Impaired fear memory, altered object memory and modified hippocampal synaptic plasticity in split-brain mice

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Article:

The hippocampus is critical for memory formation. However, the contributions of the hippocampal commissure (HC) and the corpus callosum (CC) are less clear. To elucidate the role of the forebrain commissures in learning and memory, we performed a behavioural and electrophysiological characterization of an inbred mouse strain that displays agenesis of the CC and congenitally reduced HC (BTBR T^+ t*f*/*J*; 'BTBR'). Compared to a control strain, BTBR mice have severely impaired contextual fear memory, with normal object recognition memory. Interestingly, continuous environmental "enrichment" significantly increased object recognition in BTBR, but not in control C57BL/6 ('BL/6') mice. In area CA1 of hippocampal slices, BTBR displayed intact expression of long-term potentiation (LTP), paired-pulse facilitation (PPF) and basal synaptic transmission, compared to BL/6 mice. However, BTBR hippocampal slices show an increased susceptibility to depotentiation (DPT), an activity-induced reversal of LTP. We conclude that the HC and CC are critical for some forms of hippocampal memory and for synaptic resistance to DPT. Agenesis of the CC and HC may unmask some latent ability to encode, store or retrieve certain forms of recognition memory. We suggest that the increased susceptibility to DPT in BTBR may underlie the memory phenotype reported here.

Keywords: Hippocampal commissure, Corpus callosum, Memory, Synaptic plasticity, LTP, Mouse strain

Article:

1. Introduction

Systematic surveys of multiple inbred strains have identified extreme phenotypes that can enhance our understanding of brain function. Unique among all mouse strains studied to date (Wahlsten et al., 2003b), the BTBR T^+ t*f*/*J* ('BTBR') strain always lacks a corpus callosum (CC) and has severely reduced hippocampal commissure (HC). Two X chromosome regions contribute to the anatomical defect of the BTBR forebrain (Kusek et al., 2006). Although there is a significant but small increase in the number of unmyelinated axons in the anterior commis-sure when the CC is absent (Livy et al., 1997), this compensatory increase is dwarfed by the massive loss of connectivity between the hemispheres in BTBR mice.

Here we report the first electrophysiological study of the hippocampus in these animals and find that most measures are remarkably normal. On two very different tests of mouse memory, BTBR mice show substantial deficits in contextual fear conditioning but not object recognition memory. These findings indicate that the commissural system is not important for most aspects of normal ipsilateral function of the hippocampus but may nonetheless be involved in the formation of certain kinds of long-term memories in these mice.

Table 1 – Summary of contextual fear memory in BL/6 and BTBR

	BL/6	BTBR	*p*
Pre-CS	2±1	0.3±0.2	<0.05
Post-US	15±3	8±2	<0.05
1 h	29±6	8±2	<0.01
24 h	46±4	5±1	<0.001
48 h	26±4	2±1	<0.001

All values are mean±SEM percentage freezing. *n* values for each group are given in Fig. 1C. *p* values are derived from Student's *t* test. Significance was considered $p<0.05$.

