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Silent learning

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23

24

25 SUMMARY

26

27 **We introduce the concept of 'silent learning' - the capacity to learn despite**
28 **neuronal cell-firing being largely absent. This idea emerged from thinking about**
29 **dendritic computation[1, 2] and examining whether the encoding, expression and**
30 **retrieval of hippocampal-dependent memory could be dissociated using the intra-**
31 **hippocampal infusion of pharmacological compounds. We observed that very**
32 **modest enhancement of GABAergic inhibition with low-dose muscimol blocked**
33 **both cell-firing and the retrieval of an already formed memory, but left induction**
34 **of long-term potentiation (LTP) and new spatial memory encoding intact (silent**
35 **learning). In contrast, blockade of hippocampal NMDA receptors by**
36 **intrahippocampal D-AP5 impaired both the induction of LTP and encoding, but had**
37 **no effect on memory retrieval. Blockade of AMPA receptors by CNQX impaired**
38 **excitatory synaptic transmission and cell-firing, and both memory encoding and**
39 **retrieval. Thus, in keeping with the synaptic plasticity and memory hypothesis [3],**
40 **the hippocampal network can mediate new memory encoding when LTP induction**
41 **is intact even under conditions in which somatic cell-firing is blocked.**

42

43 RESULTS

44

45 Encoding and retrieval of declarative memory are the two sides of a coin with
46 respect to the neural mechanisms of learning and memory. Encoding refers to the
47 acquisition of new information, whereas retrieval involves the reactivation of previously
48 learned memory traces. Identifying the neural activity associated with specific memory
49 processes such as these is a necessary step to understand of how information-processing
50 circuits operate. The present study tests whether (a) memory retrieval requires cell-
51 firing, enabling information transfer within and between networks; and (b) memory
52 encoding may minimally require the induction and expression of synaptic plasticity, with
53 little or no somatic cell-firing. The occurrence of learning would not be observable, but
54 we argue that recent advances in the physiology of dendritic computation predict such
55 'silent learning' could occur.

56

57 Loss-of-function manipulations such as lesions, drugs and molecular
58 interventions have long been deployed to look for 'learning impairments' in specific tasks.
59 Many studies focus on misleading learning curves during an intervention[4, 5] that likely
60 reflect impacts on encoding, storage, consolidation and/or retrieval without dissociating
61 their relative contributions. Lesions can also cause 'performance' effects (i.e. deleterious
62 effects upon sensorimotor processes or motivation). Specific protocols to dissociate
63 encoding and retrieval definitively include: for *retrieval* - monitoring performance on the
64 first trial of new training *before* any new learning can take place[6]; and for *encoding* -
65 application of the intervention *during* the new training, but testing *later* in its *absence*
66 when retrieval should be operating normally[5]. A suitable protocol for the watermaze is
67 the delayed-matching to place (DMP) or 'everyday memory' procedure which involves
68 learning a new daily spatial location of the escape platform during each session[7]. The
69 principle is that, on each session, the animals first retrieve a memory of where escape
70 was possible during the *previous* session, and then update their memory by encoding
71 where the escape platform is now located during the *current* session. The effectiveness
72 of this memory encoding is tested on a *subsequent* session in which the animals should
73 again demonstrate memory of the preceding session.

74

75 The main study we examined the impact of drugs over 5 successive series of 3
76 linked sessions (hereafter called s1, s2 and s3) in a within-subject manner (Figure 1A). It
77 followed animal handling, bilateral drug cannula implantation and initial training over 8–
78 10 sessions, during which the 16 animals learned the DMP task well each day with a new
79 platform location chosen for each session of 4 learning trials (Figures 1A,B,E). An animal
80 might be trained to encode that the escape platform is in the NW (North-West) quadrant
81 on all 4 trials of session 1 (s1, Figure 1B). On the next session (s2), using an Atlantis
82 Platform procedure [8] in which memory retrieval is assessed during the first 60 s of the
83 first trial before any new learning takes place (Figures 1B,C,D and S1), the animal should
84 remember this NW location (red dotted circle) by searching there (during the first 60 s)
85 before learning that the hidden platform had been moved to SSW (South-South-West).
86 Encoding of this new location occurs during the four escape trials of s2, updating and
87 over-writing the memory acquired in s1. The platform is moved again for s3, again
88 allowing an analogous test of memory during the first 60 s. In this protocol, *memory*
89 *retrieval* is procedurally dissociated from new *memory encoding*.

