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Effects of NGF and BDNF on baseline glutamate and dopamine release in the hippocampal formation of the adult rat

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Abstract

It has been shown using in vitro techniques that BDNF and NGF evoke neurotransmitter release in the hippocampus but this phenomenon has not been demonstrated in vivo to date. We therefore performed in vivo microdialysis in urethane-anesthetized Fischer 344 rats. The microdialysis probe was implanted stereotaxically into the CA1 area of the hippocampus. Three hours after the implantation of the probe, glutamate (Glu) and dopamine (DA) levels had reached a stable baseline. Four baseline samples were collected every 15 min at a flow rate of 1 μ L/min. The growth factors were delivered (1 μ L/10 min) using a microinjector attached to the microdialysis probe. We found that BDNF and NGF, when administered into the hippocampus, evoked dopamine and glutamate release in a dose-dependent fashion. NGF produced a biphasic response in the release of Glu, and a uniphasic response in the release of DA, both of which were calcium dependent. The neurotransmitter release induced by NGF was blocked by tetrodotoxin, indicating neuronal origin of this response. The BDNF induced release of DA and Glu was decreased in low calcium conditions, indicating that it is at least partially calcium dependent. Furthermore, BDNF-induced neurotransmitter release was partially blocked by pre-treatment with K252a, an antagonist for tyrosine kinase receptors, indicating that BDNF is acting through Trk receptors to induce neurotransmitter release. These results demonstrate a close relationship between the growth factors BDNF and NGF and the neurotransmitters DA and Glu in the hippocampus of intact animals.

Keywords

Dopamine; Glutamate; Hippocampal formation; Neurotrophin; Transmitter release; Growth factor

1. Introduction

While it has been known for a long time that neurotrophins affect neuronal migration and differentiation during development, it has recently been discovered that they are also instrumental for a number of rapid processes occurring both in the developing and adult central nervous system (CNS). Much more is known about rapid transmitter-like effects of brain-derived neurotrophic factor (BDNF) than about the first discovered nerve growth factor (NGF). BDNF elicits action potentials in central neurons (Kafitz et al., 1999) and using freely moving

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rats, Bramham et al. (1996) found that the induction of long term potentiation (LTP) at medial perforant path-granule cell synapses triggers a delayed and sustained increase in BDNF mRNA expression in granule cells (Bramham et al., 1996). BDNF has also been found to increase potassium-evoked glutamate (GLU) release in different brain regions, using tissue culture or slice preparations (Kafitz et al., 1999; Numakawa et al., 1999; Pascual et al., 2001; Canas et al., 2004; Pereira et al., 2006; Matsumoto et al., 2006). Effects of BDNF on glutamate neurotransmission are blocked by K252a, a tyrosine kinase receptor blocker, indicating that they are mediated by the high affinity TrkB receptor (Pascual et al., 2001). BDNF interacts primarily with presynaptic mechanisms of transmitter release and does not appear to affect postsynaptic processing of information (Wu et al., 2004) even though recent studies have shown that BDNF can increase phosphorylation of the glutamate receptor NMDA postsynaptically (Lessmann, 1998). One mechanism for the presynaptic stimulation seen with BDNF is that this neurotrophin increases mitogen-activated protein kinase-dependent synapsin I phosphorylation and acutely facilitates evoked glutamate release in the hippocampus (Jovanovic et al., 2000) and increases levels of synaptobrevin and synaptophysin, as well as the number of both dense core and clear vesicles in nerve terminals or varicosities (Takei et al., 1997). BDNF causes a rapid depolarization which, depending on the dose, eventually results in a cascade of action potential firing. Kafitz et al. (1999) have shown that this excitatory response is mediated by tyrosine kinase receptors of the TrkB family. Further, BDNF increases the stimulation-evoked release of GLU and the levels of exocytosis-associated proteins in cultured cortical neurons from embryonic rats (Takei et al., 1997). Despite these earlier findings in tissue culture or slice preparations, little has been reported about the short-term effects of BDNF on neurotransmitter release *in vivo*.