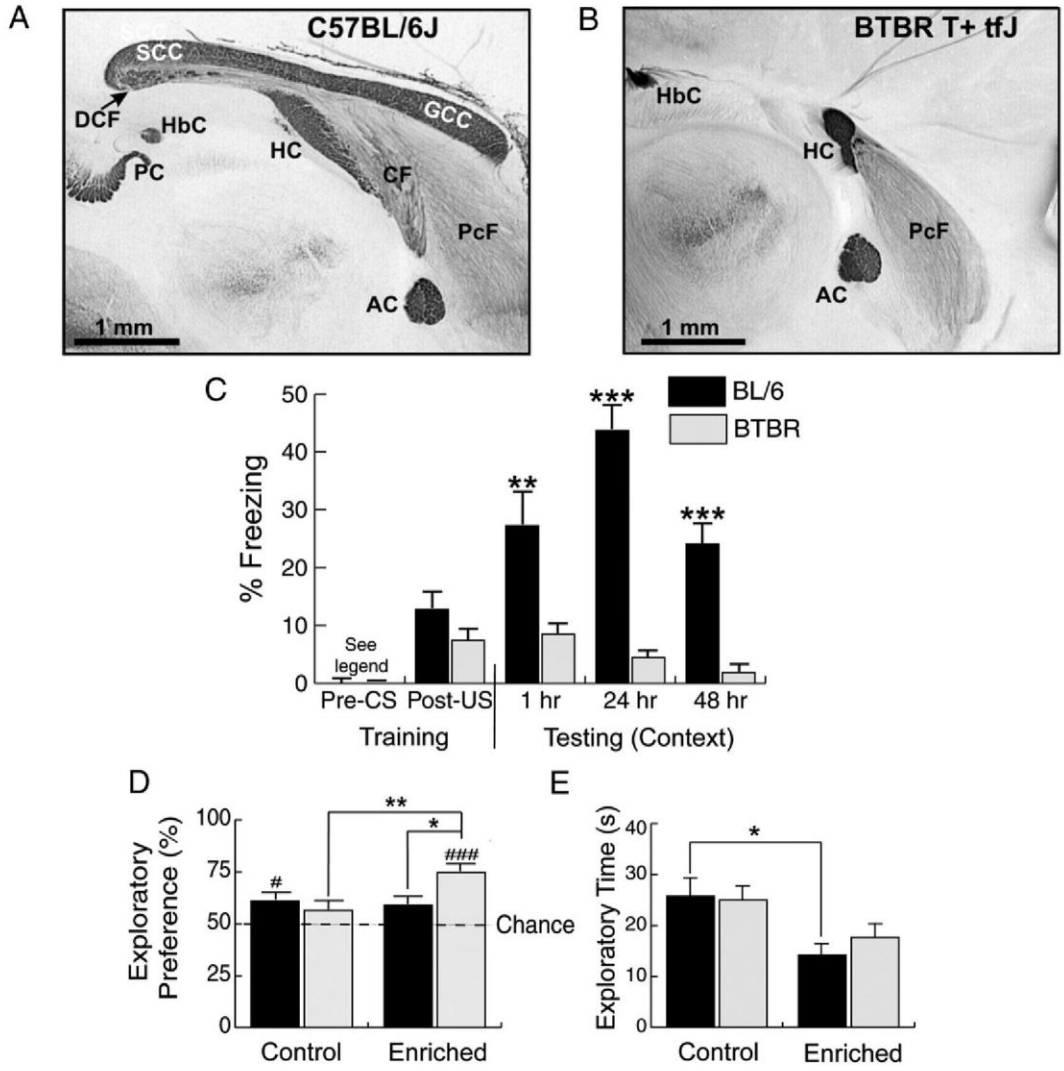

Fig. 1 – Brain morphology and behavioural phenotype of BTBR and BL/6. A and B, sagittal sections showing brain morphology of normal and acallosal mice. A, BL/6: the corpus callosum extends rostrocaudally along the midline of the brain. B, BTBR: there is no corpus callosum and the hippocampal commissure is severely reduced in size. Most of the 58 BTBR brains in this study had an HC considerably smaller than the one shown in B. Abbreviations: AC, anterior commissure; CF, columns of the fornix; DCF, dorsal commissure of the fornix; GCC, genu of the CC; HC, hippocampal commissure; HbC, habenular commissure; PC, posterior commissure; PcF, precommissural fornix; SCC, splenium of the CC. C, BTBR mice have deficient short- and long-term contextual fear memories. Pooling all training intervals in C, BL/6 ($n=53$) froze slightly, but significantly, more than BTBR ($n=41$) both before the tone (*Pre-CS*) and after the shock (*Post-US*). In order to compensate for the difference in Pre-CS freezing, the Pre-CS freezing value was subtracted from each time interval presented in C (see 'Behavioural analysis'). The original data are presented in Table 1. Following training, BL/6 displayed robust contextual fear learning at all intervals whereas BTBR showed only modest learning. BTBR ($n=10$) froze substantially less than BL/6 ($n=11$) at 1 h. BTBR ($n=21$) was similarly impaired at 24 h compared to BL/6 ($n=31$). BL/6 ($n=11$) displayed greater freezing behaviour than BTBR ($n=10$) at 48 h as well. D and E, Novel object recognition testing. D, 8 days of continuous environmental "enrichment" (EE) enhance object recognition memory at 24 h in BTBR but not in BL/6. No significant difference in object recognition existed between BL/6 and BTBR in the absence of the modified "enrichment" protocol. BTBR mice showed a heightened recognition of novel objects following "enrichment" whereas the performance of BL/6 was not significantly altered. An exploratory preference of 0.50 indicates chance performance. # denotes a significant elevation above chance performance (# $p<0.05$; ## $p<0.01$; ### $p<0.001$). D, Effect of continuous "enrichment" on exploratory time in BL/6 and BTBR. Without "enrichment", BL/6 and BTBR had nearly identical levels of total exploratory time. After 8 days of continuous "enrichment", there was no significant difference between the exploratory time of BL/6 and that of BTBR. In both strains, "enrichment" seemed to attenuate exploratory behaviour. In BL/6, "enrichment" resulted in a significant decrease in exploratory time. A similar trend was observed in BTBR though the difference was not significant. Scoring of exploratory behaviour is described above. Asterisks in C indicate significance values derived from Student's t test, asterisks in D and E are significance values derived from *post-hoc* Tukey testing (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Clinically, CC agenesis in humans is associated with mental retardation, developmental delay, cerebral palsy, and schizophrenia (Serur et al., 1988; Motomura et al., 2002; Chinnasamy et al., 2006). CC size in humans show both X-linked and autosomal patterns of inheritance, and CC development depends on a number of cellular and molecular mechanisms (Richards et al., 2004). The hippocampal commissure (HC) connects the two hippocampi, structures that are critically involved in memory (Milner et al., 1998; Andersen et al., 2007). Somewhat surprisingly, patients with transection of the CC display relatively normal memory function (Ledoux et al., 1977; for review, see Clark and Geffen (1989)). It has been reported that patients whose corpora callosum and hippocampal commis-sure have been surgically sectioned show deficits in short-term memory (Zaidel and Sperry, 1974) as well as in recall memory (Phelps et al., 1991). Additionally, individuals with callosal agenesis show some specific memory deficits (Finlay et al., 2000). Therefore, the integrity of the CC and HC seem to be required for normal memory function in humans. Interpretation of the human data is difficult, however, because studies of callosal agenesis often use subjects with other serious brain abnormalities or comorbid conditions (Bloom and Hynd, 2005). Furthermore, electrophysiological study of hippocampal function in humans is currently not feasible. Hence, a mouse model may help to elucidate the role of the CC and HC in memory.

