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1 **Separate elements of episodic memory subserved by distinct hippocampal-prefrontal**
2 **connections**

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11 **Episodic memory formation depends on information about a stimulus being integrated**
12 **within a precise spatial and temporal context, a process dependent on the hippocampus**
13 **and prefrontal cortex. Investigations of putative functional interactions between these**
14 **regions are complicated by multiple direct and indirect hippocampal-prefrontal**
15 **connections. Here application of a pharmaco-genetic deactivation technique enabled an**
16 **investigation of the mnemonic contributions of two direct hippocampal-medial prefrontal**
17 **cortex (mPFC) pathways; one arising in the dorsal CA1 (dCA1), the other in the**
18 **intermediate CA1 (iCA1). While, deactivation of either pathway impaired episodic**
19 **memory, the resulting pattern of mnemonic deficits was significantly different;**
20 **deactivation of the dCA1→mPFC pathway selectively disrupted temporal order**
21 **judgements, while iCA1→mPFC pathway deactivation disrupted spatial memory. These**
22 **findings reveal a major, previously unsuspected division of function among CA1 neurons**

23 **that project directly to the mPFC. Such sub-networks may enable the distinctiveness of**
24 **contextual information to be maintained within an episodic memory circuit.**

25

26 Remembering a past episode or event depends on the successful recall of information, not
27 only about ‘what’ happened but also ‘where’ and ‘when’ the event happened¹. It has been
28 established that the hippocampus is critical for episodic memory, and specifically that the
29 hippocampus is responsible for the integration of these different types of information
30 (what-where-when)^{2,3,4}. However, successful episodic memory also requires the
31 hippocampus to operate in concert with areas of the neocortex^{5,6,7}, and of these, the
32 prefrontal cortex (PFC) is of especial interest⁸. Patient studies have shown that lesions in the
33 PFC disrupt episodic memory^{9,10,11} associative learning¹² and memory for temporal order¹³.
34 Functional imaging has revealed activation in the PFC during episodic memory encoding and
35 retrieval¹⁴. Further there are reports of co-activation of the PFC and hippocampus during
36 episodic memory¹⁵ and electrophysiological recording studies in rodents have revealed
37 increased coherence between the hippocampus and prefrontal cortex during spatial
38 learning¹⁶. Together, such findings support the hypothesis that a functional interaction
39 between the hippocampus and prefrontal cortex is critical for episodic memory. However
40 the precise neural pathways which support such an interaction and the directionality of the
41 interaction are difficult questions to address through fMRI studies or conventional lesion
42 studies and hence remain poorly understood.

43 Animals, including rodents encode and retrieve robust ‘episodic-like’ memories^{17,18} by
44 forming associations between an object and the location (where) and/or occasion (when) it
45 was last encountered and performance of such episodic-like memory tasks have been

46 shown to be impaired by lesions of the hippocampus and medial PFC (mPFC)¹⁹. In rodents,
47 a direct hippocampal projection to the mPFC arises from the CA1 subfield but, in addition,
48 there are multiple indirect projections between the hippocampus and mPFC^{20,21,22}.
49 Consequently it is not clear whether episodic memory function is maintained by projections
50 from the hippocampus to the mPFC or by those from the mPFC back to the medial temporal
51 lobe (including the hippocampus) via polysynaptic pathways. The present study therefore
52 addressed several important questions. First we determined the necessity of a
53 hippocampal-mPFC interaction for episodic memory retrieval, using an animal model.
54 Secondly we examined whether the interaction was mediated by information transfer from
55 the hippocampus to the mPFC via the direct CA1-mPFC connections.

56 The first approach taken to investigate the importance of hippocampal-mPFC
57 interactions in episodic memory was disconnection of these two regions in rats using
58 temporary lesions placed in the hippocampus and mPFC. Episodic memory in rats was
59 tested using an episodic-like memory task, previously shown to be disrupted by bilateral
60 hippocampal lesions¹⁹. The principal behind the disconnection technique is that it prevents
61 two regions from interacting in either cerebral hemisphere by producing unilateral
62 dysfunction of one of the regions in one hemisphere (e.g. in the hippocampus) and
63 unilateral dysfunction of the other region (e.g. the mPFC) in the opposite hemisphere. If
64 memory performance depends upon an interaction between the two regions, disconnecting
65 the circuit in each hemisphere should result in a deficit. To address the second question of
66 the role of direct CA1-mPFC projections in episodic memory we used a novel pharmaco-
67 genetic technique to selectively and reversibly deactivate these pathways.

68

69 RESULTS

70 Episodic memory is disrupted following disconnection of the hippocampus and prefrontal 71 cortex

72 Ten rats had cannulae implanted into both the hippocampus and mPFC. To disconnect the
73 hippocampus and mPFC, NBQX (an AMPA receptor antagonist) was infused unilaterally into
74 the mPFC in one hemisphere and unilaterally into the hippocampus in the opposite
75 hemisphere (NBQX CONTRA) in half the animals. In the other half (control group) NBQX was
76 infused unilaterally into both regions in the same hemisphere (NBQX IPSI). One week later
77 the animals were re-tested using a cross-over design, such that each animal served as its
78 own control. All infusions occurred prior to the memory test phase (see online methods).

79 The episodic-like memory task exploits rats' spontaneous preference for novel, or
80 less recently encountered stimuli or locations compared to familiar, or more recently
81 encountered stimuli or locations. This preference is expressed behaviourally as an increase
82 in the amount of exploration directed towards the less familiar stimulus²³. The task thus
83 assesses the animal's ability to recall the temporal occurrence (when) and spatial context
84 (where) of a previously encountered object (what). In the two sample phases of the task
85 rats explored two different objects in different locations. After a retention delay, the test
86 phase was conducted, all four objects were presented but the location of one object from
87 each sample phase was switched, exploration of all four objects was measured (**Fig. 1a**).
88 Successful memory of all types of information (what, where, when) will result in a pattern of
89 preferential exploration such that most exploration is directed to the object in a novel
90 location (NL) not recently encountered (temporally distant; TD), i.e. the novel location
91 temporally distant object (NLTD). Exploration of the familiar location-temporally distant

92 object (FLTD) or the novel location-temporally recent object (NLTR) is less than for the NLTD
93 object, while the least exploration is directed to the familiar location-temporally recent
94 object (FLTR)¹⁸.