There is evidence of a relationship between the release of endogenous GLU *in vivo* and neural expression of mRNAs for BDNF and its receptor TrkB in the hippocampus (Lindfors et al., 1992; Falkenberg et al., 1996). Thus, it appears that there is a reciprocal relationship between the transmitter GLU and BDNF in terms of transmitter release and neuronal activity. In regards to BDNF's mechanism of release, it has been suggested that activation of the Trk B receptor activates phospholipase C and production of IP(3) to initiate intracellular calcium release from stores, which subsequently potentiates transmitter release (Numakawa et al., 2001, 2002; Matsumoto et al., 2001; Neal et al., 2003; Suzuki et al., 2004), and in addition, event ligand-induced TrkB translocation into lipid rafts may represent a signaling mechanism selective for synaptic modulation by BDNF in the central nervous system (Suzuki et al., 2004). To our knowledge the close relationship between GLU transmission and BDNF described above has not been evaluated in the hippocampus of intact animals.

NGF rapidly increases the basal firing rate of basal forebrain cholinergic neurons in aged rats and in transplanted cholinergic neurons (Palmer et al., 1993; Albeck et al., 1999) and has also been shown to have significant effects on GLU release in slice preparations or cultured cells (Numakawa et al., 2003; Raiteri et al., 2003). Our group has previously demonstrated that NGF administration into the hippocampal CA1 region in intact animals gives rise to a rapid up-regulation of TrkA receptors in the basal forebrain (occurring within 15 min from the time of injection) (Williams et al., 2005). This up-regulation was not altered by the retrograde transport inhibitor colchicine, suggesting a rapid stimulation cascade occurring from the target area (hippocampus) to the cholinergic cell bodies in the basal forebrain, independent of "classical" microtubule-dependent retrograde transport mechanisms (Williams et al., 2005). Other rapid effects of NGF that have been reported recently include increased GLU release in visual cortex *in vitro* in a calcium and inositol (1,4,5)triphosphate dependent manner, mediated via Trk receptors (Sala et al., 1998; Raiteri et al., 2003) and increased extracellular dopamine (DA) levels following cortical lesions (Maysinger et al., 1992). Further, both NGF and BDNF have been shown to affect dopamine (DA) release in the substantia nigra (SN), striatum, and cortical regions (Goggi et al., 2003; Narita et al., 2003; Dluzen et al., 2004). Conversely, glutamate-

induced DA release in the substantia nigra was found to increase BDNF mRNA expression significantly (Bustos et al., 2004) and BDNF-mediated potentiation of DA release from striatal synaptosomes was found to be regulated by the PI3K and Ras-MEK pathways (Goggi et al., 2003). Presently, there is much less known about NGF-induced effects than about BDNF-induced effects on rapid physiological processes in the hippocampus. The data presented in the present communication shed some light on the role of NGF in neurotransmission within the hippocampus, and the relationship between NGF vs. BDNF administration and basal GLU and DA levels in this brain region.

It is clear from the literature, that both NGF and BDNF have powerful short-term effects on neurotransmission in the hippocampal formation. Since most of the previous studies had been performed on tissue cultures or slice preparations, we wanted to explore short-term effects of these two neurotrophins when injected directly into the hippocampal formation of adult animals *in vivo*. Therefore, we administered BDNF and NGF into the dorsal hippocampus (CA1) of intact animals while simultaneously performing microdialysis which allowed us to monitor the temporal effects of BDNF and NGF *in vivo* on neurotransmission.

