The Requirement of BDNF for Hippocampal Synaptic Plasticity Is Experience-Dependent

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ABSTRACT: Brain-derived neurotrophic factor (BDNF) supports neuronal survival, growth, and differentiation and has been implicated in forms of hippocampus-dependent learning. In vitro, a specific role in hippocampal synaptic plasticity has been described, although not all experience-dependent forms of synaptic plasticity critically depend on BDNF. Synaptic plasticity is likely to enable long-term synaptic information storage and memory, and the induction of persistent (>24 h) forms, such as long-term potentiation (LTP) and long-term depression (LTD) is tightly associated with learning specific aspects of a spatial representation. Whether BDNF is required for persistent (>24 h) forms of LTP and LTD, and how it contributes to synaptic plasticity in the freely behaving rodent has never been explored. We examined LTD, and related forms of learning in the CA1 region of freely dependent mice that have a partial knockdown of BDNF (BDNF+/−). We show that whereas early-LTD (<90 min) requires BDNF, short-term depression (<45 min) does not. Furthermore, BDNF is required for LTP that is induced by mild, but not strong short afferent stimulation protocols. Object-place learning triggers LTD in the CA1 region of mice. We observed that object-place memory was impaired and the object-place exploration failed to induce LTD in BDNF−/− mice. Furthermore, spatial reference memory, that is believed to be enabled by LTP, was also impaired. Taken together, these data indicate that BDNF is required for specific, but not all, forms of hippocampal-dependent information storage and memory. Thus, very robust forms of synaptic plasticity may circumvent the need for BDNF, rather it may play a specific role in the optimization of weaker forms of plasticity. The finding that both learning-facilitated LTD and spatial reference memory are both impaired in BDNF−/− mice, suggests moreover, that it is critically required for the physiological encoding of hippocampus-dependent memory. © 2015 The Authors Hippocampus Published by Wiley Periodicals, Inc.

KEY WORDS: LTP; LTD; object recognition; CA1; enrichment

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that is important for hippocampal function. Aside from its mediation of neuronal survival and differentiation (Zagrebelsky and Korte, 2014), it has been proposed that activity-dependent secretion of BDNF may support synapse-specific synthesis of proteins that are required for the stability of long-term forms of synaptic plasticity (Park and Poo, 2013). Synaptic plasticity in the hippocampus, in the forms of long-term potentiation (LTP) and long-term depression (LTD), is believed to comprise the primary cellular mechanism underlying long-term spatial memory (Kemp and Manahan-Vaughan, 2007). Several studies, where synaptic plasticity was examined in the hippocampal slice preparation, indicate that manipulations of BDNF signaling, or genetic knockdown of BDNF result in significant impairments of LTP (Korte et al., 1995, 1998; Chen et al., 1999; Pozzo-Miller et al., 1999; Patterson et al., 2001) and LTD (Novkovic et al., 2015).

The requirement of BDNF for hippocampal LTP elicited in vitro is tightly dependent upon the afferent stimulation parameters used to induce it, and thus, could be considered to be experience-dependent in the context of stimulus pattern/repetition, or stimulus strength used for induction of LTP (Kang and Schuman, 1995; Korte et al., 1998; Patterson et al., 2001; Chen et al., 2004; Sakata et al., 2013). Less is known about hippocampal LTD. In the postnatal visual cortex of rats in vitro, LTD is blocked by application of BDNF (Kinoshita et al., 1999), and in the visual cortex of adult freely behaving rats blockade of the Trk-B receptor, for which BDNF is a ligand (McKay et al., 1996), prevents the intrinsic fluctuations in the strength of field potentials that occur throughout the diurnal cycle (Tianov and Manahan-Vaughan, 2007). In hippocampal slices, application of BDNF impairs LTD (<60 min) in the CA1 region that is elicited with 1 Hz low-frequency stimulation (LFS), but has no effect on stronger LTD that is elicited with 10 Hz stimulation (Ikegaya et al., 2002). These studies, with regard to both LTP and LTD, suggest that BDNF may play a particular role in the stabilisation of less robust forms of synaptic plasticity.

These findings are also particularly striking in light of the reported role for BDNF in hippocampus-
dependent memory. Here, too, its contribution appears to be experience-dependent. In conditional Trk-B knockout mice, spatial learning in the water maze is impaired, although spatial learning in the 8-arm radial maze is largely unaffected (Minichiello et al., 1999). A graded effect in avoidance learning was also reported, whereby passive avoidance learning was unaltered in Trk-B knockout mice, but active avoidance learning was intact, albeit poorer than in wildtype controls (Minichiello et al., 1999). In mice in which promoter-IV of the BDNF gene is disrupted, whereby hippocampal BDNF expression, but not basal BDNF levels, is impaired, spatial learning in the water maze, fear conditioning, and working memory are intact, but extinction of context-dependent fear memory is impaired (Sakata et al., 2013). Thus, the requirement for BDNF in hippocampus-dependent memory may be learning event-related.