95 Histological analyses confirmed the location of the cannulae tips in the mPFC or
96 dorsal hippocampus (**Fig. 1b**). Animals in the NBQX IPSI condition (n=10) showed the
97 expected pattern of exploration (NLTD>(FLTD,NLTD)>FLTR) as described above. In contrast,
98 animals in the NBQX CONTRA condition (n=10) were significantly impaired in their ability to
99 recall both 'when' and 'where' a specific object had been previously encountered. A two-
100 way repeated measures ANOVA with treatment and object as factors revealed a significant
101 treatment by object interaction $F_{(3,27)}=11.15$, $P=0.001$ (**Fig. 1c**). Analysis of the time spent
102 exploring each of the objects in the test phase revealed that the NBQX IPSI group spent a
103 significantly greater proportion of time exploring the NLTD object than the three other
104 objects (NLTR $P=0.006$; FLTD $P=0.014$; FLTR $P=0.001$), while the NBQX CONTRA group
105 showed no differences in object exploration (NLTD v FLTR $P=0.307$. For all other
106 comparisons $P=1.0$). Finally, one-way ANOVA of the two memory components
107 (location/where and temporal/when) confirmed that the NBQX IPSI group had significantly
108 higher levels of discrimination (location $F_{(1,9)}=43.21$, $P=0.001$; temporal $F_{(1,9)}=10.55$,
109 $P=0.010$) compared to the CONTRA group (**Fig. 1d**). Total object exploration in any phase of
110 the procedure did not differ between treatments, thus two-way ANOVA of object
111 exploration across sample phase 1 and 2 revealed no significant interaction between sample
112 phase and treatment ($F_{(1,9)}= 0.65$, $P=0.44$) (**Supplementary Table 1**).

113

114

These results reveal that a rat's ability to recall both the spatial and temporal information concerning an object's prior occurrence relies on simultaneous neural activity within the hippocampus and mPFC and a functional interaction between these two regions.

Given the requirement for simultaneous information processing within the hippocampus and mPFC for episodic memory task performance, we next investigated the role of direct anatomical connections between these two regions and the directionality of information processing i.e. whether episodic memory is dependent on direct hippocampal input into mPFC. Anatomical tracing studies reveal a topographical organization of the hippocampal-mPFC pathway along its dorso-ventral and transverse axis, with efferents arising in the posterior region of the dorsal hippocampus and in the intermediate hippocampus^{21,24}. In light of this distribution of CA1 to mPFC neurons we decided to test whether both projections (dorsal CA1 region to mPFC; dCA1→mPFC; intermediate CA1 to mPFC; iCA1→mPFC) are involved in episodic-like memory formation.

Deactivation of CA1 pyramidal neurones by Daun02

To disrupt neural activity in the dCA1→mPFC and iCA1→mPFC projections we employed a new pharmacogenetic technique, a modification of the 'Daun02 inactivation' method described previously^{25,26}. These reports have shown that it is possible to identify and manipulate neuronal activity by directing expression of the reporter gene *LacZ* to specific neuronal populations, and then administering the prodrug Daun02 via intracerebral cannulae. Daun02 is converted into daunorubicin by β -D-galactosidase (β -gal), the protein

product of *LacZ*²⁷ which results in a decrease in neuronal activity (deactivation) and disruption of behaviour^{25,26}.

To confirm that Daun02 attenuates activity of CA1 neurones we first expressed *LacZ* to induce β -gal bilaterally in CA1 (see online methods). In each animal Daun02 was delivered into CA1 in one hemisphere and vehicle into the other hemisphere and horizontal hippocampal slices were prepared three days later to perform *in vitro* whole cell recordings. In CA1 neurones from the Daun02 hemisphere, compared to neurones from the vehicle hemisphere (see **Fig. 2** and Table 1), there was a decrease in the number of action potentials in response to depolarising voltage steps (**Fig. 2a**; main effect of treatment: $F_{(1,33)}=12.83$, $P=0.001$), an increase in action potential threshold (**Fig. 2b**; $F_{(1,33)}=7.68$, $P=0.009$) and an increase in the magnitude of the after hyperpolarising potential (**Fig. 2c**; main effect of treatment: $F_{(1,33)}=11.30$, $P=0.002$). Collectively, these effects will reduce the excitability of CA1 pyramidal neurones and provides a possible physiological explanation for how connectivity between regions may be disrupted by Daun02.

Selective deactivation of two direct hippocampal-prefrontal cortex pathways disrupts episodic memory

To assess the behavioural consequences of selective deactivation of the dCA1→mPFC and iCA1→mPFC projections a VSV-G/rabies-G fusion envelope protein pseudotyped EIAV-based lentiviral vector, expressing *LacZ* was injected into the mPFC resulting in retrograde transport of EIAV vector to the nucleus and the subsequent expression of *LacZ* in a number of cell populations which project directly to the mPFC including the hippocampus (**Fig. 3a left**). Daun02 was infused into the HPC subregions via intracerebral cannulae aimed at the dCA1 (n=12) or iCA1 (n=12). Thus, only those neurons expressing β -gal within the direct

hippocampal-mPFC pathways were deactivated (**Fig. 3c,d**) leaving other neuronal populations unaffected.

Infusate spread through dorsal and intermediate hippocampus.

In a separate set of animals, we confirmed the location and spread of infusate into posterior dorsal and intermediate HPC. Infusions of fluorescent conjugated muscimol (FCM) into the dorsal CA1 revealed of drug spread in dorsal and medial areas of the CA1 which extended from -3.6 mm to -5.6 mm relative to bregma along the anterior posterior axis and 1.0mm to 3.4mm from the midline along the mediolateral axis (**Supplementary Fig. 1**). Calculation of the total area affected was 0.45 mm^3 . There was additional drug spread into the dentate gyrus and the overlying somatosensory cortex, however as these areas did not express β -gal due to absence of their direct projections mPFC (**Fig. 3b**), there is unlikely to be an effect Daun02 on neurons in these areas. Infusions into the intermediate CA1 resulted in drug spread in posterior and lateral areas of CA1 (**Supplementary Fig. 1**) which extended from -5.2 mm to -7.0 mm relative to bregma along the anterior posterior axis and extend 3.8 mm to 5.8 from the midline along the mediolateral axis. In total the infusion was spread across an area of 0.35 mm^3 . There was additional drug spread into the dentate gyrus and the overlying posterior parietal and auditory cortical regions again areas which did not show expression (**Fig. 3c**). Importantly the results reveal non-overlapping patterns of fluorescence around the dorsal and intermediate infusion sites.

In this context we considered the boundaries of these regions to be in line with those described by Dong et al.²⁸ where at the anterior level of the hippocampus (in our study,

using rats 4.5-5.6mm posterior to bregma) the border between the dorsal and intermediate CA1 is parallel to the ventral edge of the lateral blade of the dentate gyrus.

Mnemonic impairments following deactivation of the dorsal HPC and intermediate HPC projections to the medial prefrontal cortex

Two way repeated measures ANOVA of episodic memory performance following infusion of Daun02 into the dorsal or intermediate HPC or revealed significant treatment by object interactions in both conditions (dCA1→mPFC, **Fig. 3d**, $F_{(3,30)}=3.06$, $P=0.043$, $n=11$; iCA1→mPFC, **Fig. 3f**, $F_{(3,33)}= 8.35$, $P=0.001$, $n=12$). Further post hoc comparisons revealed that deactivation of the dCA1→mPFC pathway disrupted the animals' ability to discriminate the temporal order ('when') aspect of the episodic-like memory task. Thus in the dCA1 deactivation group, post hoc analyses revealed that the Daun02 treated animals showed a significant increase in exploration of the recently encountered object in a novel spatial location (NLTR) ($P=0.037$) compared to control animals indicating that the Daun02 animals detected the novel spatial location of the object irrespective of the recent exploration of that object. In addition the Daun02 group showed significantly less exploration of the temporally distant object in a familiar location (FLTD) compared to the control group (**Fig. 3d**, $P=0.001$). Separate calculations of the discrimination ratios for the temporal and location components of the episodic memory revealed no significant difference between the Daun02 and control animals in the performance of the temporal component of the task (**Fig. 3e** $F_{(1,10)}=1.77$, $P=0.213$), but a significant enhancement in the performance of the

204 location component of the task (**Fig. 3e** $F_{(1,10)} = 9.02, P=0.013$). Further analyses, comparing
205 the discrimination ratios against chance performance, revealed that the control animals
206 showed significant discrimination of both the location and temporal components (location
207 $t_{(10)} = 3.71, P=0.004$; temporal $t_{(10)}=4.07, P=0.002$) while the Daun02 animals showed
208 significant discrimination of the location component ($t_{(10)}=6.05, P=0.001$) but not the
209 temporal component ($t_{(10)}=0.30, P=0.771$).

