Contributions of area Te2 to rat recognition memory

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Ablations and local intracerebral infusions were used to determine the role of rat temporal association cortex (area Te2) in object recognition memory, so that this role might be compared with that of the adjacent perirhinal cortex (PRH). Bilateral lesions of Te2 impaired recognition memory measured by preferential exploration of a novel rather than a familiar object at delays ≥ 20 min but not after a 5-min delay. Local infusion bilaterally into Te2 of (1) CNQX to block AMPA/kainate receptors or (2) lidocaine to block axonal transmission or (3) AP5, an NMDA receptor antagonist, impaired recognition memory after a 24-h but not a 20-min delay. In PRH all these manipulations impair recognition memory after a 20-min as well as a 24-h delay. UBPS02, a GluK1 kainate receptor antagonist, impaired recognition memory after a 24-h but not a 20-min delay, contrasting with its action in PRH where it impairs only shorter-term (20 min) recognition memory. Also in contrast to PRH, infusion of the muscarinic receptor antagonist scopolamine was without effect. The Te2 impairments could not readily be ascribed to perceptual deficits. Hence, Te2 is essential for object recognition memory at delays > 5 or 20 min. Thus, at long delays both area Te2 and PRH are necessary for object recognition memory.

Lesion and recording studies in the non-human primate and the rat have demonstrated the importance of the perirhinal cortex (PRH) for object recognition memory (Meunier et al. 1993; Mumbry and Pinel 1994; Zhu et al. 1995a; Xiang and Brown 1998; Winters et al. 2004; Brown et al. 2010). The visual association cortex dorsally adjacent to PRH—here termed Te2 (Shi and Cassell 1997; Sia and Bourne 2008)—is strongly interconnected with PRH, in particular providing PRH with visual information (Shi and Cassell 1997; Burwell and Amaral 1998; Sia and Bourne 2008). Repetition-sensitive neurons signaling information concerning relative familiarity that provide a potential neural substrate for recognition memory (Brown and Xiang 1998) occur in rat Te2 as well as PRH (Zhu et al. 1995a). Similarly, in the monkey such responses occur not only in PRH but also in adjacent visual association cortical area TE (Brown and Xiang 1998; Xiang and Brown 1998). Immunohistochemical imaging for Fos (Zhu et al. 1995b; Wan et al. 1999) or CAMKII (Tinsley et al. 2009) has indicated that rat area Te2 as well as PRH is activated more in response to novel than to highly familiar objects.

Ablation studies have yet to define the role of Te2 in recognition memory. Combined lesions to area Te2 and PRH in rats (Aggleton et al. 1997) led to a greater and more robust disruption of object recognition memory than found for rats with lesions confined to PRH (Ennaceur et al. 1996). However, although rats with lesions restricted to area Te2 were unable to learn one version of a non-matching to sample task, they were able to solve another even when delays were introduced (Kolb et al. 1994). It was suggested (Kolb et al. 1994) that this difference in performance between the two tasks arose because of differences in the ease of perceptual discriminability of the stimuli or the usage of non-visual cues. In the monkey, TE lesions have been reported to impair visual recognition memory (Mishkin 1982; Ma´lkova´ et al. 1995), though others have reported only perceptual deficits (Buckley et al. 1997; Buffalo et al. 1998, 1999, 2000). Given the above listed indicative but indefinite evidence, the present study was designed to elucidate the contribution of area Te2 to object recognition memory in the rat.

Selective excitotoxic lesions have established the necessity of PRH for object recognition memory (Mumbry and Pinel 1994; Winters et al. 2004). Moreover, transient inactivation produced by local delivery of pharmacological agents to PRH via intracerebral cannulae has established roles in recognition memory for particular neurotransmitter receptors in PRH, including AMPA, kainite, and NMDA glutamate receptors (Winters and Bussey 2005a; Barker et al. 2006a,b) and muscarinic cholinergic receptors (Warburton et al. 2003; Winters et al. 2006). In addition, blocking axonal transmission by local infusion of lidocaine into PRH has been shown to impair object recognition memory (Winters and Bussey 2005b). In order to establish the role of Te2 in comparison to PRH in recognition memory, the effects of excitotoxic lesions and of local infusions into Te2 of these compounds already tested in PRH are now reported here. The results establish an important role for area Te2 in long-term recognition memory.

Results

Experiment I: Do lesions to area Te2 impair object recognition memory?

Histological extent of lesions

Sections were examined for damage between 5.1 and 7.8 mm behind bregma in the eight lesioned rats. The extents of the largest and smallest lesions are shown in Figure 1. Across both
Area Te2 and recognition memory

Figure 1. Diagramatic representations showing the cases with the largest (grey) and smallest (black) lesions of area Te2 on sections at the marked distances behind bregma (Paxinos and Watson 1998). Area Te2 defined by Sia and Bourne (2008) and Shi and Cassell (1997) is bounded by the black arrowheads. The PRH and secondary visual cortical area (V2) lie immediately ventral and dorsal to area Te2, respectively. The dorsal boundary of V2 is marked by the grey arrowheads. The dorsal boundary of area Te1 (auditory cortex) located at anterior levels is marked by black arrows. Area Te1 is not present caudally from >6.3 mm behind bregma (Shi and Cassell 1997; Sia and Bourne 2008).

Figure 2. Object recognition memory is impaired by Te2 lesions when tested at delays ≥20 min but not at a 5-min delay.