Synaptic plasticity and hippocampus-dependent memory may comprise one and the same activity-dependent phenomenon, and the requirement of BDNF for both may thus relate to the history and/or precise nature of synaptic changes within the encoding synapses. Long-term hippocampus-dependent memory is tightly associated with the expression of persistent forms of LTP (Kemp and Manahan-Vaughan, 2004; Whitlock et al., 2006; Nabavi et al., 2014) and LTD (Kemp and Manahan-Vaughan, 2004; Erkin et al., 2006; Goh and Manahan-Vaughan, 2013a), whereby LTP and LTD may, on the one hand, encode very specific memory forms: for example, evidence exists that context-dependent fear memory is encoded by LTP (Whitlock et al., 2006; Nabavi et al., 2014), whereas object-place memory is encoded by persistent (>24h) LTD (Goh and Manahan-Vaughan, 2013a). On the other hand, LTP and LTD may interact together to form complex spatial representations (Kemp and Manahan-Vaughan, 2004, 2007; Hagen and Manahan-Vaughan, 2012).

The forms of LTP and LTD that are associated with the acquisition and retention of spatial memory last for days and weeks (Manahan-Vaughan and Brauneewell, 1999; Kemp and Manahan-Vaughan, 2004). To our knowledge, all studies thus far, that have explored the role of BDNF in hippocampal synaptic plasticity, have been conducted in the form of acute studies using the hippocampal slice preparation. The role of BDNF in LTP has thus been followed for maximally 5 h after induction (Patterson et al., 2001). Furthermore, the frequency-dependency of LTP and LTD has been described in vivo, does not reflect the frequency-dependency of LTP and LTD in vivo (Buschler and Manahan-Vaughan, 2012), particularly in the case of mice, in which all of the studies of the effects of transgenic BDNF modifications, to date, have been conducted. In freely behaving mice, the frequency-range with which persistent (>24 h) LTP can be induced is extremely narrow (Buschler and Manahan-Vaughan, 2012) and late-LTD cannot be induced by afferent stimulation alone (Buschler and Manahan-Vaughan, 2012; Goh and Manahan-Vaughan, 2013a,b).

In the present study, we therefore explored the involvement of BDNF in LTP and LTD that occurs in the CA1 region of the freely behaving mouse. We observed that BDNF is particularly involved in forms of synaptic plasticity that endure for less than 4 h in vivo. Furthermore, we observed that not only is object-place memory impaired in BDNF+/− mice, but the typical triggering of LTD through object-place learning (Goh and Manahan-Vaughan, 2013a) is absent. Furthermore, spatial reference memory that is linked to LTP (Morris et al., 1986) is also impaired.

Taken together, these data suggest that the requirement of BDNF for synaptic information storage may be very tightly related to the conditions and nature of the information and experience to be encoded. We propose that the contribution of BDNF is particularly important under conditions where spatial learning is tightly related to encoding through LTP and LTD.

### MATERIALS AND METHODS

This study was carried out in accordance with the European Communities Council Directive of September 22nd 2010 (2010/63/EC) for care of laboratory animals and after approval of the local government ethics committee (Bezirksamt, Arnsberg). All efforts were made to minimize the number of animals used.

### Animals

The BDNF+/− mouse strain we used, was originally established by Korte et al. (1995). On one allele the BDNF protein-coding exon was replaced by a neomycin-resistant gene surrounded by a glyceral kinase gene promoter and a polyadenylation signal. The neomycin resistant gene served as a marker. This replacement leads to a deletion of most of the mature BDNF coding sequences. To produce wildtype and heterozygous BDNF (+/−) mice, mutant BDNF +/− male mice were crossed with wildtype C57BL/6 female mice. This was done to avoid for abnormalities, such as e.g. retarded growth and neuronal loss, which were observed in homozygote (−/−) BDNF mice. Polymerase chain reaction (PCR) from tail tissue was used to detect the transgene. PCR primers were used to identify BDNF (5′-AAC ATA AGG CGG ACT TGT AC-3′) and neomycin (5′-GAT TCG CAG CGC ATC GCC TT-3′). Three weeks after birth, newborn mice were separated from their mother. Only male mice were used for the study, and were housed individually and in a separate room from female mice.

### Surgery

Male mice (7–8 weeks old) were anaesthetized (sodium pentobarbital 60 mg/kg, i.p.) and underwent stereotaxic chronic electrode implantation into the hippocampus of the right hemisphere, as described previously (Goh and Manahan-Vaughan, 2013b). The coordinates for the stimulating electrode comprised: anterioinferior (AP): −2.0 mm; mediolateral (ML): 1.4 mm from bregma; dorsoventral (DV): ~1.2 mm.
Measurement of Evoked Potentials