90

91 This procedure enabled effective memory encoding of each daily location, with
92 performance typically characterised by a stable monotonic decline in first-crossing
93 latency across trials within each session (Figure 1E; Data S1). The swim-latency on trial
94 1 (\approx 50-70 sec) was stable across sessions, as was the ‘savings’ in latency of
95 approximately 30 s between trials 1 and 2 of each session. Asymptotic performance over
96 trials 2-4 reflects the effectiveness of memory updating.

97

98 **In vivo Hippocampal Physiology**

99 Previous studies have established that blocking hippocampal N-methyl-D-
100 aspartate (NMDA) receptors, via pharmacological [D-2-amino-5-phosphonopentanoate
101 (D-AP5)] or molecular-genetic interventions, limits memory encoding without effect on
102 retrieval[7, 9-12]. This is observed with 1-2 μ l bilateral infusions into dorsal hippocampus,
103 with autoradiography indicating substantial spread along the longitudinal axis following
104 a 2 μ l infusion[13]. This behavioural pattern is mechanistically linked to blockade of
105 NMDA receptor-mediated activity-dependent synaptic plasticity[3, 14]. Blocking α -
106 amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors pharmacologically
107 limits encoding, consolidation and retrieval [15]. Prior focus on AMPA and NMDA
108 receptors left uninvestigated the possible contribution of γ -aminobutyric acid (GABA-
109 ergic) inhibition which is known to regulate long-term potentiation (LTP) induction[14].
110 The importance of dynamic patterns of inhibitory activity is now recognised as
111 functionally important[16, 17], along with learning-associated changes in inhibitory
112 circuitry that can affect the fidelity of memory[18, 19].

113

114 Using *in vivo* electrophysiology in male Lister Hooded rats (n=20) to identify
115 appropriate drug concentrations using and the time course of their effects (Figure 2), we
116 sought drug doses that would differentially affect (a) cell-firing, (b) fast synaptic
117 transmission, and/or (c) activity-dependent synaptic plasticity *in vivo* in the hippocampal
118 formation. We chose to monitor the dentate gyrus electrophysiologically, while
119 recognising that an infusion targeting the outer molecular of the dorsal dentate gyrus
120 would diffuse throughout dorsal CA1 and CA3 as well. In the case of muscimol, it should
121 incur reasonably widespread binding to somatic and dendritically located GABA_A
122 receptors.

123

124 A key new finding is that low-dose muscimol blocked cell-firing but not LTP
125 induction. Infusion of 0.38 nanomoles of muscimol caused a modest 35% decrease of the
126 field excitatory postsynaptic potential (fEPSP) (Figures 2A,B; [Data S2](#)), but the dentate
127 population spike ceased almost completely from 30 min post-infusion for 2 hr (measured
128 at 1.0 to 1.5 mm from the infusion cannula; Figures 2C,D). Strikingly, low-dose of
129 muscimol infusion left induction of LTP intact (Figures 2E,F; [Data S2](#)). Enhanced
130 GABAergic inhibition normally blocks the induction of LTP[14], but our dose titration
131 down to 0.38 nanomoles achieved a situation in which LTP induction was intact despite
132 the absence of pre-induction cell-firing. This low dose of muscimol did not prevent a very
133 small population spike post-LTP (Figure S3; <3 mV), a change that is unlikely to be
134 relevant to the more distributed patterns of learning-associated dendritic and somatic
135 neural activity in the freely-moving animal (see below).

136

137 In contrast, D-AP5 (60 nanomoles) caused a transitory disruption of the fEPSP
138 before a return to baseline within 30 min, and a partial albeit more sustained inhibition
139 of the population spike (Figures 2A–D; [Data S2](#)). However, as expected, it blocked LTP
140 induction (Figures 2E,F; [Data S2](#)). CNQX (6 nanomoles) caused the fEPSP to be completely
141 inhibited within 30 min with respect to both synaptic activation and cell-firing (>90%
142 decrease for over 1 hr, Figures 2A–D). In the absence of a measureable fEPSP, CNQX was
143 not tested with respect to LTP.