Infusions are largely confined to area Te2

To determine the spread of infusions methylene blue was infused in two rats. Staining was found to be largely confined to area Te2 (Supplemental Fig. 2). In one rat the cannula tip was placed approximately −6.1 mm relative to bregma; the diffusion of methylene blue in the anteroposterior (AP) axis was estimated at 1 mm; medial-lateral (ML) diffusion was observed through all layers; the maximal diffusion in the dorsoventral (DV) axis was estimated at 1.3 mm. In the second rat, the cannula tip was placed approximately −6.7 mm relative to bregma; diffusion of methylene blue was estimated at 1.5 mm in the AP axis; in the ML axis methylene blue was observed in layers 2 to 6; diffusion in the DV axis was estimated at 1 mm. In both rats methylene blue was estimated to have diffused to ~25% of the area Te2. In both cases, the white matter of the external capsule appeared to limit diffusion medially and diffusion extended into dorsal area 36 but encompassed <5% of area 36 of the PRH; there was minimal diffusion dorsally into V2 (<5% V2).

Experiment 2: Pharmacological blockade of glutamate and muscarinic receptor-dependent mechanisms in area Te2

Experiment 2 sought the effects of local infusion of drugs into area Te2 via chronically implanted intracerebral cannulae. The drugs chosen were those that had effects on recognition memory when infused into PRH (Warburton et al. 2003; Winters and Bussey 2005a,b; Barker et al. 2006b). With the exception of CNQX, these drugs have been shown to impair acquisition mechanisms but not retrieval mechanisms in PRH. Therefore the drugs were infused into Te2 so as to be active during acquisition. All rats had cannula infusion tips in area Te2 (Fig. 3).

Object recognition memory is sensitive to lesions of area Te2

Recognition memory was measured by the preference of rats for a novel rather than a previously experienced (familiar) object after delays of 5 min, 20 min, 1 h, or 24 h using the discrimination ratio (DR: the proportion of the total exploration time in the test phase a rat spent exploring the novel minus that spent exploring the familiar object). Mean DRs were compared for Te2 lesion and sham-operated control rats (Fig. 2). This analysis of DRs revealed significant main effects of lesion (ANOVA; F(1,12) = 16.3, P < 0.001) and delay (F(3,47) = 6.2, P < 0.001). The delay by lesion interaction was not significant (F(3,47) = 1.6, P = 0.2).

For the Te2-lesioned rats, object recognition memory was impaired at delays of ≥20 min (DR > 0; 20 min: t(12) = 0.8, P > 0.1; 1 h: t(12) = 0.6, P > 0.1; 24 h: t(12) = 1.2, P > 0.1), but not at a delay of 5 min (DR > 0; 5 min: t(12) = 3.5, P < 0.05). Sham controls preferentially explored the novel object at all delays (DR > 0; 5 min: t(6) = 4.8, P < 0.01; 20 min: t(6) = 10.3, P < 0.001; 1 h: t(7) = 3.3, P < 0.05; 24 h: t(6) = 5.1, P < 0.01). The difference between the mean DRs of the sham-operated and lesioned groups was not significant at the 5-min delay (F(1,12) < 1, P > 0.1), but was significant at the 20-min (F(1,10) = 7.4, P < 0.05), 1-h (F(1,13) = 5.8, P < 0.05), and 24-h (F(1,12) = 10.6, P < 0.01) delays (Fig. 2).

Area Te2 lesions had no significant effect on general exploratory times when compared with sham-operated controls (Supplemental Table 1). Furthermore, the impairment could not readily be ascribed to a perceptual deficit as the same lesioned rats were not impaired in a spontaneous oddity preference task (see Supplemental Material, including Supplemental Fig. 1).

In summary, object recognition memory was impaired in Te2-lesioned rats when tested at delays ≥20 min but not at a 5-min delay.
To assess the contribution of AMPARs to object recognition memory, CNQX (9 mM) was infused locally into area Te2 15 min prior to the sample phase of the NOP task so as to be active during acquisition (Fig. 5).

Novel object preference was measured after delays of 20 min \( (n = 9) \), 1 h \( (n = 12) \), and 24 h \( (n = 9) \). ANOVA of DRs for CNQX and vehicle control conditions revealed a significant interaction between drug treatment and delay \( (F_{2,27} = 3.3, P = 0.05) \). Object recognition memory in the rats infused with vehicle was intact \( (DR > 0) \) at delays of 20 min \( t_0 = 2.8, P < 0.05 \), 1 h \( t_{11} = 5.3, P < 0.001 \), and 24 h \( t_8 = 2.8, P < 0.05 \). When CNQX was infused, the rats preferentially explored the novel object after a 20-min delay \( t_{10} = 3.0, P < 0.05 \) and did not differ significantly from the vehicle control condition \( t_{11} = 1, P > 0.05 \). In contrast, at delays of 1 h or 24 h, the CNQX-infused rats did not show significant preferential exploration of the novel object \( (1 \text{ h}: t_{11} = 0.8, P > 0.05; 24 \text{ h}: t_8 = 0.5, P > 0.05) \) and the difference from the mean DR for the vehicle condition was significant \( (1 \text{ h}: F_{1,11} = 13.2, P < 0.01; 24 \text{ h}: F_{1,8} = 6.9, P < 0.05) \) (Fig. 5).