The field excitatory postsynaptic potential (fEPSP) was employed as a measure of excitatory synaptic transmission in the CA1 region. To obtain these measurements, an evoked response was generated in the Schaffer collaterals at low frequency (0.025 Hz) with single biphasic square waves of 0.2 ms duration per half-wave, generated by a constant current isolation unit. This signal was amplified using a differential AC amplifier and digitalized through a data acquisition unit. For each time-point measured during the experiments, 5 consecutively-evoked, fEPSP responses, obtained at 40 s intervals, were averaged. The first six time-points, which were obtained at 5 min intervals, were averaged and all time-points were expressed as a mean percentage (± standard error of the mean) of this value. Patterned afferent stimulation, or the behavioral task (when appropriate), was applied immediately after the sixth time-point and synaptic transmission was recorded for the subsequent 4 h (240 min). A further 1 h of recordings was performed the next day, roughly 24 h after the experiment began, to determine the degree of persistence of any changes in synaptic strength. The fEPSP was quantified by measuring the slope obtained on the first negative deflection of the evoked potential. By means of an input-output curve determination conducted before every experiment, the largest obtainable fEPSP was found for each individual animal (maximum intensity used 150 μA). The intensity that elicited 40% of the maximum fEPSP was used for recordings. Electroencephalography (EEG) activity was monitored throughout the course of the experiment for the occurrence of seizure activity. No behavioral changes, or EEG activity, indicating seizures were observed.

To verify the stability of the recordings, all animals were first tested in a "baseline" experiment where fEPSPs were evoked by test-pulse stimulation over a monitoring period that was equivalent to plasticity experiments. Animals that exhibited evoked responses that did not deviate by more than 10% of the baseline reference value were included in the subsequent plasticity experiments. To induce synaptic depression two different low frequency protocols (LFS) were used. Both protocols were applied at a frequency of 1Hz and consisted of 900 pulses delivered as paired-pulses (PP) with a PP-interstimulus interval of either 25ms or 50ms. Where LTD was explored in the context of synaptic depression that endured for 1–3 hours as early(E)–LTD, in line with the terminology proposed for LTD by Frey et al (1993). Short-term depression (STD) was defined as synaptic depression that endured for less than 45min.

Spatial Object Recognition Protocol

We used an object-place recognition task to assess for spatial recognition (SOR) memory. This type of learning is strongly associated with the expression of hippocampal LTD in mice (Goh and Manahan-Vaughan, 2013a). The protocol was previously described by Goh and Manahan-Vaughan (2013a). After 30 min of baseline recordings, mice were exposed to two novel objects for 10 minutes that were removed from the recording chamber after the presentation. During this time evoked potentials were recorded from the CA1 region. One day later the animals were re-exposed to the same objects in the same spatial locations, and evoked potentials were monitored anew. A further one day later, the same objects were presented to the animals whereby the position of the objects was changed. Here again, evoked potentials were monitored.

Exploration of the objects was t analyzed post-hoc using the within-object area scoring system which was defined as sniffing of the object (with nose contact or head directed to the object) within ~2 cm radius of the object (Bevins and Besheer, 2006; Goh and Manahan-Vaughan, 2013a). Object exploration time was expressed as a percentage of the total exploration time (Clarke et al., 2010; Goh and Manahan-Vaughan, 2013a). The results across animals were expressed in terms of mean ± s.e.m. The data were then statistically assessed using the Student’s t-test by comparing group means with the fixed value of 50%, which represents no differentiation between objects. The significance level was set at P < 0.05 (Clarke et al., 2010).

Spatial Reference Memory Test

Spatial reference memory may be enabled by hippocampal LTP (Morris et al., 1986). Here, we used the ‘Cookie finding test’ as described by Prochnow et al. (2012). This paradigm minimizes stress in the mice and capitalizes on their innate ability to locate and remember food locations. During the initial learning phase, each animals was trained to find a cookie that was concealed under the bedding of a large animal box. One
wall of the cage contained a constant visual cue card. The mice were trained in seven consecutive training trials. Two trials were performed per day separated into a morning and an afternoon session whereby on day 4 the animals performed a training trial during the morning session and the test trial during the afternoon session. In all training trials a cookie (1 g) was hidden 4 cm beneath the bedding. The size of the cookie was gradually reduced during the learning phase and in trials 6 and 7 the cookie was replaced by mouse chow, to minimise a potentially additional olfactory component to localisation of the reward. The cookie was always hidden at the same position and the mice were allowed to seek the treat during a 10 min time period. The time-point at which the mouse first held the cookie/chow in his front paws was defined as the time to find the goal. After the last training trial, a probe test was conducted whereby mouse exploration was recorded in the absence of a food reward. The exploration time was limited to 1 min. The movement of the animals were recorded with a camera mounted above the cage and tracked with TSE VideoMot2 (TSE systems). The distance travelled by the animal to the precise (former) location of the food was used to assess the spatial memory of the mice.

Data Analysis

The genotype of each mouse was analyzed by PCR prior to analysis of the data. To analyze electrophysiological data a factorial analysis of variance (ANOVA) with repeated measures was used. This was followed by a post hoc Fisher LSD test. To assess the differences in synaptic plasticity that derived from the afferent stimulation protocols, potentials evoked after HFS or LFS were compared. The study was conducted in an "experimenter-blind" manner.

During the SOR experiments, total amount Exploration time was analyzed by using the within-object area scoring system, as described above. The data were expressed as total amount of exploration in seconds and were plotted across animals in terms of mean ± SEM. For statistical analysis a student’s t-test was used.

In the spatial reference memory experiments, ANOVA with repeated measures was used to assess differences in performance between the two animal groups during the 7 days of task acquisition. A Student’s t-test was used to assess differences in distance travelled by the animals to the goal location, or in animal velocity, during the probe trial.