2. Results

2.1. BDNF and GLU

A two-way mixed model analyses of variance was used to analyze Glutamate release {[Drug (7): Vehicle, 0.01, 0.1, 0.3, 0.3 low calcium, 0.3 + k252a, 0.3+TTX] $\times$ [Time (13): -45 to 135 min]} and revealed a dose-dependent increase in GLU in the dialysate which began 15 min following the local application of BDNF and remained significantly increased over baseline for an additional 30 min [$F(27, 324) = 5.07, p < 0.05$] (see Fig. 1A). As illustrated in Fig. 1B, BDNF elicited gradually elevated responses as the dose of BDNF increased when the area under the curve (AUC) was analyzed, suggesting a dose-response relationship to administered BDNF, with the 0.3 μ g dose of BDNF producing significantly higher responses overall compared to all other conditions (Tukey's Multiple Comparison Test $p < 0.01$). The addition of TTX to the aCSF significantly reduced the effect of BDNF, suggesting that the effect was neuronal activity-dependent ($p < 0.05$). When the effect of BDNF was examined in the presence of low calcium, there was a significant reduction in the amount of GLU in the dialysate ($p < 0.05$). In addition, the TrKB antagonist K252a (Fig. 1) attenuated the response, albeit not to baseline levels (Figs. 1A and B).

2.2. BDNF and DA

A two-way mixed model analyses of variance was used to analyze DA release and revealed a significant interaction between drug and time [$F(27, 324) = 6.144, p < 0.05$]. These data demonstrate that DA release was also enhanced by BDNF in a dose-dependent fashion (see Figs. 2A and B). A Tukey's Multiple Comparison Test revealed that all doses tested, except 0.01 μ g BDNF, produced significantly greater AUC compared to vehicle ($p < 0.05$). Both low calcium and K252a significantly attenuated the response to 0.3 μ g BDNF ($p < 0.01$). The addition of TTX to the aCSF significantly reduced the effect of 0.3 μ g BDNF suggesting that this effect was also neuronal activity-dependent ($p < 0.05$). Interestingly, TTX administration delayed the BDNF response approximately 30 min, suggesting that other indirect actions of the trophic factor may be at play in the hippocampus in terms of the biological effects of BDNF administration on DA neurotransmission (see Fig. 2A). As shown in Fig. 2B, a dose-response relationship was established with 0.1 μ g and 0.3 μ g BDNF yielding a greater release response than 0.01 μ g as well as 0.3 μ g producing greater AUC than 0.1 μ g ($p < 0.05$).

2.3. NGF and GLU

As depicted in Fig. 3A, NGF administration elicited a biphasic response in GLU release; the first peak began 30 min post-injection, and the second peak began 75 min post-injection. This biphasic response was not seen in glutamate levels after the BDNF injections, or in any of the BDNF or NGF effects on DA release. Both of these peaks were dose-dependent, with the largest response seen with the 0.3 μ g dose and at the second (delayed) peak. Glutamate release was examined using a two-way mixed model analyses of variance which resulted in a significant interaction between drug and time [$F(23, 276) = 3.862, p < 0.05$]. A one way analysis of variance of AUC of GLU release between treatments also yielded significance [$F(5,35) = 10.32, p < 0.05$]. As shown in Fig. 3B, a Tukey's Multiple Comparison Test revealed that 0.3 μ g NGF produced significantly higher responses compared to all other conditions ($p < 0.05$). The GLU response to NGF administration was completely abolished by both low calcium and TTX pretreatment, again suggesting calcium- and impulse-dependent release ($p < 0.05$). The dose-response relationship of NGF to GLU is demonstrated in Fig. 3B.

2.4. NGF and DA

In addition to increased GLU release with the NGF administration, this neurotrophin also increased extracellular basal levels of DA (Fig. 4). A two-way mixed model analyses of variance demonstrated a significant interaction between drug and time [$F(18, 216) = 4.567, p < 0.05$]. A one way analysis of variance of AUC of DA release between treatments yielded significance [$F(5,23) = 7.65, p = 0.0005$] (see Fig. 4A). As shown in Fig. 4B, a Tukey's Multiple Comparison Test revealed that all doses of NGF produced significantly higher DA release than vehicle, except when TTX was added to the aCSF ($p < 0.05$). Furthermore, 0.3 μ g NGF produced significantly higher responses overall compared to all other conditions ($p < 0.05$). The effect of 0.3 μ g NGF to induce DA release was significantly decreased by TTX and low calcium ($p < 0.05$; see Fig. 4A), albeit not to baseline levels, indicating a partial role for calcium and sodium channels on this effect. Contrary to the effects of NGF on GLU as reported above, the DA response to this factor was not biphasic. As shown in Fig. 4A, this response was more rapid than the GLU response yielding a significant peak beginning at 30 min following initiation of the administration.