210 Post hoc analyses of the effects of disruption of the iCA1→mPFC pathway revealed
211 impairments in the spatial aspect of episodic-like memory. Thus in the iCA1→mPFC
212 deactivation group the Daun02-treated animals showed a significant decrease in their
213 exploration of the recently encountered object in a novel spatial location (NLTR, $P=0.018$)
214 compared to control animals and an increase in exploration of the FLTD object ($P=0.001$),
215 indicating that following Daun02 treatment the animals could not discriminate the change in
216 the position of the object in the arena (**Fig. 3f**). Further separate one-way ANOVA of the
217 location and temporal components (**Fig. 3g**) confirmed that there was a significant
218 difference in the discrimination performance of the location component between the
219 control and Daun02 treated animals ($F_{(1,11)}=18.17, P=0.001$) but no significant difference in
220 the discrimination of the temporal component ($F_{(1,11)}=1.71, P=0.217$). Total object
221 exploration in any phase of the procedure, under either treatment did not differ (**Table 2**).

222

223 **Temporal order and object-in-place memory are mediated by distinct hippocampal-**
224 **prefrontal cortex pathways**

225 In the episodic memory task, exploration of the four objects cannot be considered
 226 completely independent, thus reduced exploration of one object may be either a cause or
 227 consequence of higher exploration of another object. In this study disruption of the
 228 dCA1→mPFC pathway reduced exploration of the FLTD object and increased exploration of
 229 the NLTR object, and significantly disruption of the iCA1→mPFC pathways produced the
 230 opposite pattern of exploration. Hence the next experiment further examined this
 231 dissociation using a battery of behavioural paradigms that would assess object spatial
 232 memory (using an object-in-place task), object temporal order memory and spatial temporal
 233 order memory selectively (**Figs. 4a,b,c**). Previous studies have shown that object-in-place
 234 and object temporal order memory depend on a hippocampal-mPFC interaction²⁹ and here
 235 we confirmed that spatial temporal order memory was also disrupted by disconnection of
 236 the hippocampus and mPFC (**Fig. 4d** one way ANOVA $F_{(1,9)}=24.14$, $P=0.001$, $n=10$). Further
 237 analyses revealed that the IPSI infused animals showed a significant preference for exploring
 238 the location the object occupied earlier in the sequence ($t_{(9)}=7.93$, $P=0.001$) whereas
 239 CONTRA infused animals explored both locations occupied by the object equally ($t_{(9)}=-0.83$,
 240 $P=0.427$) (**Fig. 4d**) Overall object exploration levels were unaffected (**Supplementary Table**
 241 **2**). Deactivation of the dCA1→mPFC projection significantly impaired performance in the
 242 object temporal order task (**Fig. 4e**, one-way ANOVA main effect of drug, $F_{(1,11)}=51.75$,
 243 $P=0.001$, $n=12$) whereas object-in-place and spatial temporal order memory were
 244 unaffected (**Fig. 4e**, one-way ANOVA object-in-place $F_{(1,10)}=0.05$, $P=0.833$; spatial temporal
 245 order $F_{(1,10)}=0.25$, $P=0.626$, $n=11$ for both). In direct contrast, selective deactivation of the
 246 iCA1→mPFC projection significantly impaired object-in-place performance (**Fig. 4f**, one-way
 247 ANOVA main effect of drug, $F_{(1,11)}=38.41$, $P=0.001$, $n=12$) but not object temporal order or
 248 spatial temporal order (**Fig. 4f**, one-way ANOVA object temporal order $F_{(1,11)}=0.83$, $P=0.383$;

spatial temporal order $F_{(1,11)}=0.009$, $P=0.774$, both $n=12$). Overall object exploration in the sample or test phases of any of the tasks was not affected by Daun02 infusions into either the dHPC or iHPC (**Table 3**).

Deactivation of the dCA1→mPFC projection or iCA1→mPFC projection had any effect on performance of a hippocampal-independent object recognition memory tasks²⁹ (**Fig. 5a,b,d,e**) or a hippocampal-dependent object location task²⁹ (**Fig. 5c,d,e**).

These results confirm a selective requirement for the dCA1→mPFC projection in the processing of object temporal order information and for the iCA1→mPFC projection in the processing of object spatial information. That deactivation of the dCA1→mPFC or iCA1→mPFC projections did not affect spatial temporal memory reveals that this process is critically dependent on alternative routes between the hippocampal and mPFC projections. Finally as object location and object recognition were not impaired and overall exploration levels in any of the tasks was not affected (**Supplementary Table 3**) our results cannot be accounted for by a non-specific disruption of hippocampal function nor a general reduction in sensitivity to novelty.

DISCUSSION

The construction of an episodic memory requires that information about an event (e.g. an encounter with a stimulus) be integrated with both the spatial and temporal context in which the encounter occurred. Here we have shown, for the first time in a rodent model, that the retrieval of episodic memory requires a functional interaction between the hippocampus and mPFC, consistent with evidence that spatio-temporal context-based

271 object memory formation is achieved through mPFC-hippocampal interactions^{29,30}. Using a
272 novel pharmacogenetic technique to produce neuronal deactivation we next demonstrated
273 that inputs to the mPFC from the dorsal and intermediate CA1 separately process the
274 temporal aspects and spatial aspects of episodic-like memory respectively. Hence
275 information concerning an object's temporal and spatial attributes, which both contribute
276 to episodic memory formation, is mediated by separate direct CA1→mPFC pathways.

277 Our results dissociated the contributions of dCA1 and iCA1 to object spatial and
278 object temporal memory in an episodic memory task, but crucially also in a series of
279 behavioural tasks which investigate each memory dimension separately. Single item object
280 recognition and object location memory were both unaffected, hence the dCA1→mPFC and
281 iCA1→mPFC pathways have distinct and hierarchical roles in relaying object associations.
282 These results also extend theoretical accounts of the neural basis of episodic memory
283 integration to include a role for the mPFC as object-spatial and object-temporal information
284 appear to be distinct in CA1. The segregation of inputs into the mPFC may allow for
285 additional cognitive flexibility in the top down processing of the mPFC, specifically in the
286 construction of contextual representations used to guide subsequent retrieval. Indeed
287 functional interaction between the hippocampus and mPFC has been implicated in both
288 encoding and retrieval⁸ but the relatively poor temporal specificity of the Daun02 technique
289 means that it is not possible in the present study to draw conclusions concerning the
290 relative contribution of the dCA1→mPFC and iCA1→mPFC pathways to these two processes.
291 The application of optogenetic techniques with more precise temporal control will enable
292 such questions to be explored.