144

145 **Silent Learning**

146 The stage was now set to conduct the companion behavioural study using male
147 Lister Hooded rats (n=16) trained in the task and now subject to intrahippocampal drug
148 infusions. Each session was conducted ‘blind’ with respect to drug-assignment[7, 20],
149 using a fully counterbalanced repeated-measures within-subjects design, such that each
150 animal served as its own control across successive ‘linked’ sessions, consisting of a pre-
151 drug session (s1), drug session (s2) and post-drug session (s3) within each block (Figure
152 1A).

153

154 Our second key finding was ‘silent learning’ with low-dose muscimol.
155 Representative swim-paths show Rat-G7207 treated with muscimol searching

156 appropriately on s1, but swimming all over the pool on s2 without memory of the s1
157 platform location until it eventually found the new escape location after >60 s; on the
158 following drug-free s3, this rat searched successfully in a focused zone around the s2
159 location (Figure 3A, panel with pool shaded blue, note multiple crossings of the s2
160 location on s3). This pattern of searching behaviour implies that memory encoding was
161 intact during s2 under muscimol, despite memory expression being blocked.
162 Representative paths of other rats show respectively: good memory for each previous
163 session for aCSF; good memory retrieval but no new learning under D-AP5; and no
164 memory retrieval or new learning under CNQX. Quantitatively, we observed a double
165 dissociation between the impact of the three drugs on memory encoding and retrieval
166 (Figure 3B; two-way ANOVA: significant Drug \times Sessions interaction: $F_{6,90} = 3.65$, $p =$
167 0.003). This statistical interaction justified separate analyses of each drug condition
168 compared to aCSF vehicle, as well as planned comparisons to chance-level performance.

169
170 Vehicle sessions (aCSF) showed good above chance memory (chance = 4%, dotted
171 line) across all three sessions (Figure 3B, black bars; Data S3). With low-dose muscimol,
172 the animals were at chance on s2 with the animals failing to remember the previous
173 session, but above chance for the location trained under the drug on s2 when tested
174 during trial 1 of s3 (note U-shaped function in Figure 3B; Data S3). That is, low-dose
175 muscimol was permissive for new encoding despite causing a complete block of memory
176 retrieval. The opposite pattern prevailed with D-AP5, with above chance retrieval of s1
177 on trial 1 of s2, but chance performance during trial 1 of s3. With CNQX, the animals'
178 memory of s1 displayed on trial 1 of s2 and their memory of s2 on trial 1 of s3 were both
179 at chance. Thus, CNQX treated rats could neither retrieve nor encode.

180
181 In this protocol, the animals 'update' their memory during each session - akin to
182 the concept of 'headed records' in which human subjects often remember the last thing
183 that happened but tend to overwrite earlier events[21]. Updating should only be
184 observed if new learning occurs on s2; thus, a distinct pattern of drug effects is expected
185 on s3. The specific prediction is that D-AP5 and CNQX would block memory updating,
186 whereas aCSF and low-dose muscimol would both be permissive of memory overwriting.
187 We quantified the swim search pattern on s3 with respect to the proportion of time spent
188 in the correct zone for s1 (i.e. *2 sessions back*; Figures 3C,D) and compared these values

189 to those for s2 (*1 session back*). The overall ANOVA was thus a 2×4 analysis for the time
190 spent searching during s3 in the locations used on s1 and on s2 as a function of the 4
191 drugs, revealing a highly significant Session-Memory \times Drug interaction ($F_{3,45} = 7.35$, $p =$
192 0.001). Unpacking this triple interaction using planned comparisons revealed that the
193 average level of memory measured on s3 after CNQX or D-AP5 during s2 was higher for
194 the s1 location than for s2 (i.e. minimal updating); whereas, with aCSF and muscimol, the
195 opposite pattern prevailed. The orthogonal comparison for this contrast was significant,
196 but a graphically simpler analysis is to look at the absolute level of memory for the s1
197 location that is expressed during s3 (Figure 3C). The ANOVA for these data also showed
198 a significant drug effect ($F_{3,45} = 3.64$, $p < 0.05$). As predicted, memory for the s1 location
199 after aCSF or muscimol had been infused on s2 was lower than when D-AP5 or CNQX
200 were infused ($F_{1,45} = 8.65$, $p < 0.01$; Figure 3C). This successful updating under muscimol
201 on s2 argues against the retrieval deficit displayed under the drug on s2 being a mere
202 'performance', 'off-target' or 'state-dependent' effect, as it is unclear how such effects
203 could *selectively* affect retrieval but not memory encoding. Illustrative paths are shown
204 following the administration of aCSF (successful updating) and CNQX (no updating) on
205 different s2 sessions (Figure 3D).