3. Discussion

In the present study, we found that microinjection of BDNF and NGF into the hippocampus induced a rapid (20–30 min post-injection) increase in the extracellular levels of GLU and DA in vivo, as evaluated with microdialysis coupled with HPLC analysis. These effects lasted for approximately 30–60 min. In addition, we found that NGF elicited a biphasic response in glutamate release, while all other effects investigated were only one phase. We also found that all the reported transmitter responses could be blocked by TTX and low calcium pretreatment; suggesting that they were dependent on neuronal activity and extracellular calcium and that the BDNF responses could be blocked by the tyrosine kinase receptor blocker K252a. The DA response to NGF was only partially reversed with low calcium and TTX, suggesting that other mechanisms may be involved in this response, either directly or indirectly via other brain regions or cell layers. These data extend the knowledge of the rapid actions of growth factors, establishing an effect on neurotransmission in the hippocampal formation of the adult intact rat.

3.1. Biological mechanisms of the neurotrophin-induced transmitter release

In the experiments herein, we attempted to address potential mechanisms by repeating the experiment in the presence of low calcium, TTX, or the tyrosine kinase receptor blocker K252a. TTX and low calcium did indeed block both NGF and BDNF effects, at least partially, and the K252a application gave rise to a significant reduction in both glutamate and DA response to

BDNF. Thus, it appeared from our experiments that both glutamate and DA responses to the neurotrophins were dependent on appropriate levels of extracellular calcium and neuronal activity (TTX). Interestingly, TTX pretreatment delayed the peak response of DA to BDNF, suggesting perhaps that this particular treatment (blockage of sodium channels) in the vicinity of the recording probe gave rise to a local, restricted blockade of neuronal activity that initially retarded the response. However, since the peak eventually occurred (30 min delayed and with lesser maximal release), this suggests that the DA response may be primarily dependent on local neuronal activity but can be “rerouted” via other systems (or signaled via other cells such as glial cells) when the primary pathway is blocked. It is of course also possible that the partial blockade of neurotrophin responses could be caused by differences in the perfusion kinetics of the neurotrophins compared to the low calcium and TTX solutions, respectively, which are difficult to control for in the *in vivo* situation, and would require further studies using for example slice preparations. The calcium-dependency of the BDNF and NGF responses reported here is consistent with previous findings *in vitro* and in tissue culture, where investigators have shown that both NGF and BDNF-induced transmitter release are calcium-dependent (Lessmann, 1998; Griesbeck et al., 1999). Neurotransmitter release is postulated to be mediated by extracellular calcium mobilization to the cytosol or by release from intracellular stores (Numakawa et al., 1999). A study by (Kleiman et al., 2000) demonstrated that activation of the TrkB receptor activates phospholipase C to initiate intracellular Ca^{2+} release from stores which subsequently potentiates transmitter release, at least in myocytes in the periphery. Other investigators have found that NGF has a direct potentiating effect on spontaneous and depolarization-evoked release of glutamate from hippocampal nerve endings, accompanied by an increase in the excitatory postsynaptic potential of CA1 neurons. This is correlated with increased incorporation of [^{32}P]phosphate into synapsin I, suggesting that NGF or BDNF stimulation elicits a presynaptic phosphorylation cascade leading to enhanced neurotransmission. BDNF modulates synaptic efficacy in a variety of preparations. Studies demonstrate a presynaptic mechanism for neurotrophin-mediated changes in synaptic strength and plasticity (Jovanovic et al., 2000). An augmentation of post-synaptic efficiency (phosphorylation of the NMDA receptor) has also been reported after administration of BDNF (Lessmann, 1998), suggesting that the response may not only be presynaptic but may also have postsynaptic consequences in this brain region. Since neurotrophins are known to be transported intracellularly in hippocampus and other brain regions (see e.g. Mufson et al., 1995; Miller and Kaplan, 2001), the delayed response could potentially be caused by intracellular transport to the site of action as well. It has been reported that the neurotrophin-induced glutamate release was dependent on intracellular Ca^{2+} mobilization, and pretreatment with K252a and also TrkB-IgG completely blocked glutamate and aspartate release elicited by BDNF, but not by NGF (Numakawa et al., 1999, 2002). The authors concluded that these results suggest that while BDNF induced release via TrkB receptors, NGF-elicited release was not mediated by the high affinity tyrosine kinase receptors, suggesting that this type of rapid release may be generated via p75 receptors for NGF instead. Further studies *in vivo* will reveal whether p75 or trkA receptors are involved in the glutamate- and DA-response. Reciprocal positive feedback between production of trophic factors in target cells by neurotransmitter released from afferent neuronal inputs and the modulation of presynaptic neurotransmitter release by target-derived trophic factors provides a novel mechanism for short-term control over synaptic communication.