Our results are consistent with neuronal recording studies showing that the CA1 contains neurons which code temporal and spatial information separately^{31,32,33} but given that these studies have confined their recording to the dorsal CA1, the anatomical distribution of these different neuronal populations has yet to be fully described. How might the topographical representation of object novelty and spatial novelty arise? One possible explanation may be in the diversity of inputs from the entorhinal cortex which terminate directly or indirectly in CA1. The distal CA1, i.e. the CA1 closest to the subiculum, and the regions targeted by the dCA1 cannulae in the present experiment, receives a direct input from the lateral entorhinal cortex (LEC)^{34,35,36}. Functionally the distal CA1 is critical for discrimination of the relative novelty of objects³⁷ and direct inputs from LEC to CA1 are important for recency memory³⁸ consistent with the finding in the present study that the dCA1→mPFC projection facilitates object temporal order memory. The intermediate hippocampus, here shown to mediate the object-spatial discrimination, receives input from cells in the MEC which are highly tuned to spatial information³⁹ and functionally the intermediate hippocampus controls precise place learning⁴⁰. Hence our data extend the notion of a topographically organised cortico-hippocampal processing systems for object spatial and object temporal order memory beyond the hippocampus to the mPFC.

Given the necessity for a functional HPC-mPFC interaction for spatial temporal order memory, it was intriguing that selective deactivation of either of the direct CA1-mPFC pathways had no effect on performance of this task. This apparent dissociation suggests that the information processing demands of the spatial temporal task are such that other routes between the hippocampus and mPFC are critical. While there are no direct projections from the mPFC back to the hippocampus, the mPFC may exert top-down

control of the hippocampus indirectly, for example via the LEC⁴¹ or via nucleus reuniens (NRe) of the thalamus²². Indeed, recent studies have revealed a role for the NRe in contextual memory⁴² and we have shown that selective excitotoxic lesions of the NRe impair spatial temporal memory (Barker and Warburton, *unpublished*). Further experiments are now required to trace the precise anatomical pathways through which the NRe may affect hippocampal and mPFC processing. It is also possible that the spatial temporal order task requires further hippocampal cortical interactions not explored here. In this task the same object is encountered in different locations within the same arena and hence depends on processing of competing local and global cues. Evidence suggests that the CA1 receives local cue information, such as that associated with the object and its immediate location in the arena from LEC via the CA3^{43,44}, while global cue information, such as that associated with the experimental room or the testing arena, is provided directly to CA1 from the MEC⁴⁵. For the resolution of conflict between the local cue and global cue information, as might be required during the spatial temporal task, on-going interactions between CA1 and MEC are necessary^{44,45} but not direct interactions between the hippocampus and mPFC, further studies will be required to examine this hypothesis.

The present demonstration of a separation of mnemonic processing within the hippocampus fits with current models of functional segregation within the primate and rodent brain along the longitudinal axis of the hippocampus⁴⁶. Models initially suggesting a sharp delineation between a dorsal 'cognitive' and ventral 'emotional' hippocampus⁴⁷, have been refined and now include multiple functional subdivisions of the hippocampus^{48,49,50} based on gene expression, and electrophysiology as well as anatomical connections. This topographical division of hippocampus function originating within parahippocampal cortex, is now clearly shown to extend beyond CA1 to mPFC and further our finding of a separation

of function within the output pathways of CA1, suggests that integration of information for episodic memory is not purely a function of the hippocampus .

The demonstration of parallel processing networks, for the spatio-temporal context of an object, from CA1 to mPFC thus addresses important questions concerning the nature of information processing via hippocampal-mPFC interactions, as well as broader issues concerning the structure of episodic memory formation and retrieval. Our results support the view that the hippocampus is critical for the formation of representations of an object's spatial and temporal context, and hence the hippocampus acts as a key hub for episodic memory. However episodic memory performance also depends on these representations being relayed to the mPFC via functional subnetworks which importantly enable differentiation of the spatial and temporal contexts in which an item is represented.

Thus our data provide a novel insight into the complexity of hippocampal-mPFC interactions. Such organisation has not been previously described, and may lead to a new understanding of the anatomy of episodic memory.

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486

487 **Author Contributions** E.C.W., G.R.I.B., Z.I.B. and J.B.U. contributed to the study design,

488 G.R.I.B., E.C.W., H.S. contributed to the behavioural experiments and data collection, J.B.U.,

489 L.F.W., G.S.R., K.A.M. designed, optimised and provided the viral constructs, G.R.I.B.

490 conducted the surgery, P.J.B. performed and analysed electrophysiology. E.C.W. and G.R.I.B.

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499

500 **Competing Financial Interests**

501 The authors declare no competing financial interests.

Figure Legends

Figure 1. Episodic memory depends on a functional interaction between the hippocampus and mPFC

a, Scheme of the episodic memory task. In each sample phases each animal is allowed to explore two different objects each located in a unique position in the arena. In the test phase the animal is presented with all four objects but the location of one object from each sample phase is changed such that each object has a particular spatial-temporal association: novel location-temporally distant (NLTD), novel location-temporally recent (NLTR), familiar location- temporally distant (FLTD), familiar location temporally recent (FLTR). **b**, Cannula localisation in mPFC and hippocampus. **c**, Episodic memory performance was significantly impaired in the NBQX CONTRA, compared to the NBQX IPSI group (Two-way ANOVA treatment by object interaction $F_{(3,27)} = 11.15$, $P=0.001$; main effect of object $F_{(3,27)} = 5.42$, $P=0.005$) **d**, Disconnection of the mPFC-HPC significantly impaired both the location and temporal components of episodic-like memory. Thus NBQX IPSI infused animals showed significantly higher levels of discrimination for both memory components compared to the NBQX CONTRA infused animals. Data presented as mean + sem. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Figure 2. Daun02 attenuates activity of CA1 pyramidal neurones.

a, CA1 pyramidal neurones fire significantly fewer action potentials (APs) in response to 500 ms current injections when treated with Daun02 compared to vehicle (Two-way ANOVA main effect of treatment $F_{(1,33)} = 12.83$, $P=0.001$; main effect of current injection $F_{(3.9, 1.8)} = 778.61$, $P=0.0001$; interaction between treatment and current injection $F_{(3.9, 1.8)} = 1.47$, $P=0.239$) . Inset shows representative traces following 100 pA injection (scale bars = 200 ms, 20mV). **b**, The AP threshold of pyramidal cells treated with Daun02 was significantly more depolarised than vehicle treated neurons (One-way ANOVA $F_{(1,33)} = 7.68$, $P=0.009$). **c**, 5-25 brief (2 ms) current injections, each resulting in a

single action potential, were applied at 50 Hz and resulted in medium afterhyperpolarising potentials (mAHP) which were significantly larger in Daun02 treated neurons than vehicle (Two-way ANOVA main effect of treatment $F_{(1,33)}=11.30$, $P=0.002$, main effect of AP number $F_{(2.0, 66.8)}=8.61$, $P=0.0001$, interaction between treatment and AP number $F_{(2.0, 66.8)}=4.47$, $P=0.015$). Insets show representative mAHPs following 10 APs (scale bars = 200ms, 2 mV). N = 17 cells from 10 slices from 4 animals vehicle, Daun02: 18 cells from 10 slices from 4 animals. Data presented as mean + sem.