206

207 A concern was that intact memory encoding with low-dose muscimol is some
208 artefact of differential spread of the drug, the most likely possibility being that the
209 infusion was restricted to a *small* region of the dorsal HPC. This might have been sufficient
210 to disrupt cell-firing during retrieval and pattern completion, but insufficient to affect
211 new learning within a larger volume of *unaffected* dorsal and intermediate hippocampus.
212 The problem with this interpretation is that the diffusion of D-AP5 and CNQX is likely to
213 have been similar to that of muscimol (MWs = 197, 232 and 114 respectively), and thus
214 new encoding should also have occurred for these drugs - which it did not. This suggests
215 that our deliberate choice of a 2 μ l infusion volume achieved substantial spread along the
216 longitudinal axis. Anticipating this, we conducted additional electrophysiological studies
217 with recording in the intermediate zone of the longitudinal axis of hippocampus following
218 infusion of muscimol into the dorsal/septal region (infusion 2.5 mm from the recording
219 electrode, Figure S2B). Inhibition of the fEPSP (Figures S2C,E, circa 23%) was slightly
220 less than at the more proximal recording site (35%) but, importantly, cell-firing remained
221 almost completely blocked (circa 86%, Figures S2D,E). We also attempted to look at drug

222 diffusion directly using fluorescent muscimol [22]. The analysis of 3 animals subject to
223 bilateral 2 μ l infusions of 0.19 mM fluorescent muscimol bodipy, indicated diffusion along
224 the longitudinal axis of up to 3.5 mm (Figure S2F,G), with minimal spread into
225 retrosplenial cortex (RSC) or overlying parietal cortex. But this measure is conservative
226 as the molecular weight of fluorescent muscimol (MW = 607) is much larger than that of
227 muscimol itself (MW = 114), and it is more lipophilic by virtue of the fluorescent label.

228

229 A third but unlikely possibility is that drug diffusion reaches beyond hippocampus
230 to retrosplenial cortex, long implicated in spatial memory[23]. On this view, muscimol in
231 RSC may be contributing to the impaired memory retrieval. We had hoped that data on
232 diffusion of fluorescent muscimol could definitively address this issue, but it is unclear
233 how this account would could explain effective new memory encoding in RSC. Extensive
234 diffusion of muscimol itself is surely unlikely as the closely packed, myelinated fibres of
235 the overlying alveus and corpus callosum would restrict this from happening. As in our
236 earlier autoradiographic and regional cerebral blood flow studies of glutamate receptor
237 antagonists [15, 24], there were non-spherical ‘rugby-ball’ shaped diffusion volumes
238 within hippocampus, also observed with fluorescent muscimol [22]. Some disruption to
239 cell-firing in RSC might nonetheless contribute to the retrieval deficit seen with muscimol
240 and CNQX, possibly by affecting the translation of memory representations from
241 allocentric to egocentric to enable accurate heading to the remembered escape location.

242

243 **DISCUSSION**

244 The present findings point to a new concept which we shall refer to as ‘silent
245 learning’ - new memory encoding in the absence of cell-firing. Silent learning
246 corresponds behaviourally to new episodic-like memory encoding in the absence of
247 memory retrieval. We suggest that this can sometimes occur if LTP induction is intact
248 during cellular silence, allowing activity-dependent synaptic potentiation to encode a
249 new spatial memory as a distributed pattern of potentiated synapses in the hippocampus
250 (dentate, CA3 and/or CA1). Cell-firing would not always be necessary.

251

252 From a behavioural perspective, the concept of ‘silent learning’ is distinct from the
253 classical concept of ‘latent learning’ which refers to successful learning in the absence of
254 reward [25]. Latent learning was a challenge for Hull’s drive reduction theory[26] which

255 supposed that animals had to be motivated to learn ('drive') and that stimulus-response
256 connections were 'stamped-in' by 'drive-reduction' following reward. Silent learning is
257 different, being more consonant with Tolman's 'cognitive map' theory[25] which
258 asserted that learning could occur in the absence of reward, resurrected in O'Keefe and
259 Nadel's theory of hippocampal function[27].