Post-hoc pair-wise analyses revealed that the CNQX-infused rats were significantly impaired at the 1-h delay compared with the 20-min delay \( (P < 0.01) \). The mean DR for CNQX-infused rats at the 24-h delay was lower than at the 20-min delay but the difference did not quite reach significance \( (P = 0.07) \). Infusions of CNQX were without effect on overall exploratory behavior compared to vehicle infusions (Supplemental Table 2).

Hence, disruption of AMPARs/KARs in area Te2 by infusion of CNQX impaired the acquisition of object recognition memory when tested at delays of 1 h and 24 h but not 20 min.

Consolidation and retrieval mechanisms

When infused into PRH, CNQX impairs retrieval (unlike the other drugs tested in Experiments 2). Therefore the effects of infusions into area Te2 of CNQX (9 mM) were sought on retrieval and early consolidation mechanisms underlying object recognition memory (Fig. 6). As CNQX remains active for \( > 1 \) h (but << 24 h) after infusion (Day et al. 2003), it may be expected to be active during both acquisition and retrieval when memory is measured after a 20-min delay.

Rats infused with CNQX immediately after the sample phase of the NOP task spent more time in the choice phase exploring the novel than the familiar object after both a 20-min \( (n = 8) \) and a 24-h delay \( (n = 9) \). After the 20-min delay, the DR value \( (0.24 \pm 0.10) \) was not quite significantly greater than zero \( (t_7 = 2.3, P < 0.06) \), but it was after a 24-h delay \( t_8 = 2.8, P < 0.05 \) (Fig. 6).

When vehicle was infused, the rats preferentially explored the novel object after a 20-min \( t_7 = 3.2, P < 0.05 \) or a 24-h delay.
of GluK1 subunit containing KARs (Fig. 7; More et al. 2004). One-way ANOVA revealed no significant differences in exploration times in the choice phase between the CNQX and vehicle conditions (Supplemental Table 3).

Hence when CNQX was infused into Te2 after acquisition there was no significant impairment of recognition memory at delays of 20 min or 24 h, even though Te2 would have been still inactivated during retrieval at the 20-min delay.

For the 24-h delay, effects of CNQX on retrieval mechanisms were investigated by infusions 15 min before the start of the test phase of the NOP task (n = 8). When the infusion before retrieval was CNQX, the rats showed no preference for the novel object (t(7) = 0.7, P > 0.1); however, the rats preferentially explored the novel object when vehicle was infused (t(7) = 2.7, P < 0.05). The mean DR for the CNQX infusions differed significantly from the vehicle control condition (F(1,7) = 5.6, P < 0.05) (Fig. 6). Overall exploration times for the rats did not differ significantly between the CNQX and vehicle infusion conditions (Supplemental Table 3).

Therefore, CNQX infusion into Te2 impaired the retrieval of longer-term (24-h delay) but not shorter-term (20-min delay) recognition memory. No evidence was found for an effect of CNQX infusion into Te2 on early consolidation mechanisms.

Area Te2 and recognition memory

The contribution of area Te2 kainate receptors (KARs) to object recognition memory was investigated through local infusion of the broad spectrum muscarinic receptor antagonist scopolamine (23 mM) (Fig. 9).

Novel object preference was measured after delays of 20 min (n = 8) and 24 h (n = 12). ANOVA of DRs in the test phase showed a significant interaction between drug treatment and delay (F(1,11) = 7.5, P < 0.05). At a 20-min delay, rats preferentially explored the novel object when infused with vehicle (t(7) = 2.4, P < 0.05) or D-APS (t(7) = 3.1, P < 0.05), and there was no significant difference between the two conditions (F(1,7) < 1, P > 0.05). At a 24-h delay, rats preferentially explored the novel object when vehicle-infused (t(11) = 6.5, P < 0.001), but not when infused with D-APS (t(11) = 1.5, P > 0.05); the difference between the mean DRs was significant (F(1,11) = 15.3, P < 0.01). Post-hoc analyses showed that rats infused with D-APS were significantly impaired at the 24-h delay compared to the 20-min delay (P < 0.05) (Fig. 8). Infusions of D-APS were without effect compared to vehicle on overall exploration times (Supplemental Table 2).

Therefore, MNDARs in area Te2 are necessary for longer-term (24 h) but not shorter-term (20 min) object recognition memory.

Area Te2 NMDARs are necessary for longer-term object recognition memory

The contribution of area Te2 NMDARs to object recognition memory was investigated through local infusion 15 min before acquisition of the NMDAR antagonist D-AP5 (25 mM) (Fig. 8).

Novel object preference was measured after delays of 20 min (n = 8) and 24 h (n = 12). ANOVA of DRs in the test phase revealed a significant interaction between treatment and delay (F(1,11) = 7.5, P < 0.05). At a 20-min delay, rats preferentially explored the novel object whether infused with vehicle (t(7) = 2.4, P < 0.05) or D-APS (t(7) = 3.1, P < 0.05), and there was no significant difference between the two conditions (F(1,7) < 1, P > 0.05). At a 24-h delay, rats preferentially explored the novel object when vehicle-infused (t(11) = 6.5, P < 0.001), but not when infused with D-APS (t(11) = 1.5, P > 0.05); the difference between the mean DRs was significant (F(1,11) = 15.3, P < 0.01). Post-hoc analyses showed that rats infused with D-APS were significantly impaired at the 24-h delay compared to the 20-min delay (P < 0.05) (Fig. 8). Infusions of D-APS were without effect compared to vehicle on overall exploration times (Supplemental Table 2).