2. Results

2.1. Histology

No BTBR T^+ t*f*/*J* brain had any CC present at midplane (Fig. 1B) and only 1 of 58 brains had a HC of normal adult size. Most BTBR brains had either no detectable HC or a commissure that was greatly reduced in size to less than 25% of the normal cross-sectional area.

2.2.Behavioural analysis

2.2.1. BTBR shows impaired fear memory at all intervals

Are callosal and commissural inputs required for fear learning? Contextual fear conditioning depends on the hippocampus (Kim and Fanselow, 1992; Chen et al., 1996) and the amygdala (Phillips and Ledoux, 1992; Kim et al., 1993).

The contextual fear data are summarized in Table 1 and discussed below. In the present study, BTBR mice displayed deficient short-term and long-term memories for contextual fear (Fig. 1C). In the fear conditioning chamber, BL/6 froze slightly, but significantly, more than BTBR both before the tone (Table 1, Pre-CS; $p < 0.05$) and after the shock (Post-US; $p < 0.05$) when all data from Table 1 were pooled. BTBR froze significantly less than BL/6 when re-exposed to the training chamber at 1 h for a short-term memory test ($p < 0.01$). Long-term fear memory was also impaired in BTBR at 24 h ($p < 0.001$) as well as at 48 h ($p < 0.001$). Whereas the BL/6 displayed robust contextual fear learning, BTBR mice displayed only very modest levels of freezing at these time points.

The difference in Pre-CS values suggests that there might be a slight difference in activity level or anxiety behaviour between the two strains. In order to compensate for this potential confound, we subtracted the Pre-CS freezing values for each strain from subsequent intervals as in Schimanski et al. (2002). This correction is shown in Fig. 1C. Following this correction, BL/6 still froze significantly more than BTBR at 1, 24 and 48 h, but there was no significant difference between the Pre-CS and Post-US values. In fact, the significance levels of the testing intervals shown in Fig. 1C were unaltered by the correction. Additionally, Wahlsten et al. (2006) reported nearly identical open field activity levels for BL/6 and BTBR. Thus, the freezing deficits observed cannot be attributed to a difference in activity levels. BL/6 and BTBR also display similar behaviour in the elevated plus maze and do not differ significantly in thigmotactic behaviour in an open field test (D. Wahlsten, Mouse Phenome Database, www.jax.org/phenome). This suggests that there are no significant differences in anxiety levels between BTBR and BL6. We therefore conclude that BTBR shows marked deficits in contextual fear memory compared to BL/6.

2.2.2. Object recognition memory is spared in BTBR and continuous environmental "enrichment" enhances object recognition in BTBR but not in BL/6

The novel object recognition task is a one-trial memory test in rodents. In this test, memory of a familiar object is manifested as preferential exploration of novel objects. This task quantifies a naturalistic rodent behaviour in a non-stressful environment without primary reinforcing stimuli and is similar to visual recognition tests used in non-human primates (Ennaceur and Delacour, 1988). The neural substrates of the object recognition task have been reviewed elsewhere (see Dere et al. (2007)).

The object recognition results are summarized in Tables 2 and 3 and discussed below. One-way analysis of variance (ANOVA) showed that the four groups of mice presented in Fig. 1D and E differed significantly in terms of exploratory preference and exploratory time ($p < 0.05$ for both comparisons).

Table 2 – Summary of exploratory preference values in BL/6 and BTBR

	BL/6	BTBR
24 h	61±4 (*n*=9)	56±5 (*n*=10)
24 h EE	59±4 (*n*=10)	75±4 (*n*=10)

All values are mean±SEM percentage exploratory preference. Significant differences are indicated in Fig. 1D and discussed in the 'Behavioural analysis' section of the results. 'EE' denotes mice that received the continuous environmental "enrichment" protocol.

Table 3 – Summary of exploratory time values in BL/6 and BTBR

	BL/6	BTBR
24 h	26±4 s (*n*=9)	25±2 s (*n*=10)
24 h EE	14±2 s (*n*=10)	18±3 s (*n*=10)

All values are mean±SEM exploratory time. These data are presented in Fig. 1E and discussed in the 'Behavioural analysis' section of the results. 'EE' denotes mice that received the continuous environmental "enrichment" protocol.

What is the role of the CC and HC in object recognition memory? We found that BTBR and BL/6 displayed comparable but rather weak evidence of object recognition memory at 24 h in the absence of enrichment ($p > 0.4$; Fig. 1D). Therefore, intact HC and CC are not required for object recognition memory in mice. Compared to chance performance (hypothetical mean of 50%), BL/6 displayed significant object recognition memory at 24 h. In BTBR, there was a tendency towards exploration of the novel object at 24 h, but this did not reach significance ($p = 0.18$). Thus, both strains seemed to preferentially explore the novel object at 24 h, but only BL/6 performed significantly above chance.

Wild-type mice placed in a large, "enriched" environment for several hours each day display enhanced performance in the object recognition task (Tang et al., 2001). In contrast, we examined the effect of continuous environmental "enrichment" (EE) presented in the home cage for 8 days prior to training.