3.2. Direct or indirect effects of NGF on glutamate and DA release

Previous studies have shown that NGF-responsive neurons in the CNS include primarily the cholinergic neurons of the basal forebrain, cholinergic neurons of the striatum and some neuronal populations of the brainstem and cerebellum (Maisonpierre et al., 1990; Michael et al., 1997; Sala et al., 1998; Ferrer et al., 1998). Therefore, it may seem puzzling that we found rapid effects of exogenously administered NGF on glutamate and DA levels in the

hippocampus, since neither hippocampal neurons nor dopaminergic neurons which innervate the hippocampus are known to be NGF-responsive or are known to contain receptors for this growth factor (Kokaia et al., 1995). It is possible therefore that the NGF effects on glutamate and DA release described here are due to an indirect effect exerted via BDNF, since NGF has been shown to rapidly elevate endogenous BDNF expression, both in the periphery and the brain (Michael et al., 1997; Karchewski et al., 2002; Xiong et al., 2002). TrkB receptors are present in hippocampal neurons and BDNF has been shown to be effective in increasing survival of hippocampal neurons in culture (Ferrer et al., 1998). It is even possible that this indirect effect could be mediated via the activation of glutamate receptor systems in the hippocampus, since both AMPA and NMDA receptors appear to be involved in this NGF-mediated increase in BDNF expression (Xiong et al., 2002). It is also possible that an initial stimulation of DA or GLU release by the growth factors can stimulate further release of the other transmitter with time, since at least DA has been shown previously to affect release of GLU in other studies (Dani and Zhou, 2004). On the other hand, NGF may indeed have direct effects on both of these transmitter systems, at least based on evidence from tissue culture studies. Blochl and Sirrenberg (1996) found that both BDNF and NGF administration to midbrain DA neuronal cultures elicited DA release; BDNF via TrkB receptors, and NGF via p75 receptors. A recent study on PC12 cells *in vitro* demonstrated that DA release can be elicited by NGF also via TrkA receptors (Amino et al., 2002), suggesting that the receptor system involved may be dependent on the cell system and/or brain region studied. The pathways involved in BDNF-induced DA release were recently found to be the PI3K and the Ras-MEK pathways (Goggi et al., 2003), but further examination into these pathways in the *in vivo* hippocampus formation still have to be undertaken. Other studies have shown that NGF has direct effects on glutamate neurotransmission, at least in the developing cerebellum (Yamada et al., 2002). NGF, by interacting with TrkA, triggers a sequence of tyrosine kinase-dependent phosphorylation steps that modulate glutamate release and calcium influx and impact the expression of LTP in the dentate gyrus (Maguire et al., 1999; Numakawa et al., 1999; Raiteri et al., 2003). However, the above findings were demonstrated in synaptosome preparations, and have not yet been verified *in vivo*. Therefore, it is not possible at the present time to determine whether the NGF effects observed here are indeed, direct effects of this growth factor or simply a secondary effect via other transmitter systems or neurotrophins. Future studies to explore these biological effects could include the use of BDNF knockout mice or BDNF anti-sense in combination with NGF administration to explore these possibilities more completely.