Area Te2 muscarinic cholinergic receptors are not necessary for longer-term object recognition memory

The contribution of area Te2 muscarinic receptors to recognition memory was investigated through local infusion 15 min prior to acquisition of the broad spectrum muscarinic receptor antagonist scopolamine (23 mM) (Fig. 9).

Novel object preference was measured after delays of 20 min (n = 12) and 24 h (n = 11). ANOVA of DRs in the test phase showed no significant interaction between drug treatment and delay (F(2,21) = 1.7, P > 0.05), and no significant main effect of drug treatment (F(2,21) = 2.2, P > 0.05). There was a significant effect of delay (F(1,21) = 8.8, P < 0.01) with the mean DRs of

Area Te2 GluK1 KARs are necessary for longer-term object recognition memory

The contribution of area Te2 kainate receptors (KARs) to object recognition memory was investigated through local infusion 15 min prior to acquisition of UBP302 (1.5 mM), an antagonist of GluK1 subunit containing KARs (Fig. 7; More et al. 2004).

Novel object preference was measured after delays of 20 min (n = 7) and 24 h (n = 8). ANOVA of DRs during the choice phase showed a significant interaction between drug treatment and delay (F(1,13) = 5.6, P < 0.05). When vehicle-infused, rats preferentially explored the novel object at delays of 20 min (t(6) = 2.8, P < 0.05) and 24 h (t(7) = 4.2, P < 0.05) (Supplemental Table 2). Infusion of UBP302 did not impair recognition memory after a 20-min delay (DR = 0.29 ± 0.07; t(6) = 3.6; P < 0.05) and the mean DR did not differ significantly from that for vehicle infusions (t(6) < 1, P > 0.05). However, after a delay of 24 h, infusions of UBP302 did impair recognition memory: UBP302-infused rats failed to show a preference for the novel object (t(7) = 0.9, P > 0.05) and the difference from the mean DR for the vehicle infusions was significant (F(1,7) = 6.7, P < 0.05) (Fig. 7). Post-hoc analyses showed that discrimination after UBP302 infusion was impaired at the 24-h delay compared to the 20-min delay (P < 0.05). Infusions of UBP302 were without effect compared to vehicle on overall exploration times (Supplemental Table 2).

Therefore, GluK1 KARs in area Te2 are necessary for the acquisition of longer-term (24 h) but not shorter-term (20 min) object recognition memory.
both the vehicle- and scopolamine-infused rats being greater at the 20-min delay than at the 24-h delay. When infused with vehicle or scopolamine, rats preferentially explored the novel object at the 20-min delay (vehicle: $t_{11} = 4.3, P < 0.001$; scopolamine: $t_{11} = 8.8, P < 0.001$) and at the 24-h delay (vehicle: $t_{10} = 3.7, P < 0.01$; scopolamine: $t_{10} = 4.0, P < 0.01$). There was no significant difference between the mean DRs for the vehicle and scopolamine conditions at the 20-min ($F_{1,11} = 3.2, P > 0.05$) or 24-h delays ($F_{1,10} < 1, P > 0.05$) (Fig. 9). Infusions of scopolamine were without effect compared to vehicle on overall exploration times (Supplemental Table 2).

Therefore, infusion of scopolamine into area Te2 had no effect on the acquisition of shorter-term or longer-term object recognition memory.

**Discussion**

Lesioning area Te2, or interference with axonal or glutamatergic synaptic transmission within Te2 during acquisition, impaired object recognition memory measured after a delay of 24 h, but not when the delay was 5 min or 20 min. In addition, infusion of CNQX (an AMPA/kainate glutamatergic antagonist that blocks most excitatory transmission) prior to retrieval impaired recognition memory when the delay was 24 h, though not 20 min. These findings establish that area Te2 is essential for the acquisition and retrieval of long-term (24 h) object recognition memory but suggest that it is not necessary for shorter-term object recognition memory. They provide the first unequivocal evidence for the importance of rat area Te2 to long-term visual object recognition memory. The findings accord with reports of impairment of visual recognition memory following lesions of anterior visual association cortex (area TE) in the monkey (Mishkin 1982; Málková et al. 1995), albeit a deficit was found at short delay intervals. They challenge the view that cortex adjacent to medial temporal lobe structures has only perceptual functions (Buckley et al. 1997; Buffalo et al. 1998, 1999, 2000; Squire et al. 2004). They establish that PRH is not the only region essential for the single object familiarity discrimination component of recognition memory.

Area Te2 is part of visual association cortex; however, the memory deficits found in the current experiments could not easily be explained as being solely the result of visual perceptual impairment. Firstly, the lesioned rats demonstrated preferential exploration of the odd object in an oddity discrimination task, which used objects similar to those used in the recognition memory task (see Supplemental Material). Hence rats with Te2 lesions were still capable of discriminating between objects similar to those used in the recognition memory task. These objects were not perceptually ambiguous, differing in many features, and so were not similar to the ambiguous stimuli that have been shown to produce perceptual impairments following perirhinal lesions (Murray et al. 2007). Indeed, previous work has shown that after Te2 ablation rats can still form a variety of visual perceptual tasks without impairment (Williams and McDaniel 1999) and are unimpaired in spatial tasks which also require the processing of visual information (Kolb et al. 1994; Aggleton et al. 1997). Secondly, the lesioned rats in the present study were unimpaired when object recognition memory was measured after a 5-min delay. As there was impairment after a 24-h delay, the impairment was delay-dependent, i.e., mnemonic. Moreover, the rats receiving local infusions prior to acquisition of CNQX (that blocks most excitatory transmission) or lidocaine (that blocks axonal conduction) were unimpaired when recognition memory was measured after a 20-min delay, again demonstrating that the rats must have been able to perceptually discriminate between the objects. Nevertheless, these comments leave open the possibility that rats where Te2 is lesioned or temporarily inactivated by drug infusion suffer from greater mnemonic interference during the 24-h delay period than control animals (McTighe et al. 2010).