In BL/6, continuous environmental "enrichment" did not significantly alter performance in the object recognition task ($p>0.7$). The discrepancy between this result and other reports of increased object recognition memory following "enrichment" (e.g. Tang et al., 2001) is likely attributable to the marked differences in "enrichment" protocols used. The "enrichment" protocol used here presented mice with much less stimulation than many reports in the literature, over a longer period. Interestingly, BTBR displayed a marked enhancement of object recognition memory after "enrichment" (Fig. 1D). After "enrichment", BTBR had a significantly higher exploratory preference than the "enriched" BL/6 group ($p<0.05$). Continuous "enrichment" also significantly improved the object memory of BTBR mice from the non-"enriched" level ($p<0.01$). Additionally, "enriched" BTBR mice performed significantly above chance ($p<0.001$). In comparison, BL/6 mice seemed to preferentially explore the novel object, but this tendency did not reach statistical significance ($p=0.06$). Our environmental "enrichment" paradigm therefore selectively increased object recognition memory in BTBR, but not in BL/6.

Without continuous "enrichment", BTBR and BL/6 displayed similar exploratory times at 24 h ($p>0.8$). After 8 days of enrichment, both strains seemed to display an attenuation of exploratory time (Fig. 1E). The exploratory time of the "enriched" BL/6 group was significantly lower than their non-"enriched" counterparts ($p <0.05$). The tendency towards an attenuation of exploratory time in the "enriched" group was not significant in BTBR ($p>0.1$). Continuous exposure to novelty therefore seems to diminish the time spent exploring objects.

2.3. Electrophysiological analysis

Synaptic plasticity, including LTP, can regulate some forms of learning and memory in the mammalian brain (for reviews, see Bliss and Collingridge (1993), and Martin et al. (2000)). LTP is an activity-dependent enhancement of excitatory synaptic strength that is induced by high-frequency electrical stimulation (Bliss and Lømo, 1973). Recently, learning was demonstrated to induce LTP in vivo (Whitlock et al., 2006). We used various electrical stimulation protocols in the Schaeffer collateral (SC)-CA1 pathway of the hippocampal slices of BTBR mice using BL/6 slices as a control.