Figure 3. Selective deactivation of the dCA1-mPFC projection disrupts the temporal component of episodic memory while selective deactivation of the iCA1-mPFC projection disrupts the spatial component of episodic-like memory.

a, Expression of the *LacZ* construct in mPFC (left) and diagram of the injection site of the viral construct and cannula placement (right). Injection of a VSV-G/rabies-G fusion envelope protein pseudotyped lentiviral EIAV vector, expressing the reporter gene *LacZ* was injected into the mPFC transduces neurons in anatomically connected regions including the hippocampus. Bilateral cannulae in the hippocampus enabled the direct infusion of Daun02 to selectively deactivate the hippocampal-mPFC projection as indicated. **b,c** Histological sections showing cannulae tract (CT) and transduced neurons in dCA1 (**b**) and iCA1 (**c**). Hippocampal areas CA3 and dentate gyrus (DG) are also shown. Numbers refer to relative position from bregma and black scale bars are shown on each image (1000 μ m). **d**, Episodic memory was significantly disrupted by deactivation of the direct dCA1 $\rightarrow$ mPFC projection (Two-way ANOVA treatment by object interaction $F_{(3,30)}=3.06$, $P=0.043$; main effect of object $F_{(3,30)}=14.93$, $P=0.001$) (n=11). **e**, Analysis of location and temporal order components of the episodic memory performance clearly shows that deactivation of the dCA1 $\rightarrow$ mPFC projection impaired discrimination of the temporal component and enhanced discrimination of the location component. **f**, Episodic memory was significantly disrupted by deactivation of the CA1 $\rightarrow$ iHPC projection (Two-way ANOVA treatment by object interaction ($F_{(3,33)}=8.35$, $P=0.001$; main effect of object $F_{(3,33)}=65.03$, $P=0.001$)

551 (n=12). **g**, Analysis of location and temporal order components of the episodic memory
552 performance shows that deactivation of the iCA1→mPFC projection significantly impaired the
553 location, but not the spatial component. Data presented as mean + sem. * $P<0.05$; ** $P<0.01$;
554 *** $P<0.001$.

555

556 **Figure 4. Deactivation of the dCA1-mPFC projection selectively impaired object-temporal**
557 **order memory whereas selective deactivation of the iCA1-mPFC projection impaired**
558 **object-in-place memory.**

559 **a**, Object-in-place task with a 1h retention delay. **b**, Object temporal order task. **c**, Spatial temporal
560 order task. **d**, Spatial temporal order memory was significantly impaired following infusion of NBQX
561 into the mPFC and HPC in opposite hemispheres (NBQX CONTRA) **e**, Deactivation of the
562 dCA1→mPFC pathway by Daun02 impaired object temporal order performance (n=12) without
563 affecting object-in-place or spatial temporal order memory (n=11 for both). **f**, Deactivation of the
564 iCA1→mPFC projection by Daun02 produced a selective impairment in object-in-place performance
565 but not object temporal order or spatial temporal order (n=12). Data presented as mean + sem.
566 ** $P<0.01$; *** $P<0.001$.

567

568 **Figure 5. Deactivation of either the dCA1-mPFC or iCA1-mPFC projection did not alter**
569 **either object recognition or object location memory.**

570 **a**. Object recognition task based on object temporal order task. **b**. Object recognition task based on
571 object-in-place task. **c**. Object location task with a 4h retention delay. **d**. Deactivation of the direct
572 dCA1→mPFC projection did not affect object recognition (one-way ANOVA $F_{(1,10)}=0.49$, $P=0.501$,
573 n=11) or object location performance (one-way ANOVA $F_{(1,11)}=0.05$, $P=0.829$, n=12). **e**. Deactivation

574 of the direct iCA1→mPFC projection did not affect object recognition (one-way ANOVA $F_{(1,11)}=0.04$,
575 $P=0.854$, $n=12$) or object location performance (one-way ANOVA $F_{(1,11)}=0.01$, $P=0.909$, $n=12$). Data
576 presented as mean + sem.

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583 **ONLINE METHODS**

584 **Subjects.** All experiments were conducted in naive adult male Lister Hooded rats (Charles
585 River, UK, weighing 300-350 g at the start of the experiments). The animals were housed, in
586 pairs, under a 12-h/12-h light/dark cycle (light phase 18:00 – 6:00 h). Behavioural training
587 and testing were conducted during the dark phase of the cycle. Food and water were
588 available *ad libitum* throughout the experiment. All animal procedures were performed in
589 accordance with United Kingdom Animals Scientific Procedures Act (1986) and associated
590 guidelines. All efforts were made to minimize any suffering and the number of animals used.
591 Each rat was randomly allocated to an experimental group prior to surgery.

592

593 **Stereotaxic surgery for cannula implantation.** Each rat was anaesthetised with isoflurane
594 (induction 4%, maintenance 2-3%) and secured in a stereotaxic frame with the incisor bar
595 set at 3.3 mm below the interaural line. Four stainless steel guide cannulae (26 gauge,
596 Plastics One, Bilaney, UK) were implanted bilaterally into the hippocampus and medial
597 prefrontal cortex of each rat through burr holes in the skull at the co-ordinates relative to
598 skull at bregma, hippocampus: anterior-posterior (AP) -4.3mm; medial-lateral (ML) $\pm$ 2.5mm;
599 dorsal-ventral (DV) -2.8mm from dura; medial prefrontal cortex (AP+3.2mm ; ML $\pm$ 0.75mm;
600 DV-3.5mm). The cannulae were anchored to the skull by stainless steel skull screws (Plastics
601 One, Bilaney, UK) and dental acrylic. Following surgery, each animal was given fluid
602 replacement therapy (5ml saline, s.c.) and analgesia (0.05 ml Vetgesic, i.m.), and was housed
603 individually for one week post-surgery and were subsequently housed in pairs for the
604 duration of the experiments. The animals were allowed to recover for at least 14 days

before habituation to the testing arena began. Between infusions 33 gauge obdurators (Plastics One, Bilaney, UK) were used to keep the cannulae patent.