The impairments reported in the present study are not readily explicable as being due to incidental damage to the PRH. Previous work has indicated that perirhinal lesions need to be extensive for behavioral deficits to be found (Bucci et al. 2000; Burwell et al. 2004). Damage to the PRH in the present study was either unilateral or <5%, with the exception of one rat. Even this rat preferentially explored the novel object at the 5-min delay; at this short delay object recognition memory has been reported to be impaired rats with lesions centered on the PRH (Norman and Eacott 2004; Barker et al. 2007).

When Te2 was lesioned, memory was impaired after a 20-min but not a 5-min delay whereas all drug infusions left memory intact after a 20-min delay. It needs to be noted that although the majority of Te2 was compromised, neither the lesions nor the infusions encompassed the whole of Te2. The difference in the effect of lesioning or infusion at the 20-min delay may have been due to the precise regions compromised within and adjacent to Te2; however, all manipulations tested left recognition memory unimpaired at either a 5-min or a 20-min delay. The preservation of memory after short delays indicates that another region can sustain recognition memory at such delays, the most obvious region being PRH. Lesions (Norman and Eacott 2004; Winters
et al. 2004) of PRH or infusions of CNQX (Winters and Bussey 2005a; Barker et al. 2006b) or lidocaine (Winters and Bussey 2005b) into PRH produce deficits in object recognition memory after delays of 5 min or 20 min. In contrast, the results establish that PRH cannot sustain object recognition memory after a 24-h delay if Te2 is not fully functional during acquisition and retrieval. As a functional PRH during acquisition and retrieval is also necessary for object recognition memory after a 24-h delay (Winters and Bussey 2005b; Barker et al. 2006a,b; Mumbry et al. 2007), both regions are required during acquisition and retrieval for such long-term recognition memory.

Visual information is fed forward from area Te2 to PRH (Burwell and Amaral 1998; Shi and Cassell 1999; Sia and Bourne 2008), though there is also direct input to PRH from occipital visual areas (Burwell and Amaral 1998). Accordingly, interference with processing in Te2 by ablation or infusion may produce an effect on recognition memory by preventing visual information transfer to PRH. However, in this case impairments would be expected in both perception and in short-term recognition memory and as discussed above such impairments were not found. Moreover, total exploration was unaffected by any of the interferences with Te2. While reduction in visual information transfer to PRH may still account for the memory deficit at the 24-h delay (when the task is more difficult because of the longer delay), the amnesia might also arise from disruption to information fed back from PRH to Te2. Evidence for such feedback signals has been provided in the learning of visual paired associates in the monkey where a gradual recruitment of neuronal activity from PRH to area TE was interpreted as backward propagation of learned information at retrieval from PRH to adjacent visual association cortex (Naya et al. 2001, 2003). There is suggestive evidence for such signals in recognition memory processing. When an inhibitor of the phosphorylation of CAMKII is infused into PRH, CAMKII phosphorylation is reduced in Te2 as well as in PRH (Tinsley et al. 2009), suggesting that CAMKII processing in Te2 is dependent on perihinal cortical activity.

In comparison to the recognition memory impairments produced by ablation or drug infusion involving PRH, those for Te2 are characterized by occurring at only longer (24 h) delays. Thus the effects of infusions of CNQX and lidocaine differed between area Te2 and PRH in their lack of effect on recognition memory measured at the shorter delays, but were the same for a 24-h delay (Winters and Bussey 2005a,h). Pre-acquisition infusion of the NMDA receptor antagonist AP5 into Te2 produced memory impairment at a 24-h but not a 20-min delay, the same effect as found for perirhinal infusions of D-AP5 (Barker et al. 2006b). However, as Te2 is not essential for recognition memory measured after a 20-min delay, this finding cannot exclude an action of AP5 in Te2 on shorter-term recognition memory mechanisms (but if so, these are not essential to task performance). That Te2 is unnecessary for recognition memory measured after a 20-min delay may explain the lack of effect of infusion of the muscarinic receptor antagonist scopolamine; pre-acquisition infusions of scopolamine into PRH impair recognition memory at a 20-min but not a 24-h delay (Warburton et al. 2003; Tinsley et al. 2011). In contrast, GluK1 kainate receptor antagonism by UBP302 impaired recognition memory after a 24-h delay when infused prior to acquisition into Te2 but left such long-term memory unimpaired when infused into PRH (where it impaired memory after a 20-min delay; Barker et al. 2006b). This result establishes a clear difference in recognition memory processing mechanisms between Te2 and PRH. Establishing the biochemical and/or physiological basis for the difference is an important area for future research.