In all cases and the significance level was set to $P < 0.05$.  

RESULTS

Early Long-Term Depression, but Not Short-Term Depression Is Impaired in Freely Behaving BDNF$^{+/-}$ Mice

Recently we reported that in an in vitro hippocampal slice preparation, LTP as well as LTD are impaired in BDNF
partial-knockout mice (BDNF$^{+/−}$) (Novkovic et al., 2015). Here, we explored to what extent plasticity is altered in freely behaving mice of the same transgenic strain. We compared LFS-induced synaptic depression in wild-type and BDNF$^{+/−}$ mice. We used two different low frequency stimulation (LFS) protocols, whereby 1Hz LFS (900 pulses) was applied with a PP-interstimulus interval of either 25 ms or 50 ms.

Wild-type mice ($n = 7$) responded to LFS given at the 25 ms interval with STD that persisted for 45 min (Fig. 1A,C). Equivalent responses were elicited in BDNF$^{+/−}$ mice ($n = 6$). Thus, STD was significant in both wild-type (WT) (ANOVA...
F_{22,264} = 10.4928, P < 0.001) and BDNF\textsuperscript{+/-} mice (ANOVA F_{22,220} = 6.6576, P < 0.001) (Fig. 1A,C) compared with responses evoked by test-pulse stimulation only (not shown). The magnitude of depression lasted for \( \sim 45 \) min in both mouse cohorts (post hoc Fisher’s test: WT, \( P < 0.05 \) at \( t = 45 \) min; \( P = 0.120495 \) at \( t = 60 \) min; BDNF\textsuperscript{+/-} \( P < 0.05 \), at \( t = 45 \) min; \( P = 0.194973 \) at \( t = 60 \) min compared with test-pulse stimulated controls, \( n = 7 \), not shown). The profile of STD was equivalent in WT and BDNF\textsuperscript{+/-} mice (ANOVA F_{1,11} = 0.2634, \( P = 0.61791 \); Interaction effect F_{22,242} = 0.5201, \( P = 0.964448 \) compared with test-pulse stimulated controls, \( n = 6 \), not shown) (Fig. 1A,C).

Low frequency stimulation (LFS) of the Schaffer collaterals of freely behaving mice evoked leads at best to E-LTD in the CA1 region that lasts for maximally 90 min (Goh and Manahan-Vaughan, 2013b). LTD (>24 h) only results if afferent stimulation is coupled with novel learning about spatial content (Goh and Manahan-Vaughan, 2013a). Here, we assessed whether E-LTD that is elicited by LFS given at a 50 ms interval is affected by transgenic knockout of BDNF. WT animals (\( n = 7 \)) responded to LFS with E-LTD that lasted for \( \sim 90 \) min (Fig. 1B,D) (post hoc Fisher’s test: \( P < 0.05 \) at \( t = 75 \) min; \( P = 0.916589 \) at \( t = 90 \) min, compared with test-pulse stimulated controls, \( n = 7 \), not shown). By contrast, synaptic depression in BDNF\textsuperscript{+/-} mice was evoked by LFS (50 ms PP-interval) that lasted for maximally 10 min (Fig. 1B,D) (post hoc Fisher’s test, \( P < 0.01 \), \( t = 5 \) min; \( P = 0.05 \), \( t = 10 \) min; \( P = 0.216944 \), \( t = 15 \) min; \( n = 6 \)).

Furthermore, the synaptic plasticity profile induced by this LFS protocol in BDNF\textsuperscript{+/-} mice was significantly impaired compared with wild type mice (ANOVA F_{22,242} = 3.4318, \( P < 0.001 \) (Fig. 1B,D).

These data suggest that the dependency of synaptic depression on BDNF may specifically relate to forms of plasticity that require a more robust form of encoding. This begs the question as to whether forms of hippocampus-dependent learning that rely on LTD may also be affected.

**Spatial Object Recognition Memory Results in Persistent Synaptic Plasticity That Is Altered in BDNF\textsuperscript{+/-} Knockdown Mice**

When mice engage in spatial object recognition (SOR, also known as object-place recognition) during test-pulse stimulation of Schaffer collaterals, robust LTD is expressed in the CA1 region (Goh and Manahan-Vaughan 2013 a,b,c,d). We explored whether this type of learning-facilitated synaptic plasticity is altered in BDNF\textsuperscript{+/-} mice.

Firstly, animals were allowed to explore two novel objects during test-pulse stimulation of the Schaffer collaterals. In WT mice (\( n = 6 \)) this resulted in robust LTD that lasted for at least 24 h (Fig. 2A) (ANOVA F_{1,10} = 29.66; \( P < 0.001 \), compared with test-pulse stimulated controls that did not undergo object-exposure, \( n = 6 \) (Fig. 2A,C). By contrast, novel object exploration failed to elicit LTD in BDNF\textsuperscript{+/-} mice (Fig. 2D,F; \( n = 6 \)) (ANOVA F_{1,10} = 2.242; \( P = 0.165173 \)).