Vector constructs. Lentiviral vectors based on the equine infectious anaemia virus (EIAV) were produced by transient transfection of human embryonic kidney 293T cells with three plasmids ((i) vector genome, encoding the *LacZ* gene, (ii) optimized *gag-pol* packaging component and (iii) the pseudotyping plasmid encoding a VSVG/rabies-G fusion envelope glycoprotein) using Lipofectamine 2000 (Invitrogen, UK) according to the manufacturer's instructions. Cell supernatants were harvested 24-48 hours following transfection and concentrated by 2000-fold using two centrifugation steps comprising a low speed centrifugation at 6,000 $\times g$ for 16 hours at 4°C and ultracentrifugation at 50,000 $\times g$ for 90 mins at 4°C. The vectors were resuspended in a buffer containing tromethamine, NaCl, sucrose and mannitol. The titre of the vesicular stomatis virus VSV-G/rabies-G pseudotyped EIAV-*LacZ* viral vector as determined by an integration (DNA) titre assay was 4×10^8 transducing units/mL.

Stereotaxic surgery for deactivation of specific projections. Rats were anaesthetised and secured in a stereotaxic frame as described above. Viral particles were delivered bilaterally into the medial prefrontal cortex (AP+3.2mm; ML $\pm$ 0.5mm; DV-4.3mm) 2.0 μ l per hemisphere at a rate of 200nl/min. Cannulae were implanted to target either the dCA1, n=12 (AP - 4.3mm, ML $\pm$ 2.5mm, DV -2.6mm) or the iCA1, n=12 (AP-6.3mm, ML $\pm$ 5.3mm, DV-4.0mm), and secured to the skull as described. Animals were allowed to recover for 5 weeks before behavioural testing commenced.

Histology. Following completion of the experiments each rat was anaesthetised with Euthetal (Rhône Mérieux) and perfused transcardially with phosphate buffered saline followed by 4% paraformaldehyde. Following removal the brain was post-fixed in paraformaldehyde for either 2 h (X-gal histochemistry) or 24 h (cresyl violet staining) before being transferred to 30% sucrose in 0.2 M phosphate buffer for 48 h. Sections were either incubated in reaction buffer for X-gal histochemistry (see below) or stained with cresyl violet to verify cannulae locations against standardised sections of the rat brain⁵³. Any mounting artefacts were removed from the histology images (**Fig. 3b & 3c**).

X-gal histochemistry. Coronal sections (50 µm) were incubated in the X-gal reaction buffer (5 mM K₃[Fe(CN)₆], K₄[Fe(CN)₆] · 3H₂O, 2 mM MgCl₂, CA-630 0.02 %, C₂₄H₃₉NaO₄ 0.01 %, and phosphate buffered saline) for 6h at 37 °C. Sections were washed three times with PBS and mounted onto chrom-alum-coated slides. Slides were left to dry and then counter stained with Eosin and coverslipped with Vectashield (Vector laboratories, Burlingame, California).

Infusate spread through dorsal and intermediate CA1. Fluorophore-conjugated muscimol (FCM) (Muscimol, BODIPY® TMR-X conjugate; Invitrogen UK) was infused to establish that the dorsal and intermediate CA1 infusions targeted distinct neuronal populations within the hippocampus. Animals (n=3) were implanted with bilateral guide cannula (as outlined in surgery) targeting either the dorsal or intermediate CA1 region of the hippocampus (one in each hemisphere). Animals were allowed 2 weeks to recover from surgery. The infusion procedure was identical to that used for all other infusions (see infusion procedure), briefly 0.5µl of FCM (0.5 mg/ml, 5% DMSO) was infused into each hemisphere at a rate of 0.25

653 $\mu\text{l}/\text{min}$. Twenty minutes after the start of the infusion the animals were anaesthetised with
654 Euthetal (Rhône Mérieux) and transcardially perfused (as described in histology section).
655 Brains were sectioned ($40\mu\text{m}$), mounted onto slides, counterstained with dapi and
656 coverslipped with Vectashield (Vector laboratories, Burlingame, California). Sections were
657 imaged using a Leica DM500b microscope and Leica DFC300FX camera, fluorescence images
658 of FCM spread were overlayed onto images of dapi stain to allow anatomical localisation of
659 the FCM. Area of the hippocampus filled with FCM was measured (Qwin, Leica) every
660 $160\mu\text{m}$ along the anterior posterior axis of the hippocampus and the total volume of the
661 hippocampus filled with FCM was calculated. In addition the extent of FCM spread in the
662 anterior posterior and the mediolateral axes was assessed for each infusion site.

663

664 **Infusion procedure.** General infusion procedures were performed as previously
665 described^{51,52} NBQX (Ascent Scientific) dissolved in sterile 0.9% saline solution was infused
666 at $1\text{mM}/\text{side}$ at a volume of $0.5\mu\text{l}$, over a 2 min period immediately prior to the test phase.
667 Daun02 (Ascent Scientific custom synthesis) was dissolved in a 45% w/v (2 Hydroxypropyl)-
668 β -cyclodextrin (Sigma) and 2% DMSO solution (Fischer Scientific), and infused at $4\text{mg}/\text{ml}$, a
669 volume of $0.5\mu\text{l}$ over a 2min period. Thus each hippocampus received $2\mu\text{g}$ of Daun02.
670 Vehicle infusion consisted of a 45% w/v (2 Hydroxypropyl)- β -cyclodextran and 2% DMSO
671 solution. Daun02 was infused 3 days prior to the start of behavioural testing, as described
672 previously²⁵.

673

674 **Behavioural Protocols (Figs 1-4)** Exploration occurred in a wooden open-topped arena $90 \times$
675 100 cm , walls 50cm high and was video recorded for subsequent analysis. The stimuli
676 presented were copies of objects composed of 'Duplo' (Lego UK Ltd, Slough, UK) that varied

677 in shape, colour and size ($9 \times 8 \times 7$ cm to $25 \times 15 \times 10$ cm) too heavy for the animal to
678 displace.

679 *Habituation:* For four days prior to the commencement of behavioural testing the animals
680 were placed in the arena for 5min.

681 *Episodic memory task (Fig1a):* The task involved three phases, two sample phases and one
682 test phase with a one hour delay between each phase. In each sample phase the animals
683 explored two different objects in a unique location in the arena for 10 min. In the test
684 phase (5 min) animals were presented with all four objects but one object from each sample
685 phase had switched location and thus possesses a unique spatial-temporal representation:
686 novel location- temporally distant (NLTD); familiar location-temporally distant (FLTD); novel
687 location-temporally recent (NLTR); familiar location-temporally recent (FLTR). The time
688 spent exploring each of the objects was recorded.

689 *Object-in-place memory task (Fig 4a):* This task comprised a sample and test phase
690 separated by a 1 hr delay. In the sample phase the rats explored four different objects for 5
691 minutes. In the test phase (3 min), two of the objects e.g. B and D exchanged positions. The
692 time spent exploring the two objects that had changed position was compared to the time
693 spent exploring the two objects that had remained in the same position.

694 *Object temporal order memory task (Fig 4b):* This task involved four sample phases (S1-S4)
695 and a test phase each separated by 1h. In each sample phase the rats explored two copies
696 of the sample object for four minutes. Different objects were presented in each sample
697 phase. In the test phase (3 min) the rats were presented with objects from S2 and S3 and
698 the time spent exploring each object was recorded.