3.3. Is the second wave of NGF-induced glutamate release elicited via direct or indirect mechanisms?

Contrary to what was found in the other experiments described herein, NGF elicited a biphasic response for GLU release (see Fig. 3A). It would be interesting to determine whether the second wave of effects of administration of NGF on glutamate (occurring up to 50–60 min after initiation of neurotrophin administration) may be due to the fact that NGF stimulated BDNF or TrkB expression, and thus caused a second response that occurred within 60 min of initiation of drug response. This is quite likely, based on previous reports showing that secondary TrkB expression which occurs in the hippocampal formation following an injection of BDNF into the brain (Ferrer et al., 1998). Since NGF administration can elicit increased BDNF expression (see above), it is not unlikely that such a series of events could be the origin of the second wave of the biphasic response. It is also possible that the glutamate response in itself could have given rise to an increase in BDNF expression, thus leading to the second wave, since this phenomenon has indeed been demonstrated in the hippocampus as well (Lindfors et al., 1992). Thus, the present experiments give rise to a series of interesting questions regarding rapid effects of neurotrophins and how they may affect other systems within the hippocampal

formation. The model described in this communication, microdialysis coupled with growth factor reverse dialysis, lends itself to this type of systemic studies on the intact animal.

In summary, this study demonstrates pharmacological effects of the neurotrophic factors BDNF and NGF on release of GLU and DA in the hippocampal formation of the adult anesthetized rat. These results have implicit physiological significance regarding hippocampal synaptic transmission and behavioral processes related to this brain region, such as learning and memory. Since both BDNF and NGF levels in the hippocampus have been correlated with cognitive performance in animal models (for example see Bimonte et al., 2003; Hunter et al., 2004) and administration of these factors gives rise to attenuated memory loss in aging or animal models for Alzheimer's disease (Kordower and Fiandaca, 1990; Haller and Saltzman, 1998), these factors are clearly important for memory processing to occur. Together these data suggest that the role of BDNF and NGF in memory formation may be mediated through the modulation of the synaptic release of neurotransmitters such as GLU and DA, although more direct evidence for this hypothesis should be provided in future experiments. Nonetheless, the data clearly demonstrate the importance of these two factors, not only in neuroprotection in the hippocampus, but also in rapid processes such as neurotransmission.

4. Experimental procedures

4.1. Animals and surgery

Adult male (Fischer 344) rats weighing 350–400 g were individually housed on a 12–12 h light–dark schedule (lights off 2000–0800 h) with food and water available ad libitum, and the room temperature was kept at 22 °C. The rats were anesthetized with urethane (1.25 g/kg, i.p.) and placed in the stereotaxic apparatus to implant the microdialysis probe at the following coordinates: A –6.30 mm, L 5.40 mm, DV 6.0 with respect to bregma, the mid-sagittal sinus, and the surface of the level skull (Paxinos et al., 1985). The microdialysis probes were laboratory-made (Hernandez et al., 1986) with 31 gauge steel tubing attached to the microdialysis body probe which was used as a guide for the capillary tube (75 µ I.D., 104 µ O.D.; Polymicro Technologies, Phoenix, AZ) used to inject the growth factors. The substances injected by this attached device were delivered at the center of the active membrane zone which was 3 mm length. All procedures were approved by the local IACUC committee and adhered to the NIH approved standard treatment of laboratory animals.

4.2. Drugs

Recombinant Human BDNF (R&D Systems, Minneapolis MN; catalog number 248-BD) and Recombinant Human NGF (R&D Systems, Minneapolis MN; catalog number 256-GF) was reconstituted in sterile phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin to prepare a stock solution. K-252a (M.W. 467.5; catalog number 420297) was obtained from Calbiochem® a brand of EMD Biosciences Inc., La Jolla, CA. Tetrodotoxin (TTX) with citrate (M.W. 319.28; catalog number 00061) was purchased from Biotium Inc., Hayward, CA.