Re-exposure of WT mice (\( n = 6 \)) to the same objects in the same locations failed to trigger LTD (Fig. 2A,C) (ANOVA F_{1,10} = 1.97; \( P = 0.1950547 \), compared with WT animals that received test-pulse only, \( n = 6 \)). This aligns with previous reports that it is the novel learning about the objects that drives LTD (Goh and Manahan-Vaughan, 2013a), and this interpretation is supported by our observation that re-exposure to the objects was associated with a significant drop in exploration of the objects compared to novel exploration (Fig. 2B) (\( P < 0.05 \)). Testing under the same conditions also failed to significantly alter the profile of responses evoked in BDNF\textsuperscript{+/-} mice (\( n = 6 \), Fig. 2D,F) (ANOVA F_{1,10} = 0.225; \( P = 0.645332 \), compared with BDNF\textsuperscript{+/-} animals that received test-pulse only, \( n = 6 \)). Here, it was notable that assessment of exploratory behavior revealed that the level of object exploration during novel exposure and re-exposure was not significantly different in BDNF\textsuperscript{+/-} mice (Fig. 2E) (p=0.31099). This suggests that the failure to elicit LTD was associated with a failure to create a memory of the novel object-place experience.

In the past, it has been shown that the triggering of LTD by object exploration is dependent on object-place learning. Here, we first verified this by exposing WT mice to the familiar objects once more, whereby both objects had been placed in a new location. Here, test-pulse stimulation coupled with SOR resulted in de novo LTD in WT mice (Fig. 2A,B, \( n = 6 \) (ANOVA F_{1,10} = 14.605; \( P < 0.01 \), compared with WT animals that received test-pulse only, \( n = 6 \)). This response was coupled with a significant increase in exploration of the objects compared to re-exposure of the familiar objects in familiar locations (Fig. 2B) (\( P < 0.05 \)). Exploration levels were equivalent to this seen when the animal encountered the objects for the first time (Fig. 2B) (\( P = 0.08516 \)).

When the object-place relationship of the familiar objects was changed, BDNF\textsuperscript{+/-} mice expressed synaptic depression (Fig. 2D,E) (ANOVA F_{1,10} = 12.324, \( P < 0.01 \), compared with BDNF\textsuperscript{+/-} mice that received test-pulse only, \( n = 6 \)). Effects were highly unstable however, and did not differ from responses recorded during novel object exploration (Fig. 2D,E) (ANOVA F_{1,10} = 3.092, \( P = 0.109200 \)). Effects were paralleled by a tendency towards an increase in exploration levels compared to novel object exposure and object re-exposure (Fig. 2F). However effects were not significant (\( P = 0.31099 \) compared with novel object exploration, and \( P = 0.10768 \) compared with object re-exposure).

Examination of the individual exploration performances of the animals (scatter plot, Fig 2B,E), revealed that all WT animal exhibited the characteristic ‘V-pattern’ of higher-lower-higher exploration in the novel exposure vs. re-exposure vs. novel configuration conditions, regardless of the time spent with the objects during novel exposure, and in line with successful object-place learning. By contrast, only 2 (arguably 3) of the 6 BDNF\textsuperscript{+/-} mice tested showed the same evidence of successful learning. This indicates that the failure to elicit LTD was associated with a failure to create a memory of the novel object-place experience. The fact that two of the BDNF\textsuperscript{+/-} mice learned the object-place relationships and that the novel
configuration of the objects provoked a small and transient synaptic depression in the TG mice, reinforces the possibility that the involvement of BDNF in effective synaptic encoding and functional learning is experience-dependent.

Taken together, these data suggest that the impairment of learning-facilitated LTD seen in BDNF<sup>+/−</sup> mice is tightly coupled to deficits in spatial object recognition memory.

### The Afferent Stimulation Protocol Determines the Requirement of BDNF for LTP In Vivo

In the hippocampus in vitro, the BDNF-dependency of LTP is tightly dependent on the afferent stimulation protocol (Kang et al., 1997; Bramham and Messaoudi, 2005; Novkovic et al., 2015), whereby LTP elicited with strong afferent protocols is unaffected, but less robust LTP that elicited using TBS is impaired.

Here, application of high-frequency stimulation (HFS, 100Hz) to the Schaffer collaterals of freely behaving mice, led to significant LTP in WT mice that lasted for at least 24 h (Fig. 3A,D, n = 7 (ANOVA F<sub>1,10</sub> = 15.83, P < 0.01, compared with test-pulse stimulated controls, n = 7, not shown). The same stimulation protocol, when applied to BDNF<sup>+/−</sup> mice (n = 6) resulted in an equivalently robust LTP (Fig. 3A,D) (ANOVA F<sub>1,10</sub> = 63.87, P < 0.001). The profile of LTP elicited in BDNF<sup>+/−</sup> mice did not significantly differ from LTP evoked in WT mice (ANOVA F<sub>1,11</sub> = 0.2395, P = 0.634185).

These findings are in agreement with reports as to the BDNF-dependency of LTP in the mouse hippocampus in vitro.
responded to this TBS protocol with impaired LTP (Fig. 3C,F). Two mice were significantly impaired in their memory of the reward location of the (now absent food reward) was measured. BDNF was the same (Fig. 4C (p < 0.05)). These data indicate that BDNF+/- mice are impaired in spatial reference memory.