699 *Spatial temporal order memory task (Fig 4c):* This task was identical to the object temporal
700 order memory task, except that the rats were exposed to the same object in a series of

different spatial locations in four sample phases (S1-4). In the test phase (3 min) the rats were presented with two copies of the object, one object was in the S2 location, the other object was in the S3 location and the time spent exploring each object was recorded.

Object recognition task: To assess recognition memory the dCA1 group were tested for recognition memory performance in the temporal order memory task (*Fig 5a*) thus the dCA1 group were presented with the object presented in sample phase 2 and a novel object. In the iCA1 group novel object recognition was tested in a modified version of the object-in-place task (*Fig 5b*), i.e. four objects were presented in the sample phase, and two objects were replaced by novel objects at test, thus the iCA1 group were presented with two novel objects and two familiar objects. As for the other tasks the time spent exploring each object was recorded.

Object location task (Fig 5c): In the sample phase the rat was exposed to two objects for 3 min. After a delay of 4 h the rat was placed back in the arena which contained an identical object from the sample phase in the same position as in the sample phase and a fourth identical object was in a novel location. The time spent exploring each object was recorded.

Experimental design. A within subject design was used to assess the effect of disconnecting the hippocampus and mPFC or deactivation of a specific pathway on each memory task tested. The cohort of animals which undertook the NBQX disconnection study was tested in the episodic-like memory task, and the spatial temporal order task. Both cohorts which undertook the deactivation studies (dCA1→mPFC and iCA1→mPFC) performed each of the memory tasks described. Each experiment consisted of two trials separated by a minimum of 7 days, each animal received either a control infusion (NBQX IPSI or vehicle) or a

treatment infusion (NBQX CONTRA or Daun02) in the other. Choice of infusate for each experimenter was determined randomly by the person conducting the infusions. On the second trial each animal received the alternative treatment. Infusates were counterbalanced across animals so that equal numbers of animals received control and treatment infusions in each run of a task. Within each behavioural experiment the location and/or order of object presentation was also counterbalanced.

Assessment of exploration in behavioural tasks. For all the spontaneous exploration tasks, the proportion of time each animal spent exploring each object was analysed. Exploratory behaviour was strictly defined as the animal directing its nose towards the object at a distance of < 2 cm and was scored by the experimenter, blind to the infusion status of each animal.

In the episodic memory task for analysis of the location memory component within the episodic memory task the following formula was used $(NLTD+NLTR)/(FLTD+FLTR)/(NLTD+NLTR+FLTD+FLTR)$. To analyse the temporal component within the episodic memory task the following formula was used $(NLTD + FLTD)/(NLTR+FLTR)/(NLTD+NLTR+FLTD+FLTR)$. For the object-in-place, object temporal order and spatial temporal order tasks, object discrimination was determined using a discrimination ratio, calculated as the difference in time spent by each animal exploring the novel compared to the familiar object divided by the total time spent exploring all objects.

Acute slice preparation. Four Animals were injected with 2 µl of virus containing equal parts EIAV-LacZ and EIAV-GFP into iCA1 implanted with bilateral cannulae into iCA1 as described above. Following at least 5 weeks recovery each animal was given an infusion of Daun02

into one hemisphere and vehicle into the other. 3 days later animals were anaesthetised with 4 % isoflurane and decapitated. Brains were rapidly removed and placed in 4 °C oxygenated (95 % O₂, 5 % CO₂) sucrose solution (in mM: 189 sucrose, 10 D-glucose, 26 NaHCO₃, 3 KCl, 5 MgSO₄·7H₂O, 0.1 CaCl₂, 1.25 NaH₂PO₄). 350 µm thick horizontal hippocampal slices were prepared using a vibratome (7000smz-2, Campden Instruments). Slices were kept such that each hemisphere was separate and slices were kept in order of being cut in 34 °C aCSF (124 NaCl, 26 NaHCO₃, 3 KCl, 1.4 NaH₂PO₄, 1 MgSO₄·7H₂O, 10 D-glucose and 2 CaCl₂) for 30 mins and then at room temperature for at least another 30 mins before use. The experimenter was blind to which hemisphere had received Daun02 and which vehicle.

Electrophysiology. Hippocampal slices were placed in a submerged recording chamber and perfused with 34 °C aCSF at 2ml.min⁻¹. A cannula tract was observable in stratum pyramidale of some slices – experiments were performed from slices where cannula tracts were visible or from immediately adjacent slices only so as to ensure the cells recorded from had been previously exposed to Daun02 or vehicle. Fluorescence imaging was used to visualise cells expressing GFP and recordings were made from CA1 pyramidal neurones that were either GFP positive or in a region of dense GFP labelling so as to maximise the probability that recorded cells expressed LacZ. CA1 pyramidal neurones, selected based on location of the soma within stratum pyramidale and pyramidal morphology under oblique infra-red imaging, were patch-clamped using 2-6 MΩ borosilicate glass (GC150F-10; Harvard Apparatus) electrodes filled with potassium gluconate-based solution (120 K-gluconate, 10 KCl, 40 HEPES, 0.5 EGTA, 0.3 Na-GTP, 2 Mg-ATP, 1 MgCl, 2 NaCl; pH 7.25, 295 mOsm). Current-clamp recordings were obtained using an Axon Multiclamp 700B amplifier, pClamp

10 acquisition software, filtered at 4 KHz and digitised at 100 kHz (Digidata 1322A; Molecular Devices). Following recording of the resting membrane potential current was injected such that membrane potential was -70 mV following post-hoc subtraction of the liquid junction potential. To assess neuronal firing cells were injected with 500 ms pulses ranging from +100 to +300 pA steps (**Fig. 2a, Table 2**). To measure afterhyperpolarisations a series (5-25) of brief (2 ms) +2000 pA steps were injected at a frequency of 50 Hz such that each pulse resulted in a single action potential (**Fig 2c**). To measure subthreshold membrane properties (Fig. 2b, Table 2) a -100 pA current injection was given for 500 ms.

Statistical analysis. The sample size for each experiment was determined by previous studies conducted in both our and other laboratories. Power calculations on previously reported data^{51,52} collected in our laboratory suggest that to achieve a power of 0.8, a group size of eight is required. Larger sample sizes were used to allow for maintenance of power should animals be excluded due to cannulae misplacement or blockage.

Memory performance between groups was compared using an ANOVA analyses using SPSS (IBM). Statistical analyses were designed using an assumption of normal distribution and similar variance, but this was not formally tested. Performance in the episodic-like memory task was compared using a two-way repeated measures ANOVA with treatment and object as factors, *post-hoc* comparisons used a Bonferroni correction. Performance in all the other tasks used was compared using a one-way repeated measures ANOVA with treatment as the factor. In addition to test whether each group of animals could significantly discriminate between objects or pairs of objects within each task, the discrimination ratios of each condition was compared to zero (chance performance) using a one-sample t-test (two-tailed). Cannula blockage resulted in the loss of an animal from the dCA1→mPFC group

prior to the object recognition memory test (as indicated by reduced degrees of freedom in the quoted statistical tests). The significance of the results was accepted at $P < 0.05$. Electrophysiological recordings were imported into MATLAB using code from SourceForge (<https://sourceforge.net/projects/libaxon/>) and analysed using code kindly provided by Dr Jon T. Brown, Exeter University, U.K. (Supplementary Software 1, see^{54,55} for details, values reported in Table 1 are subtracted sag and steady-state Rinput) and statistical tests using SPSS (IBM). Neuronal firing rates and after hyperpolarisation amplitude between groups were compared using a two-way mixed design ANOVA with treatment as a between subjects factor and current injection (neuronal firing) or number of action potentials (after-hyperpolarisation) as within subject factors. Action potential threshold and all of the intrinsic membrane properties were compared with a one-way between subjects ANOVA with treatment as the factor.