In sum, the findings establish an essential role for rat area Te2 in the acquisition and retrieval of long-term (24 h) visual object recognition memory. No deficits in such memory were seen at short delays (5 or 20 min). There is a difference in the role of GluK1 kainate receptors in Te2, where they are involved in acquisition processes necessary for longer-term memory, compared with PRH, where they are only involved in shorter-term memory processes. The findings are most easily interpreted as indicating the necessity of interactions between area Te2 and PRH during both acquisition and retrieval of long-term visual recognition memory.

Materials and Methods

Subjects

A total of 43 male Dark Agouti rats (230–250 g; Bantin & Kingman, Hull, UK) were housed under a controlled light/dark cycle with darkness being from 8 am to 8 pm. Experiments were carried out during the dark phase of the cycle. All procedures were in accordance with UK Home Office regulations and the Animal (Scientific) Act, 1986, and had approval from the University of Bristol Ethics Committee.

Anesthesia

For excitotoxic lesions and implantation of intracerebral cannulae, each rat was anesthetized with isoflurane (induction 4%; maintenance 2%–3%) then secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The incisor bar was adjusted so that the heights of bregma and lambda were within 0.2 mm of each other. During the surgical procedure, two doses of 2.5 mL of glucose-buffered saline were administered subcutaneously, and 0.05 mL Temgesic (Schering-Plough Ltd.) was administered intra-muscularly just before termination of anesthesia to provide post-surgery analgesia. At the completion of surgery, the skin was sutured and veterinary wound powder (Hayward and Bower Ltd.) was applied. Rats recovered for ≥14 d after which habituation to the behavioral procedures commenced.

Behavioral apparatus

Behavioral tests of recognition memory were carried out in an open topped 1 m² arena constructed of plywood with walls 50 cm high, and the floor covered with sawdust. The inside of the walls were painted grey. Black curtains surrounded the arena to a height of 1.5 m to conceal the rat's view of extra maze cues. A video camera was mounted directly above the centre of the arena to monitor and record the animal’s behavior. Objects were composed of Duplo (LEGO, Slough, UK) and were of sufficient size (10 × 5 × 15 cm to 20 × 20 × 30 cm) and weight to prevent the rat from displacing them. Duplo was used to minimize the use of textural or olfactory rather than visual or shape cues; however, the objects were constructed to differ in many visual/shape features, as in other work (Warburton et al. 2003; Barker et al.
2006a,b, 2007; Barker and Warburton 2008a,b; Tinsley et al. 2009). Objects were wiped clean with 100% ethanol to remove any odor cues after use.

Novel object preference (NOP) task

The NOP task (Ennaceur and Delacour 1988) was used to assess visual object recognition memory through the preferential exploration of a novel rather than a familiar object, as previously (Dix and Aggleton 1999; Warburton et al. 2003; Barker et al. 2006b, 2007; Barker and Warburton 2008a). In the sample phase, the rat was placed into the arena facing the centre of the opposite wall and allowed a maximum of 40 sec total exploration of the objects or a total of 4 min in the arena. Following a delay, the test phase commenced. In the test phase, the rat was allowed to explore freely for 3 min. Each rat had to complete both a minimum of 15 sec active exploration of the objects in the sample phase and a minimum of 10 sec in the test phase to be included in the analyses. The positions of the objects in the test phases and the objects used as novel or familiar were counterbalanced between the animals in a group and between the control and experimental groups. Rats were allowed at least four days rest between behavioral runs and were handled routinely.

Behavioral measures and statistical analyses

In all behavioral procedures, the experimenters was blind to the condition of the rats. Active exploration of the object was defined as the animal directing its nose toward the object at a distance <2 cm. Other behaviors such as sitting on or resting against the object were not considered as exploration.

The discrimination ratio (DR) was calculated as the proportion of the total exploration time in the test phase a rat spent exploring the novel minus that spent exploring the familiar object. One sample t-tests with comparisons against zero were used to test significance of the mean DR (a mean DR score of zero indicated equal preference for the novel and familiar object) for each group. For comparison between the lesion and sham groups across delays, a two-way analysis of variance (ANOVA) was used with condition (sham or lesion) and delay as factors. For comparisons of drug treatment in the NOP task, a repeated measures ANOVA was used with drug treatment (drug or vehicle) as a within-subjects factor and delay as a between subjects factor. Post-hoc pair-wise comparisons across delays with a Bonferroni correction for multiple comparisons were performed. All tests used a significance level of P = 0.05 and were two-tailed.

Experiment 1: Excitotoxic lesions

A total of 17 rats were used in Experiment 1. Eight rats underwent excitotoxic lesioning to area Te2 and nine rats underwent sham operations. Rats were anesthetized as described above. Craniotomies were made directly above the target regions. For the lesions, two injections, each consisting of 0.12 µL of 0.09 M NMDA (Ascent Scientific), dissolved in phosphate buffer (PB) (pH 7.4), were made in each hemisphere through a 1 µL Hamilton gas tight syringe (Hamilton Company). The stereotaxic coordinates relative to bregma (mm) were: anteroposterior (AP) −7.2, lateral (L) ±6.4, dorsoventral (DV) −6.0 and AP −8.2, L ±5.8, DV −4.8. Each injection was delivered gradually over 3 min and the needle was left in situ for a further 3 min before removal. Sham operations consisted of lowering of the Hamilton syringe into the appropriate locations with no injection of NMDA.