**DISCUSSION**

The findings of this study suggest that in behaving animal, the contribution of BDNF to information encoding in the form of synaptic plasticity, is graded and highly dependent on experience-related factors such as the stimulus pattern and the history of synaptic experience. We observed that BDNF is not required for robust (>24 h) LTP that is induced by means of high-frequency afferent stimulation at 100 Hz, but LTP that is induced using theta-burst stimulation (TBS) requires BDNF. This is in line with previous in vitro studies that reported that the requirement of BDNF for LTP induction is stimulus-dependent (Kang et al., 1997; Korte et al. 1998; Balkowiec et al., 2002). A different response profile was identified for synaptic depression: low-frequency stimulation (LFS) at 1Hz using.
paired stimuli given at 25 ms intervals, resulted in STD that was not impaired in BDNF+/− mice, whereas the same LFS protocol given at a stimulus-interval of 50 ms, resulted in (early)E-LTD that was impaired in BDNF+/− mice. This suggests that BDNF may be critically required for forms of synaptic plasticity that are elicited by mildly afferent stimulation protocols. This in turn indicates that the contribution of BDNF to synaptic plasticity is experience-dependent, whereby the pattern of incoming afferent stimuli, and perhaps even the behavioral state of the animal at the time-point of learning, determine whether BDNF is required for the synaptic plasticity that is induced. In line with this, we observed that LTD that is triggered by object-place learning (Goh and Manahan-Vaughan, 2013a) is prevented in BDNF+/− mice, as is object-place memory itself, and spatial reference memory, that has been linked to LTP (Morris et al., 1986).

The differences in the intervals between afferent stimuli were decisive with regard to the requirement of BDNF for the form of synaptic plasticity subsequently expressed. This may relate to the neurotransmitter receptor systems that are specifically activated by different interstimulus-intervals: typically, a triphasic pattern of paired-pulse responses to stimuli delivered at interstimulus-intervals ranging from 20 to 1000 ms, can be observed in vivo (Naie and Manahan-Vaughan, 2005). Short-latency intervals in the range of 10−40 ms result in transient synaptic depression that is most likely mediated by gamma-aminobutyric acid (GABA) receptors of the GABA_A subtype (Thiels et al., 1994). Intermediate intervals in the range of 40–300 ms reflect a selective N-methyl-D-aspartate receptor (NMDAR)-mediated response to further release of glutamate from presynapses (DiScenna; 1994), as well as activation of GABA_B receptors (Kahle and Cotman, 1993). Our observation that LTD that is elicited by LFS, given at 50 ms interstimulus-intervals, suggests that BDNF may modulate synaptic plasticity thresholds by influencing NMDAR and/or GABA_B-mediated effects.

The findings of this study have important implications for our understanding of how BDNF contributes to hippocampus-dependent information encoding, and suggest that experience that leads to very potent encoding by means of synaptic plasticity, may bypass the critical need for BDNF. This condition may arise in fearful situations. One-trial context-dependent fear conditioning triggers LTP-like changes in the hippocampus (Whitlock et al., 2006), and learning of this kind not only recruits encoding in the dorsal hippocampus (Misane et al., 2005), it also triggers LTP in structures such as the amygdala (Nonaka et al., 2014). It is not unreasonable to assume that this form of memory is encoded by intense afferent stimulation of hippocampal synapses, derived from a convergence of stimuli arising from the limbic, sensory and neuromodulatory systems. Fear conditioning is highly resistant to extinction (Monfils et al., 2009) and can thus be considered a very robust form of learning. Interestingly, in aged BDNF+/− transgenic mice, the acquisition of extinction memory is impaired (Psotta et al., 2013), whereas intracerebral infusion of BDNF supports extinction of condition fear (Peters et al., 2010). Although activation of Trk-B receptors by BDNF may be involved, in the abovementioned learning and memory processes, many other signaling pathways, including those mediated by activation of β-adrenoceptors, metabotropic glutamate receptors and calcium calmodulin kinase II (CAMKII) also contribute to triggering protein synthesis that underlies the consolidation and maintenance of memory (Johansen et al., 2011).

The emotional state of the animal may interfere with the requirement of BDNF in learning processes. BDNF+/− mice are impaired in learning a spatial task such as the water maze (Linnarson et al., 1997) that can be considered to be quite stressful for mice: swimming is not a typical/preferred mouse behavior. This finding is in contrast to our results that show that the BDNF+/− mice are indistinguishable from controls in the learning phase of the behavioral task we implemented in the present study. The genetic background of the mice may have impact on these results. Linnarson et al. used mice originating from 129/1 and BALB/c mice strains, whereas our mice had a C57Bl/6 genetic background. Different studies reported that the reactivity of dopaminergic neurons to stress is increased in BALB/c mice compared to C57Bl/6 mice (Hervé et al., 1979; Tassin et al., 1980). Since stress before a learning event impairs learning processes (Joëls et al., 2006), it is perhaps not surprising that this study showed different results than those obtained in our behavioral task. In line with the possibility that the backstrain of mice used in the Linnarson study could have contributed to the effects observed, aged-matched BDNF+/− mice, with the same C57Bl/6 background as ours, showed no learning impairment in a water maze task (Montkowski and Holsboer, 1997) whereas old BDNF+/− mice showed impaired spatial learning performances (Petzold et al., 2015).