4.3. Experimental design

Probes were gently inserted using the stereotaxic arm 4 h prior to sample collection to allow neurotransmitter recovery to baseline level, during which the probe was perfused with artificial cerebral spinal fluid (aCSF) containing 142 mM NaCl, 3.9 mM KCl, 1.2 mM CaCl₂, 1.0 MgCl₂, 1.35 mM Na₂HPO₄, 0.3 mM NaH₂PO₄ at pH 7.4 at a flow rate of 1.0 µL/min. 2 µL of HCl 0.1 M was added to the sample collection tubes prior to the collection of the samples to prevent DA degradation. Four baseline samples were collected at 15 min. intervals. The growth factors were delivered (1 µL) through the attached microinjector at a flow rate of 1 µL in 10 min. The co-perfusion of aCSF with low calcium concentration (0.1 mM), TTX (1 µM) or K252a (1.2 µM) was performed after collecting two baseline samples and 30 min prior to the

administration of the growth factors. A total of 4–7 rats per condition were used for the neurochemical analysis. Samples (dialysates) were collected across time and split for the analysis of Glu and DA in this experiment.

4.4. Histology

After completing the experiments, the animals were overdosed with sodium pentobarbital and decapitated and the brains were dissected, placed in 10% formalin for 24 h and transferred to 15% sucrose with 10% formalin for 24 h. The brains were then frozen, sectioned (40 μ m) on a freezing microtome and stained with Cresyl violet for verification of the probe placement and extensions of the lesion. Only animals with probe placements verified in the hippocampal CA1 region were used for data analysis.

4.5. High performance liquid chromatography (HPLC) analysis

Microdialysis samples were frozen at -80°C until analysis (within 2 weeks) with HPLC. For the analysis of DA: The mobile phase was a citrate–acetate, containing 6% (v/v) methanol at pH 4.1 at 1 mL/min flow rate (Hebert and Gerhardt, 1997). Dopamine was measured by injecting 10 μ L of the dialysate into an isocratic system (Beckman, Inc., Fullerton, CA) coupled to a dual electrode coulometric detector (model 5100A, ESA, Inc, Chelmsford, MA). Voltages of the screening and analytical electrodes were set at -350 mV and $+250$ mV respectively. Analytes in the dialysate were separated by an ODS, 4.6 mm $\times$ 100 mm, 3 μ m particle column. The mobile phase was a sodium–acetate buffer, containing 10% (v/v) methanol at pH 4.1 at 1 mL/min flow rate. Glutamate was analyzed by derivatizing it with naphthalene-2,3-dicarboxaldehyde (NDA) (for methodological background, see de Montigny et al., 1990; O'shea et al., 1992; Rawls and McGinty, 1997). The dialysate (7 μ L) was added to a mixture of 7 μ L of 12 mM sodium borate (at pH = 9.6) plus 7 μ L of 12 mM potassium cyanide. Then 4 μ L of 10 mM NDA was added. After 7 min the sample (25 μ L total volume) was injected into the isocratic system (Beckman, Inc., Fullerton, CA) coupled to an Acufuor programmable Fluorescence detector with the absorbance set at 420, and 450 nm. Glutamate was separated by an ODS, 4.6 mm $\times$ 100 mm, 3 μ m particle column. The quantification of both DA and GLU was based on peak area by comparison with an external calibration curve.

4.6. Statistical analyses

Data from experiments were subjected to separate two-way mixed model analyses of variance to analyze Drug and Time course effects for DA and Glutamate release {[BDNF Drug (7): Vehicle, 0.01, 0.1, 0.3, 0.3 low calcium, 0.3 + k252a, 0.3 + TTX] $\times$ [Time (13): -45 to 135 min] and {[NGF Drug (6): Vehicle, 0.01, 0.1, 0.3, 0.3 low calcium, 0.3 + TTX] $\times$ [Time (13): -45 to 135 min]}. Post hoc analyses (Dunnetts) were used to test for Drug and Time effects. In addition, data were normalized using the area under the curve (AUC) and analyzed using a one-way analysis of variance of the AUC for each neurotransmitter, followed by a Tukey's Multiple Comparison post hoc test to determine direct comparisons between two groups. DA and Glu levels [nM] were used as dependent measures. The significance level was set at $p < 0.05$.