Experimental design

Habituation to the empty arena preceded behavioral testing. Habituation consisted of handling for 5 min before being placed in an empty arena for 15 min. Behavioral testing began after the last day of habituation. The NOP task preceded the object preference task. The same animals (lesioned and sham) were used for the duration of the experiments.

At the start of the NOP experiments, eight Te2-lesioned rats were compared to nine sham-operated controls for their preference for a novel object. Rats were excluded from analyses if they failed to explore sufficiently as outlined above: Two, three, one, and two sham-operated rats were excluded at the 5-min, 20-min, 1-h and 24-h delays, respectively. One, two, one, and one lesions were excluded at the 5-min, 20-min, 1-h, and 24-h delays, respectively. Thus, the final group numbers for analyses at the delays tested were as follows: 5-min (sham, n = 7; lesion, n = 7), 20-min (sham, n = 6; lesion, n = 6), 1-h (sham, n = 8; lesion, n = 7), and 24-h delays (sham, n = 7; lesion, n = 7).

The spontaneous oddity preference task was conducted following the NOP experiment. One rat from the sham-operated and one rat from the lesion group were excluded from analyses as they failed to explore sufficiently as outlined above. Thus, the final group numbers were as follows: sham, n = 8; lesion, n = 7.

Histology

At the end of behavioral experiments, each rat was euthanized with an overdose of sodium pentobarbital (Euthatal, Merial Animal Health Ltd.) then perfused transcardially with 0.1 M PB (pH 7.4) followed by 4% (w/v) paraformaldehyde (PFA). The brain was then immersion-fixed with 4% (w/v) PFA for 24 h at 4°C before being transferred to 30% (w/v) sucrose in 0.1 M PB (pH 7.4), for 48 h at 4°C. Thirty-micrometer thick coronal sections were cut using a cryostat and collected for neuronal nuclei (NeuN) immunohistochemistry. Lesions to area Te2 were calculated based on the Cavalieri unbiased stereological method (Gundersen et al. 1988). Extra temporal cortical damage was estimated as the damage spread across insufficient sections to make unbiased stereological calculations.

NeuN immunohistochemistry

Immunohistochemistry was performed on free floating sections. Endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide for 15 min. After washing in phosphate buffered saline with 0.2% (w/w) Triton-X (PBST), nonspecific protein binding sites were blocked by incubation in 1.5% (v/v) normal horse serum for 1 h at room temperature (RT). Primary mouse anti-NeuN antibody (1:5000) (Millipore) was then applied for 24 h at 4°C. Following washing, sections were incubated with a biotinylated anti-mouse secondary antibody (1:200) (Vector Laboratories) for 2 h at RT. The immunoreaction product was visualized with avidin-biotin technique and 3,3’-diaminobenzidine (DAB) (Sigma-Aldrich) as the chromagen. Sections were mounted onto glass slides then counterstained for Nissl bodies using cresyl violet.

Experiment 2: Intracerebral cannulation

Rats were anesthetized as described above. A skin flap was excised for craniotomies to be made for the implantation of bilateral guide cannulae. One 10 mm deburred 26 gauge (0.22 mm internal and 0.46 mm external diameter) stainless steel guide cannula (Plastics 1, Semat) was lowered though a craniotomy bilaterally at the following coordinates from bregma: AP, −6.7 mm; L, ±4.5 mm. The cannulae were lowered 5.5 mm below the height of the skull surface at an angle of 22° to the vertical in the coronal plane (medial to lateral). The cannulae were anchored into the skull by two anchor screws and dental cement. Once secured, cannula dummies (Plastics 1, Semat) were used to obdurate the guide cannulae.

Intra-Te2 infusions

The general infusion procedure followed that of Barker et al. (2006b). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Tocris Bioscience), D-2-amino-5-phosphonopentanoate (D-AP5) (Ascent Scientific), scopolamine hydrobromide (Sigma-Aldrich), and lidocaine hydrochloride (Alamone Labs) were dissolved in sterile 0.9% saline. (S)-3,4-dihydroxyphenylalanine (UP3002) was dissolved in 0.1 M NaOH and made up to the required concentration with
sterile 0.9% saline (final concentration of NaOH, 1.5 mM). CNQX was infused at a concentration of 9 mM; lidocaine was infused at a concentration of 6.7 mM; UBP302 was infused at a concentration of 1.5 mM; D-AP5 was infused at a concentration of 25 mM; and scopolamine was infused at a concentration of 23 mM. Vehicle solutions consisted of saline or saline plus an equivalent quantity of 0.1 M NaOH. Infusions were made through a 33 gauge infusion cannula attached to a Harvard infusion pump via polyethylene tubing. A volume of 1 µL (drug or vehicle) was injected bilaterally into area Te2 at a rate of 0.333 µL/min over 3 min. The infusion cannulae were left in place for a further 4 min before being removed and the cannula dummies replaced.

Experimental design

Before behavioral experiments commenced, rats were habituated to the infusion procedure and to the empty arena over four days. Each experiment consisted of two runs and used a crossover design; in the first run half the rats received vehicle and the other half received drug; in the second run the treatments (drug or vehicle) were counterbalanced.