This latter observation raises the additional question as to why BDNF+/− mice show no learning deficits in a water maze task, but showed effects in our spatial learning task. We put forward the proposal that our behavioral assay is much more precise. In the Montkowski and Holsboer study, the search time of the animals was assessed in a quadrant area that was twice the size of the (original) location of the target platform. Thus, any changes in specific knowledge of the platform location may have been overlooked. Furthermore, crossing the (target) quadrant was designated as goal-directed search behavior, and calculated as a number of crossings. The swim speed of the animals was not reported, thus the possibility that faster swimming animals could cross the target quadrant more often during the test-period, thereby confounding the data analysis, cannot be excluded. In our study we measured the distance traveled to the precise (very small, original) location of the food reward, and only assessed this single event (as opposed to the number of crossings of the general target area), which means that we were able to detect subtle changes in learning behavior. Thus, we would argue that our more precise assessment of spatial learning was able to detect differences in learning behavior in the BDNF+/− mice that may have been overlooked by a more general assessment of spatial learning behavior.
It was striking that during the task acquisition trials, BDNF\(^{+/−}\) mice performed as well as their wild-type counterparts. In both animal cohorts, the time taken to find the food reward steadily increased from day-to-day, indicating that the mice rapidly acquired the principles of the task. The deficits in learning behavior first became apparent during the probe trial. Their performance improvements from day to day suggest that they had learned the general location of the food reward. Their precision in finding it may have been supported by olfactory cues as they neared the location of the reward. However, in the absence of the reward, they failed to remember its precise location. This suggests that BDNF\(^{+/−}\) mice are impaired in the consolidation of their memory about the location of the food-reward, as opposed to an impairment in the acquisition of the task itself. This is consistent with reports by Lee et al. (2004), who showed that memory consolidation requires of BDNF.

We observed that BDNF is required for only specific forms of synaptic plasticity, and is particularly important for enabling plasticity to persist for longer periods. Furthermore, plasticity that is induced by milder forms of stimulation is particularly dependent on BDNF. We believe that these forms of synaptic plasticity may be much more representative of how experience is encoded under naturalistic conditions. Learning under non-fearful, or less-emotionally charged, conditions rarely happens as a one-trial event. Most forms of declarative or associative learning are cumulative (Karpicke et al., 2008), and acquired either based on the duration of exposure to the experience, or by repetitions of the experience (Buchler et al., 2011; Kilb and Naveh-Benjamin, 2011). Interestingly, novel spatial learning can result in very potent hippocampal synaptic plasticity in the absence of strong afferent stimulation. In mice, novel object recognition learning or novel object-place learning result in LTD that lasts for over 24h in mice that only received test-pulse stimulation of Schaffer collaterals during learning (Goh and Manahan-Vaughan, 2013a,b,c). This suggests on the one hand, that the kinds of afferent stimulation protocols that are traditionally used to induce LTP and LTD may actually be too potent to reflect how the synapse actually encodes a learning event. On the other hand, it suggests that quite mild, and seemingly weak afferent stimulation is sufficient to promote the encoding of very long-term spatial memories.

What is interesting in the context of BDNF is that weak but not strong LTP is impaired in BDNF\(^{+/−}\) mice (Novkovic et al., 2015). In hippocampal slices, LTD also requires BDNF, and strikingly, object-recognition memory that is so tightly associated with LTD induction (Goh and Manahan-Vaughan, 2013a) is also impaired in BDNF\(^{+/−}\) mice (Novkovic et al., 2015). In the present study we observed that this also extends to object-place memory. LTP may contribute to the encoding of spatial reference memory: both phenomena are prevented by antagonists of the N-methyl-D-aspartate (NMDA) receptor (Morris et al., 1986) and by knockout or antagonism of metabotropic glutamate receptors (Aiba et al., 1994; Lu et al., 1997). Strikingly, spatial reference learning in the water maze is impaired in BDNF\(^{+/−}\) mice (Minichiello et al., 1999) and was also impaired in a different spatial reference memory test in the present study. Furthermore, impaired spatial learning in the 8-arm radial maze occurs in BDNF\(^{+/−}\) mice (Minichiello et al., 1999) or when BDNF is inhibited (Mizuno et al., 2003). This form of learning is associated with both LTP and LTD (Bohme et al., 1993; Altinbilek and Manahan-Vaughan, 2007, 2009). This suggests that forms of LTP and LTD that relate to spatial and/or cumulative learning, and may be far more representative of what happens in nature, may be particularly dependent on BDNF.