260

261 The distributed associative neural circuit of the hippocampus[28] has been
262 proposed to operate in distinct encoding and retrieval modes at different phases of the
263 theta rhythm[29]. We reasoned that it might be possible to realise a double dissociation
264 of the processes of memory encoding and retrieval as a function of task demands.
265 Blocking NMDA receptor activation is permissive for memory retrieval but prevents
266 encoding[9, 12], whereas AMPA receptor inhibition blocks both[30]. Our new finding
267 indicates that, even though memory retrieval fails to occur when cell-firing in the
268 hippocampus is blocked by low-dose muscimol, new encoding can occur provided
269 synaptic plasticity is intact and fast synaptic transmission only modestly affected.
270 Although not accompanied by electrophysiological analysis, a previous behavioural
271 pharmacology study was also suggestive of such a possibility, with spared 'extinction
272 learning' being observed in an inhibitory avoidance task during intrahippocampal low-
273 dose muscimol (approximately twice as high as we used[31]). Impaired retrieval has
274 been shown to occur with higher doses of muscimol[32], but the possibility of intact or
275 impaired learning was not investigated.

276

277 The qualification is the possibility of acute 'off-target' alteration of neural circuits
278 (e.g. RSC) that were not directly enveloped by the muscimol infusion. Alterations in the
279 level of learning-associated immediate early-gene expression in RSC are seen as a
280 network effect following lesions of the hippocampal formation [23]. We suspect instead
281 that enhanced inhibition targets the complexity of inhibitory circuitry in
282 hippocampus[16] coupled to dynamic changes of parvalbumin-positive GABAergic
283 inhibition associated with learning[19]. Our findings suggest that 'on-target' effects of
284 muscimol are a more likely explanation.

285

286 From a physiological perspective, this interpretation requires that synaptic
287 plasticity can sometimes occur in the absence of cell-firing. Indeed, this may be more

288 frequent than generally realised and has been considered since the original papers on
289 LTP [33, 34]. The conceptually interesting idea is that a network may sometimes be able
290 to change the pattern of synaptic weights on its input synapses 'secretly' from neurons
291 further downstream. Our electrophysiology focused on the perforant path input to the
292 dentate gyrus, but a similar 'silent learning' effect may occur on the entorhinal cortex
293 layer III input to CA1 and on the entorhinal cortex layer II input to CA3; it would not
294 necessarily require activation of recurrent circuitry in CA3. Understanding the detailed
295 physiological mechanism was not the primary purpose of this initial study, but comments
296 relevant to such a future project are appropriate. First, the complexities of dendritic
297 inhibition in the hippocampus might allow for a failure of memory retrieval to be caused
298 by a block of cell-firing due to muscimol activation of GABA_A receptors expressed on the
299 cell soma of hippocampal excitatory neurons innervated by parvalbumin-positive
300 GABAergic interneurons[16, 17, 35]. An impact of GABA_A receptors in the dendrites may,
301 however, reflect a differential effect on tonic rather than phasic inhibition[36, 37], arising
302 because low-dose muscimol acts preferentially (but not exclusively) at extra-synaptic
303 GABA_A sites mediating tonic inhibition. A modest increase in tonic inhibition may be
304 permissive for postsynaptic backpropagating dendritic spikes[1, 2, 38]. A further
305 possibility is that augmented GABA_A mediated inhibition in dendrites may leave intrinsic
306 changes in dendritic membrane potential unaffected, and these are now known to be
307 permissive for place-cell formation[39] and behavioural time-scale synaptic
308 plasticity[40]. Addressing these distinct possibilities will not be easy in freely-moving
309 animals. The retrieval/encoding dissociation might be examined optogenetically or
310 chemogenetically[41, 42] using appropriate promoter lines that would allow differential
311 targeting of distinct GABAergic neurons. Interestingly, the possibility that changes in
312 excitation-inhibition balance is relevant to unmasking latent memory has also recently
313 been studied in humans[43].

314

315 To summarise, the phenomenon of 'silent learning' in the awake animal is
316 compatible with dendritic computation and the complexity of inhibitory network
317 connectivity in the hippocampus. It suggests that synaptic plasticity can lurk cryptically
318 under conditions in which the network expression of new memory trace formation is
319 prevented by somatic inhibition. It has not escaped our notice that such learning may

320 occur more often than is generally appreciated and, indeed, be a characteristic of several
321 aspects of cognitive learning.
322

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336 Aarhus University, Denmark. The Centre for Cognitive and Neural Sciences in Edinburgh
337 is now assimilated into the Centre for Discovery Brain Sciences which is RM's current
338 affiliation.