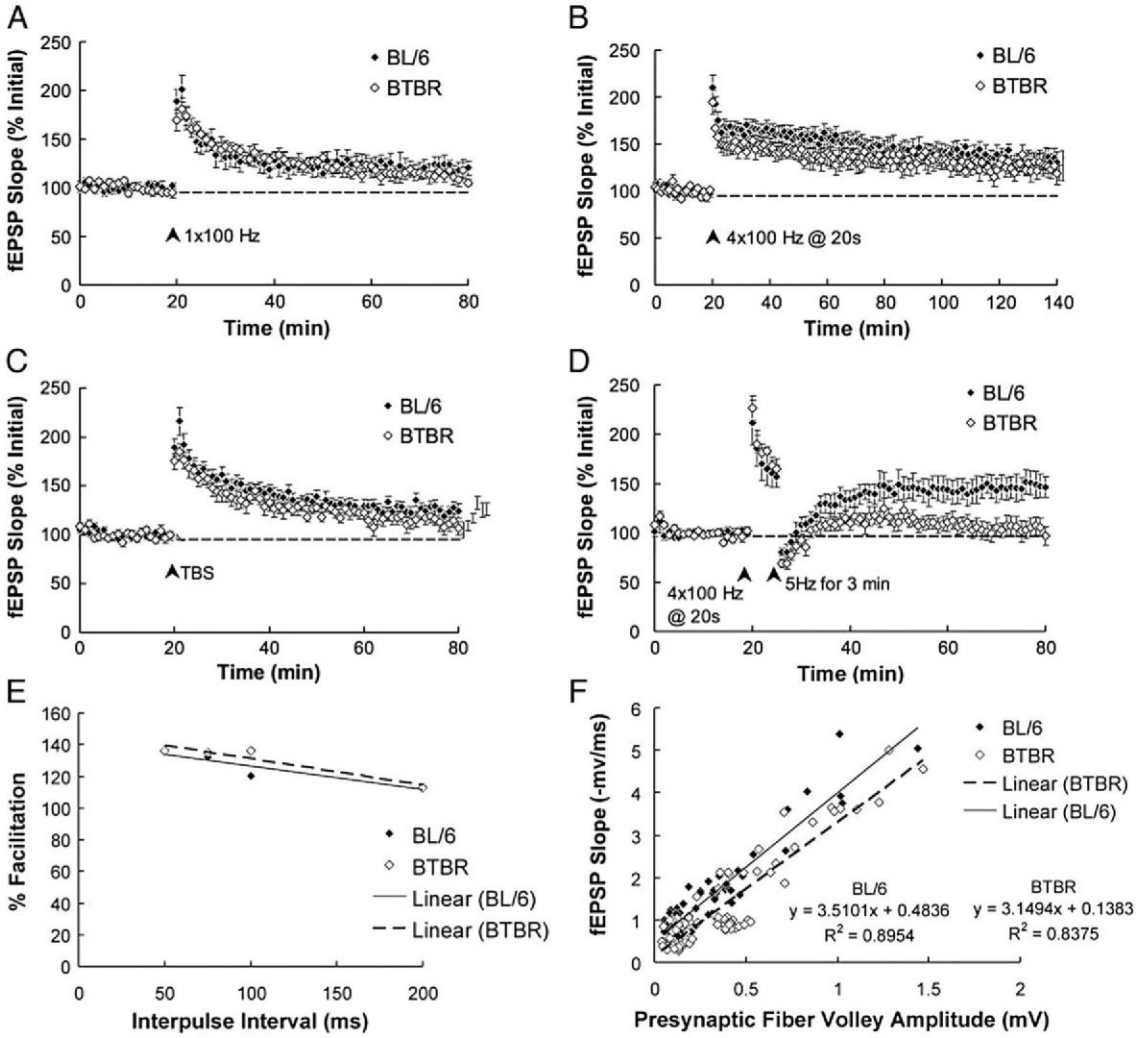

Fig. 2 – Hippocampal LTP, paired-pulse facilitation and input–output curve are normal in mice with a reduced HC, but resistance to DPT is impaired. A, Plot of fEPSP slope versus time, comparing averaged responses from BL/6 (*n*=7 slices) and BTBR (*n*=7 slices). LTP was elicited by one train of 100-Hz stimulation (1×100 Hz). B, Average responses from BL/6 (*n*=10 slices) and BTBR (*n*=7 slices) for four-train-induced LTP (4×100 Hz at 20 s). C, Average responses from BL/6 (*n*=12 slices) and BTBR (*n*=11 slices) for TBS-induced LTP (TBS). D, Average responses from BL/6 (*n*=7 slices) and BTBR (*n*=9 slices) for LFS-induced DPT (4×100 Hz at 20 s, followed by 5 Hz for 3 min). E, Paired-pulse facilitation results data. Plot of percentage of facilitation [ratio of fEPSP slopes: [(((pulse2/pulse1)−1)×100)]] versus interpulse interval for strains BL/6 (*n*=4 slices) and BTBR (*n*=3 slices). F, Input–output curves. Plot of fEPSP slope versus presynaptic fiber volley amplitude for BL/6 (*n*=3 slices) and BTBR (*n*=3 slices).

2.3.1. Three forms of LTP are intact in BTBR

In hippocampal slices, one train of HFS (100 Hz for 1 s duration) results in early-LTP (E-LTP). E-LTP decays to baseline within 1–2 h of induction, and requires activation of NMDA receptors (Collingridge et al., 1983) and certain protein kinases (Malinow et al., 1989; Silva et al., 1992), but not protein synthesis (Huang and Kandel, 1994). One-train HFS elicited very similar mean fEPSP slope values at 50 min after HFS in the two strains (118± 8%, for 7 C57BL/6 mice, 115±7% for 7 BTBR, p>0.7; see Fig. 2A).

Using four trains of HFS, late-LTP (L-LTP) can be induced (Huang et al., 1996). L-LTP can last for several hours in vitro (Andersen et al., 1977), and its maintenance requires activation of PKA (Huang and Kandel, 1994), transcription (Nguyen et al., 1994) and protein synthesis (Frey et al., 1988). At 110 min post-induction, mean fEPSP slope values were very similar (132 ± 9% in 10 C57BL/6, and 129±15% for 7 BTBR, p>0.9; see Fig. 2B).

Theta patterns of electrical activity (5–10 Hz) in the hippo-campus can occur during environmental exploration and behavioural conditioning (Green et al., 1960; Vanderwolf, 1969). The theta-burst stimulation (TBS) used in this study mimics these naturally occurring oscillations in the hippocampus. LTP evoked by TBS may be linked to the exploratory behaviour of rodents in spatially novel environments (Bland, 1986). Thus, TBS may be a more physiologically relevant experimental protocol than one-train or four-train HFS. In the present study, maintenance of theta-burst LTP was slightly less robust in the BTBR slices 50 min after LTP induction (C57BL/6 fEPSP slope 123±6%, n=12; BTBR fEPSP slope 113±7%, n=11; p>0.2; see Fig. 2C). Thus, an intact HC and CC are not required for normal E-LTP, L-LTP or theta-burst LTP.

2.3.2. Resistance to depotentiation is impaired in BTBR

Depotentiation (DPT) is the activity-dependent reversal of previously induced LTP. In vivo, rapid DPT can occur when behaving rats are placed in a novel environment following LTP induction, suggesting that DPT may be an electrophysiological correlate of some forms of novelty (Xu et al., 1998). E-LTP induced by HFS can be persistently erased by LFS (Barrionuevo et al., 1980; Staubli and Lynch,1990; Fujii et al., 1991). However, L-LTP (i.e. induced by four-train stimulation) will return to baseline immediately after LFS, but then recover to potentiated levels (Woo and Nguyen, 2003). This ‘resistance’ to DPT is dependent on protein synthesis. We induced L-LTP with four-train stimulation, and then applied LFS (5 Hz for 3 min). In BL/6 slices, mean fEPSP slopes recovered to pre-LFS potentiated levels at 50 min post-DPT (143±12%, n=7). In contrast, the fEPSP slopes in BTBR slices did not recover significantly above pre-HFS baseline (106±9%; n=9; cross-strain comparison p<0.03) (Fig. 2D). Agenesis of the HC and CC therefore results in enhanced susceptibility to DPT in area CA1.