Two groups of rats were prepared with bilateral intracerebral cannulae for local infusions into area Te2. For the first group of rats (n = 12), the first set of experiments consisted of infusions of CNQX to block AMPARs/KARs during acquisition, early consolidation, and retrieval of object recognition memory. The same group of rats was used for bilateral infusions of UBP302 before the sample phase to block GluK1 KARs during acquisition. A second group of intra-Te2 cannulated rats (n = 12) was then prepared. Experiments were carried to block axonal transmission using lidocaine; NMDARs then muscarinic receptors were blocked by infusions of D-AP5 and scopolamine, respectively, in subsequent experiments. All infusions for the second group were carried out before the sample phase. Supplemental Figure 3 summarizes the history of the behavioral experiments for the two groups of cannulated rats.

Methylene blue spread

Methylene blue (1%) dissolved in 0.9% saline was used to assess the region perfused by intra-Te2 infusions. Two rats not used for behavior were prepared with intracerebral cannulae and allowed to recover as described above. These rats were introduced to the infusion procedure on two consecutive days without any infusion taking place. On the third day, 1 µL methylene blue was infused according to the infusion procedure above. Fifteen minutes after the start of the infusion, the rat was deeply anesthetized with iso-

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References

Aggleton JP, Keen S, Warburton EC, Bussey TJ. 1997. Extensive cytotoxic lesions involving both the rhinal cortices and area TE impair recognition but spare spatial alternation in the rat. Brain Res Bull 43: 279–287.

Barker GR, Warburton EC. 2008a. NMDA receptor plasticity in the perirhinal and prefrontal cortices is crucial for the acquisition of long-term object-in-place associative memory. J Neurosci 28: 2837–2844.

Barker GR, Warburton EC. 2008b. Recognition memory using recency or object-context associations: The role of interactions between the hippocampus, perirhinal cortex and medial prefrontal cortex. Society for Neuroscience Abstract.
Naya Y, Yoshida M, Miyashita Y. 2001. Backward spreading of memory-retrieval signal in the primate temporal cortex. *Science* **291**: 2861–2871.

Naya Y, Yoshida M, Miyashita Y. 2003. Forward processing of long-term associative memory in monkey inferotemporal cortex. *J Neurosci* **23**: 2861–2871.

Norman G, Eacott MJ. 2004. Impaired object recognition with increasing levels of feature ambiguity in rats with perirhinal cortex lesions. *Behav Brain Res* **148**: 79–91.

Paxinos G, Watson C. 1998. The rat brain in stereotaxic coordinates, 4th ed. Academic Press, San Diego, CA.

Shi CJ, Cassell MD. 1997. Cortical, thalamic, and amygdaloid projections of rat temporal cortex. *J Comp Neurol* **382**: 153–175.

Shi CJ, Cassell MD. 1999. Perirhinal cortex projections to the amygdaloid complex and hippocampal formation in the rat. *J Comp Neurol* **406**: 299–328.

Sia Y, Bourne JA. 2008. The rat temporal association cortical area 2 (Te2) comprises two subdivisions that are visually responsive and develop independently. *Neuroscience* **156**: 118–128.

Squire LR, Stark CE, Clark RE. 2004. The medial temporal lobe. *Annu Rev Neurosci* **27**: 279–306.

Tinsley CJ, Narduzzo KE, Ho JW, Barker GR, Brown MW, Warburton EC. 2009. A role for calcium-calmodulin-dependent protein kinase II in the consolidation of visual object recognition memory. *Eur J Neurosci* **30**: 1126–1139.

Tinsley CJ, Fontaine-Palmer NS, Vincent M, Endean EPE, Aggleton JP, Brown MW, Warburton EC. 2011. Differing time dependencies of object recognition memory impairments produced by nicotinic and muscarinic cholinergic antagonism in perirhinal cortex. *Learn Mem* (this issue). doi: 10.1101/lm.2274911.

Wan H, Aggleton JP, Brown MW. 1999. Different contributions of the hippocampus and perirhinal cortex to recognition memory. *J Neurosci* **19**: 1142–1148.

Warburton EC, Koder T, Cho K, Massey PV, Duguid G, Barker GR, Aggleton JP, Bashir ZI, Brown MW. 2003. Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron* **38**: 987–996.

Williams LR, McDaniel WF. 1999. Visual relational ("categorical") learning and performance by rats with temporal or partial striate cortical lesions. *Psychobiology* **27**: 341–350.

Winters BD, Bussey TJ. 2005a. Glutamate receptors in perirhinal cortex mediate encoding, retrieval, and consolidation of object recognition memory. *J Neurosci* **25**: 4243–4251.

Winters BD, Bussey TJ. 2005b. Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J Neurosci* **25**: 52–61.

Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ. 2004. Double dissociation between the effects of peri-posterior cortex and hippocampal lesions on tests of object recognition and spatial memory: Heterogeneity of function within the temporal lobe. *J Neurosci* **24**: 5901–5908.

Winters BD, Saksida LM, Bussey TJ. 2006. Paradoxical facilitation of object recognition memory after infusion of scopolamine into perirhinal cortex: Implications for cholinergic system function. *J Neurosci* **26**: 9520–9529.

Xiang JZ, Brown MW. 1998. Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. *Neuropharmacology* **37**: 657–676.

Zhu XO, Brown MW, Aggleton JP. 1995a. Neuronal signalling of information important to visual recognition memory in rat rhinal and neighbouring cortices. *Eur J Neurosci* **7**: 753–765.

Zhu XO, Brown MW, McCabe BJ, Aggleton JP. 1995b. Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience* **69**: 821–829.
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