339

340

341 **AUTHOR CONTRIBUTIONS**

342

343 RM conceived the study. JR conducted the behavioural studies, AM the
344 electrophysiological studies, and MY the drug diffusion studies; LG, TT, SC and RM
345 prepared the manuscript. We acknowledge the considerable contribution of three
346 anonymous reviewers.

347

348

349 **DECLARATION OF INTERESTS**

350

351 The authors declare no competing interests.

352

353

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- 482

483 **FIGURE LEGENDS**

484

485 **Figure 1. A Novel Watermaze Protocol to Dissociate Encoding and Retrieval**

486 (A) The experimental design with animal handling, drug-cannula implantation, DMP
487 training for 8–10 sessions, followed by the series of 3 linked sessions for the drug
488 infusions interspersed with an additional interleaved training.

489 (B) Aerial drawings of the watermaze across 3 linked sessions (repeated across sessions
490 for different drug conditions), with exemplar daily locations of the single escape platform
491 (black circles; see Figure S1). The location on a specific session 1 (s1; black continuous
492 circle in NW quadrant) and that on the next session 2 (s2; black circle in SW quadrant)
493 are each shown, with the dotted line in black reflecting where the platform was available
494 after 60s, and the continuous line showing it being available (60–120 s; it was always
495 hidden below the water). The black filled parts of the cartoons are the platforms (in
496 proportion) and the surround black circle is the associated analysis zone (20 cm diameter,
497 centred on each location, 4% of area of pool). The location used on s1 is shown for s2 as
498 a red dotted line (the 'memory' location of s1, but not actually used on s2; likewise for s3
499 with respect to s2). Note the platform always stayed in the same location for all 4 trials,
500 and was available immediately for escape on trials 2–4.

501 (C) Atlantis Platform that is unavailable for the first 60 s of trial 1 of each session, but
502 then rises to near the water surface. Cartoons below (dotted and continuous line) are as
503 in panel B.

504 (D) A series of 3 linked sessions when drug (or aCSF) is administered 30 min (CNQX) or
505 40 min (muscimol and D-AP5) before s2. The same locations are displayed as in (B). Note
506 that the first 60 s of trial 1 of s2 offered the opportunity to retrieve the memory of s1 (red
507 continuous line), while trials 1-4 of s2 are the opportunities for new memory encoding[of
508 black continuous circle in SW (south-west) quadrant]. When encoding was blocked
509 during s2 by a drug, preventing updating, s3 may have offered the opportunity to retrieve
510 the location on s1 (red dotted line).

511 (E) Mean first-crossing latencies on trials 1-4 of multiple interleaved training sessions.
512 These training sessions are interspersed between the successive 3 linked sessions (grey
513 bars). Means \pm SEM.

514

515 **Figure 2. Impact and Time-Course of Distinct Drugs on Hippocampal Physiology *in***
516 ***vivo***

517 (A) Differential impact of 2 μ l infusions of muscimol (0.38 nmoles, orange), D-AP5 (60
518 nmoles, pink) and CNQX (6 nmoles, blue) on the early-rising slope of evoked fEPSPs in
519 the dentate gyrus to perforant path stimulation, normalised to the pre-drug baseline (n =
520 5 for all conditions). Arrow points to the drug infusion; yellow shading indicates when
521 the behavioural test is applied (40–70 min later).

522 (B) Normalised averaged fEPSP values for baseline, end of test period (after test), and 3
523 hr post-infusion (end), normalization to aCSF baseline.

524 (C and D) Normalized changes in the population spike (PS).

525 (E) Impact of aCSF, muscimol and D-AP5 on the induction of LTP (yellow lightning marks),
526 with the fEPSP slope re-normalised with respect to the pre-tetanus baseline (10 min) and
527 compared to the corresponding drug-treated group that was not subject to tetanisation.
528 A key new finding is intact LTP induction under low-dose muscimol as well as aCSF, but
529 not D-AP5. Yellow shading reflects the daily timing and duration of the behavioural
530 experiment. LTP data plotted for that time. For absolute PS data, see Figure S3.

531 (F) Averaged LTP data.