2.3.3. Paired-pulse facilitation and synaptic transmission are intact in BTBR

Paired-pulse facilitation (PPF) is a type of short-term plasticity easily induced at hippocampal synapses. PPF is an enhancement of synaptic transmission during the second of two closely spaced stimuli (Katz and Miledi, 1968). Residual intracellular calcium from the first stimulus summates with the calcium influx for the second stimulus, resulting in an enhancement of transmitter release during the second pulse (Katz and Miledi, 1968). We examined PPF in the SC-CA1 pathway synapse using interpulse intervals of 50, 75, 100 and 200 ms. There was no significant difference in PPF between the two strains at any time interval (Fig. 2E; p>0.2 in all comparisons). Thus, an intact HC and CC are not required for PPF.

It may be that a reduced HC or CC alters basal synaptic input-output relationships. To investigate this possibility, we measured the presynaptic fiber volley amplitude and initial fEPSP slope from single fEPSP sweeps in the SC-CA1 pathway, using a range of stimulation intensities. The fiber volley size is proportional to the number of presynaptic axons recruited by stimulation, and the initial fEPSP slope is a measure of synaptic strength (Johnston and Wu, 1995). We used linear regression analysis to plot a line of best fit for each strain (Fig. 2F). A positive slope indicates that postsynaptic responses increase in tandem with presynaptic stimulation. The slopes generated for the two strains were not significantly different from each other (C57BL/6: $y = 3.51\times+0.5$, $R^2=0.90$; BTBR: $y = 3.15\times+0.1$, $R^2= 0.84$; p>0.1). Thus, the lack of HC and CC in BTBR did not significantly affect basal hippocampal synaptic transmission and presynaptic fiber recruitment in the SC pathway.

Altogether, these findings indicate a remarkably normal hippocampal slice in BTBR mice that lack two major interhemispheric commissures. Only one of six electrophysiological indicators showed a deficit in BTBR compared with BL/6 mice.

3. Discussion

We investigated the effect of CC agenesis and HC reduction on synaptic plasticity and memory as a first step towards elucidating the roles of interhemispheric connections in memory. BTBR mice displayed severely impaired contextual fear memory but intact object recognition memory. Upon continuous environmental "enrichment", the BTBR strain displayed a marked enhancement of object recognition memory. Basal synaptic transmission, PPF, and LTP in BTBR mice were not significantly different from BL/6 mice.

We observed that BTBR mice lack the resistance to DPT that is characteristic of the late phase of LTP in BL/6 slices. We suggest that susceptibility to DPT may account for the contextual fear and object recognition results reported here. Synaptic resistance to DPT provides protection against LTP erasure, and LTP is thought to underlie hippocampus-dependent spatial memory (Morris et al., 1986; Moser et al., 1998). It has been speculated that resistance to DPT may help set the neural threshold for memory formation (Woo and Nguyen, 2003). If this is the case, the increased ease of LTP erasure in the hippocampi of BTBR mice would tend to impede memory formation. This would explain the impaired short- and long-term contextual fear memory.

In the absence of enrichment, BTBR and BL/6 exhibited similar levels of object recognition memory. Studies have found that gross hippocampal lesions (75–100% of hippocampal tissue) can impair object recognition at longer intervals (Broadbent et al., 2004; Ainge et al., 2006). It is therefore not surprising that HC reduction and CC agenesis would not result in evident deficits in this task.

A surprising finding of the present study is that an "enrichment" protocol emphasizing novelty enhances object recognition memory in BTBR but not in BL/6. This difference is unlikely to result from differences in exploratory behaviour, since the strains do not differ in exploratory time either with or without "enrichment". DPT has previously been correlated with spatial novelty (Xu et al., 1998) and environmental enrichment (Abraham et al., 2002) in behaving rodents. Interestingly, exploration of an empty novel environment induces LTP in vivo and subsequent exposure to novel objects results in DPT (Kemp and Manahan-Vaughan, 2004). The same study found that exposure to novel objects in a familiar context-induced longterm depression (LTD). We suggest that an interaction between the "enrichment" protocol emphasizing novelty and the enhanced DPT in BTBR mice may have resulted in the selective increase of object recognition memory in BTBR.

How could loss of callosal and commissural inputs lead to the increased susceptibility to DPT in BTBR? Interhemispheric fibers have been implicated in extrahippocampal synaptic plasticity (Cissé et al., 2004; Bogdanova and Sil'kis, 2002). The HC contains homotopic and heterotopic contralateral projections between the CA1 and CA3 subfields of the hippocampus (Raisman et al., 1965; Laurberg, 1979; Buchhalter et al., 1990); these HC fibers could be important for hippocampal synaptic plasticity. In brains with an intact HC and CC, contralateral commissural or callosal inputs might therefore modulate hippocampal synaptic plasticity in a manner that enhances resistance to DPT. The impairment of such a process in BTBR due to the loss of interhemispheric connectivity could lead to the susceptibility to DPT reported here. In turn, this could result in stronger encoding of novel objects and the observed enhancement of object recognition memory following "enrichment". This line of reasoning remains speculative, but it warrants further investigation.

The "enrichment"-induced increase in object memory observed in this study suggests that loss of callosal and commissural fibers may 'unlock' some latent capacity to encode, retrieve or store some forms of memory. Ledoux et al. (1977) reported that an adolescent male's memory capacity increased following complete sectioning of the CC and, for some sub-tests, became supranormal. An anecdotal case in the popular scientific press also supports this hypothesis. A'savant' with a phenomenal memory and limited capacity for abstract thought displayed callosal agenesis as well as several other brain abnormalities (Treffert and Christensen, 2005). Taken together, our results suggest that interhemispheric connections have a nuanced role in learning and memory.