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Abbreviations

BDNF

brain-derived neurotrophic factor

NGF

	nerve growth factor
GLU	glutamate
DA	dopamine
TTX	tetrodotoxin
Trk	tyrosine kinase
CNS	central nervous system
SN	substantia nigra
AUC	area under the curve
HPLC	high performance liquid chromatography

A. BDNF and Glutamate Levels

B. Glutamate Release after BDNF

Fig. 1. Effects of BDNF on GLU neurotransmission

A = time course for microdialysis results on GLU levels when BDNF was administered via reverse dialysis. X-axis depicts time in min and Y-axis depicts GLU levels in micromolar (μM). BDNF elicited a dose-dependent elevation in GLU levels, with the highest dose (0.3 μg) eliciting the highest response. The BDNF-induced peak started approximately 15 min after the onset of BDNF administration, and lasted for 45–60 min. TTX, low calcium and the TrkB blocker K252a gave rise to significant inhibition of the signal. B = Bargraph illustrating the area under the curve (AUC) analysis of the GLU elevation. BDNF (0.3 μg) gave rise to significantly higher GLU levels than all other conditions ($p < 0.05$) (indicated by *).

Fig. 2. Effects of BDNF on DA neurotransmission

A = Time course for BDNF effects on DA levels in the CA1. The onset and magnitude of this response was similar to the GLU response to BDNF (see Fig. 1A), with approximately 200% elevation of basal DA levels, beginning 15 min after BDNF and lasting approximately 45 min. B = Area under the Curve (AUC) analysis bargraph. BDNF elicited a significant increase in DA levels, with the highest dose (0.3 μ g) giving rise to the greatest increase (indicated by *). Reverse dialysis of TTX gave rise to a reduced and also delayed response, and low calcium as well as the TrkB inhibitor K252a both significantly decreased DA levels, suggesting a TrkB- and calcium-dependent response.

Fig. 3. Effects of NGF on GLU neurotransmission

A = Time course for alterations of GLU levels in response to NGF administered via reverse dialysis. NGF administration gave rise to a bi-phasic GLU response, with the first and smaller peak occurring at 15 min after the onset of NGF administration, and the second and largest peak occurring at 60 min post-injection. The second peak represented a more than 300% increase in GLU levels, which was the strongest response observed in the present study. Low calcium and TTX both ablated both of the peaks, suggesting a calcium-dependent neuronal response. B = Area under the Curve (AUC) analysis of the NGF response. AUC analysis revealed a dose-dependent relationship for NGF effects on GLU, with 0.3 μ g BDNF giving rise to the highest GLU levels ($p < 0.05$) (indicated by *) and the lowest dose not significantly

different from the baseline levels (ns). In addition, TTX and low calcium were not significantly different from the baseline levels, suggesting a complete block of the NGF effects.

Fig. 4. Effects of NGF on DA neurotransmission

A = Time course for NGF effects on DA levels in the hippocampus CA1. The magnitude and time course for the response were both similar to those observed in DA levels following a BDNF administration (see Fig. 2A). Contrary to what was observed with NGF administration on GLU levels (Fig. 3A), the DA response to NGF was not bi-phasic and lasted until about 50 min after the onset of NGF administration. B = Area under the Curve (AUC) analysis of DA levels. NGF gave rise to a significant elevation of DA levels, with the highest dose (0.3 μg NGF) representing the highest increase ($p < 0.05$) (indicated by *). There was a dose-response relationship also for DA levels after NGF administration, and the response to the lower doses of NGF were much greater than what was observed with BDNF administration (Fig. 2B).

Reverse dialysis of TTX or low calcium both blocked this effect, and neither TTX nor low calcium yielded levels that were different from baseline levels, indicating a partial block of NGF effects.