Data acquired in in vitro studies suggest that the involvement of BDNF in hippocampal synaptic plasticity may be frequency-dependent (Korte et al., 1998; Chen et al., 1999; Patterson et al., 2001; Sakata et al., 2013). In the present study, however, we observed that in the behaving animals, it was not so much the frequency with which LTP or synaptic depression was induced, but rather the precise pattern with which the frequency was applied to hippocampal afferents that determined whether BDNF was required for the subsequently induced form of synaptic plasticity. Recent work already showed a stimulation pattern-dependent involvement of BDNF secretion in synaptic plasticity in slice experiments (Edelmann et al., 2015). In the context of naturalistic information encoding that occurs as result of experience, this makes a lot of sense. It is counterintuitive to expect that a spatial memory, with all the detail and complexity it entails, will be encoded by stereotypic, unvarying or constant afferent stimulation patterns, such as it typically used to induce LTP or LTD in experimental situations. During a learning experience, particularly one that does not happen instantaneously, fluctuations in intracortical information transfer in the form of neuronal oscillations (Tsanov and Manahan-Vaughan, 2009), variations in intrahippocampal network activity (Bikbaev et al., 2008), activation of neuromodulatory structures (Hansen and Manahan-Vaughan, 2014; Lemon et al., 2009) can all be expected to exert potent influences on the patterns of stimuli that converge on the hippocampal synapses that store that experience, even assuming that the afferent frequency remains relatively constant. This unique pattern is very likely to confer the property of uniqueness to a memory engram stored within a population of synapses that will be used again and again to store hippocampus-dependent memories. In line with this possibility, an identical number of stimuli given by means of different patterns of afferent activity have been reported to trigger distinct signaling pathways that underlie LTP induction (Kang et al., 1997). Furthermore, the pattern of afferent activity determines the dependency of the resulting potentiation on TrkB receptors or BDNF (Kang et al., 1997; Chen et al., 1999; Patterson et al., 2001).

The present study indicates that BDNF is required for LTD that is triggered by patterned afferent stimulation of behaving animals, and by object-place learning. Recently, it was also reported that LTD in the CA1 region in vitro requires BDNF (Novkovic et al., 2015). This is surprising given reports that others have reported that BDNF can inhibit LTD that was induced by LFS in hippocampal slices (Ikegaya et al., 2002). However, this effect was frequency-dependent: induction of

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LTD using higher afferent frequencies was not affected (Ikegaya et al., 2002). The inhibitory effect of BDNF on hippocampal LTD appears to be dose-dependent, whereby effects are most prominent when BDNF levels are strongly elevated (Rodrigues et al., 2014). BDNF is a ligand for the low affinity p75 receptor and the high-affinity Trk-B receptor (McKay et al., 1996). BDNF-dependent LTP appears to be tightly related to the activation of TrkB receptors (Kang et al., 1997; Xu et al., 2000), whereas LTD requires activation of p75 (Rösch et al., 2005). Interestingly, BDNF actions of p75 and TrkB receptors may mediate opposing effects on dendritic spine density and the relative proportion of immature and mature spines (Chapelleau and Pozzo-Miller, 2012). An interesting corollary of this is that LTD triggers restructuring in the synapses such that weakly active spines are eliminated (Wieghert and Oertner, 2013), whereas LTP triggers restructuring such that spine growth is initiated and supported (Bosch et al., 2002). However, under certain circumstances, p75 can act as a co-receptor for TrkB receptors and generate high affinity sites for TrkB activation (Benedetti et al., 1993). Thus, the relative degree and interplay of these receptors may depend on the degree of neuronal activity (Chapelleau and Pozzo-Miller, 2012). This in turn may influence the relative degree of expression of LTP and LTD during the encoding of spatial experience. In line with this, LTP and LTD can occur simultaneously at memory-relevant synapses (Solger et al., 2004). Furthermore the maintenance of BDNF within a homeostatic range is likely to be essential for the optimal encoding of memories through LTP and LTD (Novkovic et al., 2015). Accordingly, the unstable LTD that occurred during the object-place recognition test in BDNF+/- mice, gives a hint that the first two exposures of the objects in constant locations may have created a weak representation in the hippocampus, but that (in the absence of the required levels of BDNF) this was not sufficient to create a precise spatial memory. The high variability of evoked responses during the spatial learning tests, reflect an impoverished ability of the hippocampus of BDNF+/- mice to respond to this kind of spatial experience with LTD. Nonetheless, some weak encoding may have occurred that served as a template for detection of the object-place rearrangement during the third attempt to induce LTD. The fact that stable LTD did not result suggests that BDNF is critically required for this kind of encoding.

In conclusion, the findings of this study indicate that BDNF is not required for all forms of experience-dependent synaptic plasticity: the induction of robust LTP through high frequency stimulation of hippocampal afferents circumvents the need for BDNF, and BDNF is also not required for hippocampal STD that endures for less than 45min. By contrast, BDNF is required for LTP that is induced by TBS and for early-LTD. As different forms of LTP and of LTD are associated with different forms of hippocampus-dependent learning, this suggests that BDNF is required for specific, but not all, forms of hippocampus-dependent memory. We postulate that synaptic encoding that is triggered by very strong afferent activity, and may be related to very intense experience, bypasses the need for BDNF. However, synaptic information storage is mediated by an interplay of LTP and LTD, and thus requires delicate and fine-tuning of a synaptic network, and critically requires BDNF.

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