Our results are relevant to the study of commissurotomy and callosal agenesis patients. While there appear to be no dramatic memory defects in human split-brain patients, specific, more subtle mnemonic processes may be

compromised in commissurotomy patients. In a study of callosotomy patients, individuals with transected HCs showed post-surgery declines in recall but not recognition memory whereas those with an intact HC displayed intact memory function (Phelps et al., 1991). This suggests that the forebrain commissures, in particular the HC, may be critically involved in some forms of memory. Our results support this view. Contextual fear memory in BTBR was severely compromised, whereas object recognition memory was spared. This suggests either that the HC and CC are not normally required for object recognition or that extrahippocampal structures are capable of mediating novel object recognition if the hippocampus is lesioned.

It should be emphasized that the behavioural and electrophysiological consequences of an anatomical defect depend, to some extent, on the genetic background of a particular mouse strain (Wahlsten et al., 2003b). Since the reduced HC and CC in BTBR are congenital, compensations for the anatomical defect may exist. Thus, there is the possibility that the observed phenotype results not only from the initial defect, but also from the consequent adaptations. It should be noted that another inbred mouse strain (9XCA/Wah) with a very similar, but less severe, reduction in CC and HC has been characterized (Schimanski et al., 2002). Similar to BTBR, 9XCA/Wah has intact LTP and basal transmission. However, they also have impaired PPF which we did not observe in BTBR. Furthermore, unlike BTBR, 9XCA/Wah had normal memory formation but impaired memory extinction. The comparison of these two strains with similar anatomical defects coming from different genetic backgrounds might provide an opportunity to study the genetic and molecular bases of the observed electrophysiological and behavioural phenotypes.

In summary, our results indicate that the CC and HC are required for contextual fear memory as well as resistance to DPT in some hippocampal circuits. The HC and the CC are not required for object recognition memory. Absence of the CC and HC enhances object recognition following continuous "enrichment". We speculate that susceptibility to DPT may underlie the memory phenotype of these particular acallosal mice that have a congenitally reduced HC.

4. Experimental procedures

4.1. Animals

Female mice were obtained between the ages of 5 to 8 weeks. C57BL/6 mice were obtained from Charles River (Saint-Constant, Québec, Canada); BTBR T^{+} t*f*/*J* mice were obtained from Jackson Laboratories (Bar Harbor, ME). Contextual fear memory was tested between the ages of 8 and 14 weeks. Animals were tested in the novel object recognition task between the ages of 8 and 16 weeks. The mice were group housed in plastic cages (29 × 18 × 13 cm) filled with Aspen Chip bedding (Northeastern Products, Warrensburg, NY). They had access to distilled water and solid food (Laboratory Rodent Diet 5001; Purina Mills, St. Louis, MO) ad libitum. Care and experimental procedures conformed to the guidelines of the Canadian Council on Animal Care.

4.2. Histology

Histological procedures were those used by Wahlsten et al. (2003a). Briefly, the animals' brains were rapidly removed following euthanasia with carbon dioxide gas. Brains were then fixed by immersion in 10% formalin, bisected at the midsagittal plane, stained with 0.2% gold chloride in phosphate buffer at 37 °C for about 1 h until heavily myelinated structures appeared clearly, and then fixed in 2.5% sodium thiosulfate for 5 min. Stained half brains were then stored in fresh fixative. Colour JPEG images were obtained with a HP4470c flatbed scanner at 2400 dpi according to the method of Wahlsten et al. (2003a). The result was an image of the midsagittal plane of each brain that was about 1120 × 900 pixels. The image was inspected for the presence of forebrain commissures and the original half brain was further inspected microscopically to resolve finer details when deemed necessary.

4.3.Fear conditioning

The procedures for fear conditioning were the same as used previously (Schimanski et al., 2002; Schimanski and Nguyen, 2005). Each mouse was placed in a clear Plexiglas conditioning chamber (Med Associates, E. Fairfield, VT) for training. Animals were allowed to become acclimatized to the chamber (40 × 16 × 22 cm) for 2 min. After the acclimatization period, a tone [conditioned stimulus (CS)] of 85 dB intensity was applied for 30

s. Mice received a 2 s foot-shock [unconditioned stimulus (US)] of 0.7 mA that co-terminated with the tone. The animal remained in the chamber for an additional 30 s before being removed. All testing took place between 09:00 and 12:00 to minimize circadian variations.

All mice in the present study were tested for contextual fear memory. Cued fear was not assessed. At various intervals, the mice were returned to the original chamber for a 5 min contextual fear test wherein no foot-shock or tone was applied. Three groups of animals were used in the present study to test contextual fear memory. The first group of mice was returned to the chamber one hour after training to test short-term fear memory. The second group was returned to the chamber once at 24 h as an index of long-term memory. A third group of animals was returned to the chamber at 48 h as another index of long-term memory.

Conditioning was assessed at all stages by scoring freezing behaviour every 5 s. Freezing was defined as the complete absence of motion (except for respiratory movements). Freezing was scored by a human observer. As coat colours differed between BTBR and BL/6, the observer was not blind to the animal's strain. The percentage of time spent frozen was calculated for each mouse in discrete time intervals, and these results were pooled and averaged for each strain in each interval.