Data/Code availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request. The code used to analyse the electrophysiological data is available in Supplementary Software.

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Table 1. Intrinsic membrane properties of CA1 pyramidal neurones treated with vehicle or Daun02. For all parameters vehicle n=17, Daun02 n=18. Statistical values reported for one-way between subjects ANOVA. Data presented as mean $\pm$ sem.

Parameter	Condition		t-value	p value
	Vehicle	Daun02		
Resting membrane potential (mV)	-75.6 $\pm$ 0.4	-73.7 $\pm$ 0.6	F(1,33)= 6.92	0.013*
Input resistance (M Ω)	78.9 $\pm$ 4.9	70.6 $\pm$ 5.5	F(1,33)= 0.27	0.27
Tau (ms)	18.7 $\pm$ 0.7	17.5 $\pm$ 1.2	F(1,33)= 0.72	0.40
Sag (%)	19.0 $\pm$ 0.8	21.0 $\pm$ 1.2	F(1,33)= 1.84	0.18
AP threshold (mV)	-58.3 $\pm$ 0.7	-55.2 $\pm$ 0.9	F(1,33)= 7.68	0.009**
AP peak (mV)	34.7 $\pm$ 1.6	33.4 $\pm$ 2.6	F(1,33)= 0.19	0.67
AP Width (mV)	0.75 $\pm$ 0.02	0.75 $\pm$ 0.03	F(1,33)= 0.04	0.84
AP max rate of rise (V.s ⁻¹)	516 $\pm$ 28	482 $\pm$ 27	F(1,33)= 0.76	0.39

Table 2: Object exploration in the episodic-like memory task after deactivation of either the direct dCA1-mPFC projection or the direct iCA1-mPFC projection. Two-way ANOVA of object exploration levels across sample phases 1 and 2 revealed no significant interaction between sample phase and treatment (dHPC $\rightarrow$ mPFC $F_{(1,10)} = 0.01$, $p > 0.1$; iHPC $\rightarrow$ mPFC $F_{(1,11)} = 0.08$, $p > 0.1$). Further there was no significant difference in overall object exploration levels in the test phase in either group (dHPC $\rightarrow$ mPFC $F_{(1,10)} = 0.55$, $p > 0.1$; iHPC $\rightarrow$ mPFC $F_{(1,11)} = 0.35$, $p > 0.1$). Data presented as mean $\pm$ sem.

Experiment	Condition	Exploration in sample phase (s)		Exploration in test phase (s)
		S 1	S 2	
dCA1-mPFC	Vehicle	88.3 $\pm$ 11.2	83.4 $\pm$ 11.0	46.9 $\pm$ 4.7
	Daun02	88.5 $\pm$ 8.8	83.7 $\pm$ 7.4	50.9 $\pm$ 4.5
iCA1-mPFC	Vehicle	93.9 $\pm$ 6.2	72.6 $\pm$ 4.2	54.3 $\pm$ 2.9
	Daun02	99.0 $\pm$ 4.3	77.8 $\pm$ 6.6	51.1 $\pm$ 4.8

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847 **Table 3:** Object exploration in the object-in-place, object temporal order or spatial temporal order
848 task was not affected following deactivation of either the direct dCA1→mPFC projection or the
849 direct iCA1→mPFC projection. Object-in-place sample phase exploration dHPC→mPFC $F_{(1,10)} = 0.04$,
850 $p > 0.1$; iHPC→mPFC $F_{(1,11)} = 0.22$, $p > 0.1$; test phase exploration dHPC→mPFC $F_{(1,10)} = 0.06$, $p > 0.1$;
851 iHPC→mPFC $F_{(1,11)} = 0.06$, $p > 0.1$). spatial temporal order sample phase exploration dHPC→mPFC
852 $F_{(3,33)} = 0.20$, $p > 0.1$; iHPC→mPFC $F_{(3,33)} = 0.20$, $p > 0.1$), test phase exploration dHPC→mPFC ($F_{(1,11)} = 2.96$,
853 $p > 0.1$) or the iHPC→mPFC ($F_{(1,11)} = 0.6$, $p > 0.1$) group. Data presented as mean $\pm$ sem.

Experiment	Task	Condition	Exploration in sample phases (s)				Exploration in test phase (s)
			S1	S2	S3	S4	
dCA1-mPFC	object-in-place	vehicle	91.8 $\pm$ 4.8				42.2 $\pm$ 2.5
		Daun02	92.5 $\pm$ 3.7				41.3 $\pm$ 4.5
	object	vehicle	64.3 $\pm$ 5.4	55.7 $\pm$ 4.2	60.4 $\pm$ 8.3	64.2 $\pm$ 8.3	33.8 $\pm$ 3.5
	temporal order	Daun02	67.0 $\pm$ 5.4	51.9 $\pm$ 4.5	64.6 $\pm$ 7.5	60.3 $\pm$ 4.3	26.1 $\pm$ 2.6
	spatial	vehicle	45.3 $\pm$ 3.5	38.5 $\pm$ 5.5	23.2 $\pm$ 4.0	37.9 $\pm$ 5.6	20.0 $\pm$ 3.0
	temporal order	Daun02	47.8 $\pm$ 6.2	35.8 $\pm$ 4.6	37.5 $\pm$ 7.1	43.0 $\pm$ 6.2	22.1 $\pm$ 2.3
iCA1-mPFC		vehicle	88.8 $\pm$ 3.9				36.8 $\pm$ 2.0
	object-in-place	Daun02	86.6 $\pm$ 4.1				35.8 $\pm$ 3.6
	object	vehicle	67.0 $\pm$ 6.2	54.3 $\pm$ 5.4	48.8 $\pm$ 3.9	50.2 $\pm$ 3.5	31.8 $\pm$ 4.0
	temporal order	Daun02	70.6 $\pm$ 4.7	56.7 $\pm$ 3.2	48.8 $\pm$ 4.0	53.8 $\pm$ 4.2	29.1 $\pm$ 3.1
	spatial	vehicle	48.1 $\pm$ 4.5	44.0 $\pm$ 5.7	35.8 $\pm$ 3.9	35.3 $\pm$ 3.3	29.7 $\pm$ 3.5
	temporal order	Daun02	43.9 $\pm$ 4.3	38.1 $\pm$ 2.1	35.8 $\pm$ 3.7	40.3 $\pm$ 2.8	26.0 $\pm$ 2.1

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a **b** **c** **d**

■ NBQX Contra ■ NBQX IPSI

Task

■ Vehicle ■ Daun02

Task