF has been demonstrated to control several types of input-specific long-term synaptic plasticity at central glutamatergic synapses (for review, see Lessmann 1998; Lu 2003; Lu and Chow 1999). Both pre- and postsynaptic mechanisms for persistent increases in synaptic strength have been shown to depend on BDNF (e.g., Itami et al. 2003; Zakharenko et al. 2003). Moreover, BDNF is also well known to affect the cell-wide homeostatic regulation of miniature synaptic currents. Chronic BDNF application has been shown to enhance both the frequency (Collin et al. 2001; Paul et al. 2001; Vicario-Abejon et al. 1998) and the amplitude of AMPA mEPSCs (McLean Bolton et al. 2000) in primary cultured hippocampal neurons. In addition, effects of BDNF on AMPA mEPSC amplitudes have been shown to be strongly dependent on the neuronal cell type investigated with opposing effects occurring in neocortical pyramidal cells as compared with GABAergic interneurons (Leslie et al. 2001; Rutherford et al. 1998).

In our co-culture system, we observed a strong BDNF-induced increase in the frequency of AMPA mEPSCs in ES cell-derived neurons similar to the effects of BDNF in immature hippocampal neurons (Collin et al. 2001; Vicario-Abejon et al. 1998). We found in addition an increase in mEPSC amplitudes suggesting that chronic BDNF application affects both presynaptic release properties (mEPSC frequency) and postsynaptic AMPA receptors. Although postsynaptic mechanisms acting on AMPA receptors appear more likely, changes in mEPSC amplitudes can in principle be explained also by presynaptic mechanisms such as an increased transmitter content of synaptic vesicles. Because presynaptic mechanisms would lead to an increased glutamate concentration in the synaptic cleft, a change in the rise time of mEPSCs would be expected. However, we did not observe any significant change in AMPA mEPSC rise times in our experiments.

In contrast, in mature neocortical pyramidal neurons a BDNF-dependent downregulation of the amplitude of AMPA mEPSCs has been described (Rutherford et al. 1998). Taken together, these results suggest that BDNF might affect mEPSCs differently in different types of cells: in immature (precursor) cells, BDNF appears to enhance frequency and amplitude, whereas in mature neurons, BDNF limits quantal size. To this end, our results in ES cell-derived neurons indicate that ES cell-derived neurons are capable of BDNF-dependent long-term synaptic plasticity. The effects of BDNF in our ES cell-derived neurons more closely resembled BDNF effects in immature hippocampal neurons as compared with those in mature neocortical neurons. Whether this is appropriate for synaptic integration and plasticity in neocortical circuits needs further investigation in organotypic systems at different stages of differentiation. Nevertheless BDNF appears to represent a promising cofactor in cell replacement therapies of neurodegenerative diseases that has the potential to enhance the functional synaptic integration of stem cell-derived neurons into cortical neuronal networks.

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