4.4.Novel object recognition task

The novel object recognition task took place in a large open field (59 ×48 ×25 cm). The floors were covered with Aspen Chip bedding and the walls were covered with white paper. The testing occurred in a quiet room with only indirect light. The objects used varied in colour, texture and shape but were of similar size and interest (unpublished observations). None of the objects had any apparent biological significance. The identity (novel versus familiar) and relative location (left versus right) of objects was counterbalanced across trials in order to preclude any effects based on preference for a given object or a particular part of the testing environment.

Training consisted of one 15 min exposure to two identical objects in the open field. The objects were placed 20 cm apart in the centre of the field. One day later, the animals were returned to the open field where one of the original objects had been replaced by a novel object. Each mouse was then allowed to explore the novel and familiar objects for 15 min. The testing period was video-taped and scored for exploratory behaviour.

Exploratory behaviour was defined as a behaviour lasting at least one second in which the animal's nose was pointed at the object and within a distance of 2 cm. Climbing on an object or rearing near that object was not considered exploratory behaviour. An inclusion criterion was used to exclude animals that have naturally low levels of exploratory behaviour. Animals whose total exploratory time was less than 5 s in the testing trial were excluded from the analysis. One animal was excluded for this reason.

The total time spent exploring the novel object (N) and the familiar (F) objects was tabulated. Exploratory preference (EP) was calculated using the formula $EP=[N/(N+F)]\times100\%$. At a score of 50%, neither object is preferred, indicating chance performance in the object recognition task. Scores greater than 50% indicate a preference for the novel object.

Some mice received continuous environmental "enrichment". Eight days prior to the training trial, a plastic tunnel and two small objects of varying shape, texture and colour were placed in the home cage of mice receiving “enrichment”. The objects were rotated such that there were two novel objects in the cage each day. The tunnel remained in the cage for the entire period. The objects and tunnel were removed immediately prior to the training trial and were not returned. None of the objects used in the “enrichment” period was used in object recognition testing. These mice therefore received 8 days of environmental “enrichment” in a modified paradigm that continually presented novelty.

4.5. Electrophysiology

After cervical dislocation and decapitation, transverse hippocampal slices (400 μm thickness) were prepared as described by Nguyen and Kandel (1997) (see also Nguyen (2006)). Slices were maintained in an interface chamber at 28 °C and perfused (flow rate 1–2 mL/min) with artificial cerebrospinal fluid (ACSF) composed of the following (in mM):124 NaCl, 4.4 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 10 glucose, aerated with 95% O_2 and 5% CO_2. Slices were allowed to recover for 90 min before recordings were initiated. Extracellular field excitatory postsynaptic potentials (fEPSPs) were evoked by stimulating the Schaeffer collateral (SC) pathway with a bipolar nickel–chromium electrode (130 μm diameter) positioned in the stratum radiatum. Stimulation intensity (0.08 ms pulse duration) was adjusted to evoke fEPSP amplitudes that were 40% of maximal size. Subsequent fEPSPs were elicited once per minute at this stimulation intensity. fEPSPs were recorded with a glass microelectrode filled with ACSF (resistance 2–3 MΩ). LTP was induced by applying one of the following stimulation protocols: one 100-Hz train of high-frequency stimulation (HFS) of 1 s duration at test strength (1 × 100 Hz); four 100-Hz trains of HFS of 1 s duration at test strength, with a 20 s inter-train interval (4×100 Hz); and theta-burst stimulation (TBS) consisting of 15 trains of 30 ms duration; each train consisting of four pulses delivered at 100 Hz, with an inter-train interval of 200 ms (Nguyen and Kandel, 1997). Depotentiation (DPT) was induced 5 min after four-train LTP by applying low-frequency stimulation (LFS; 5 Hz for 3 min). Paired-pulse facilitation was tested in slices at the baseline stimulation intensity at interpulse intervals of 50, 75, 100 and 200 ms. An input–output curve was created for each strain by plotting presynaptic fiber volley amplitudes and fEPSP slopes.

4.6.Statistical analysis

To assess LTP and DPT, we measured the average fEPSP slopes during stable recordings acquired over a period of 20 min before LTP induction. fEPSP slopes recorded after HFS were then normalized in relation to these baseline averages and expressed as percentages of baseline fEPSP slopes. Slopes were averaged within each strain and for each protocol, and the resulting means were compared between strains. fEPSP slopes were compared 110 min after 4× 100-Hz stimulation, and 50 min after 1 × 100-Hz, TBS and DPT stimulations. Student's t test was used for statistical comparisons of mean fEPSP slopes between two groups; significance was considered $p < 0.05$. Presynaptic volley amplitudes and fEPSP slopes, measured by varying the stimulus intensity, were plotted and a linear regression of the input–output relationship was calculated for each strain. Student's t test was used to compare linear regression slopes, and assess statistical significance.

Student's *t* test was used to compare freezing values between BTBR and BL/6. The Welch correction was applied when the difference between the standard deviations was significant. Two-tailed one sample *t* tests were used to compare exploratory preference values in the object recognition task to the theoretical mean of 0.50. All values in the present study are reported as means ±SEM. One-way analysis of variance followed by post-hoc Tukey testing was used to compare object recognition values between the four groups of mice used in Fig. 1D and E.

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