532 (G) Schematic of stimulating and recording electrodes, and drug cannula locations of rat
533 brain *in vivo*. Detail in Figure S2. Paired two-tailed t test (versus chance): **p < 0.01, ****p
534 < 0.0001. Means \pm SEM.

535

536 **Figure 3. Impact of Distinct Pharmacological Manipulations on Encoding and**
537 **Retrieval, and on Memory Updating**

538 (A) Illustrative paths of representative rats during trial 1 of 3 linked sessions for all 4
539 drug conditions. Black platform = location that session; continuous red line = location on
540 previous session; dotted red line = platform location two sessions back; small green circle
541 = start of swim path; small blue circle = end of swim-path; dotted blue line = latter part
542 of the swim path, not calculated in the memory retrieval data, after the Atlantis Platform
543 became available. The key finding of silent learning is shown for the representative
544 muscimol treated animal. Note random search all over the watermaze on s2 (during the
545 drug; middle), but focused search at the s2 location on s3 (right). With aCSF, the rat
546 always searches at or very close to the previous session location; with D-AP5, searching

547 is optimal on s2 but at chance on s3; with CNQX, the rat fails to show either memory
548 retrieval or new encoding.

549 (B) Impact of hippocampal drug infusion on encoding and retrieval. Quantitative measure
550 of search in the zone around a platform location on each trial 1 of 3 linked sessions, with
551 drug administered on s2 (drug conditions were counterbalanced and given blind). Search
552 time is plotted for the platform location of the *preceding* session. Note stable above-
553 chance performance for s1–s3 for aCSF condition (black bars), but a different pattern in
554 each of the three drugs. Following the overall Drugs × Sessions interaction (see text),
555 separate ANOVAs were conducted for each drug over s1–s3 (muscimol: $F_{1,45} = 4.49$, $p =$
556 0.029 ; D-AP5: $F_{1,45} = 4.53$, $p = 0.007$; CNQX: $F_{1,45} = 18.37$, $p = 0.001$). The key finding was
557 chance performance in muscimol condition on s2 but recovery during retrieval on s3.

558 (C) Impact of hippocampal drug infusion on memory updating. Dissociable impact of the
559 drugs on memory updating as measured on s3. In the aCSF and muscimol conditions,
560 memory of s1 during s3 was at chance (successful updating) and significantly below that
561 observed for D-AP5 and CNQX which are both above chance (no updating).

562 (D) Representative search paths on trial 1 of s3 reflecting updating (aCSF: path frequently
563 crosses continuous red line of s2) or no updating (CNQX: path frequently crossed the
564 dotted red line of s1). Paired two-tailed t test (versus chance): $**p < 0.01$. 4% chance =
565 ratio of surface areas of search zone and pool area. Means ± SEM.

566

567

568 **STAR♣METHODS**

569

570 **Silent learning**

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573

574 **CONTACT FOR REAGENT AND RESOURCE SHARING**

575 Further information and requests for resources and reagents should be directed to and
576 will be fulfilled by the Corresponding Author, Richard Morris (r.g.m.morris@ed.ac.uk).

577

578 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

579

580 **Rats**

581 All experiments were performed on adult male Lister Hooded Rats (200–300 g on arrival;
582 Charles River, UK) in accordance with the United Kingdom Home Office Animal
583 Procedures Act (1986) conducted under a Project Licence (PPL 60/4566) held by RGM
584 Morris. They were kept in a vivarium in the same building on a 12 hr lights on/off
585 schedule, in group cages of 4 rats per cage, with free access to food and water.

586

587 **METHOD DETAILS**

588

589 **Behavioural apparatus and the Atlantis Platform**

590 The watermaze is an apparatus in which a variety of distinct behavioural protocols can
591 be trained [44]. It consists of a large pool of water (2 m in diameter) from which rodents
592 learn to escape onto a hidden platform whose top surface is 1.5 cm beneath the water
593 surface (Supplementary Figure S1A). Latex solution is added to render the water cloudy
594 and thereby hide the escape platform. Water temperature is regulated at 25°C such that
595 escape is desirable, but the procedure is not stressful as this is only 12–13°C lower than
596 body temperature.

597

598 While many studies have deployed a reference memory procedure in which the escape
599 platform remains in the same location across days, an alternative is the so-called ‘delayed
600 matching to place’ (DMP) protocol [7] in which the escape platform moves from one
601 location to another. This ‘everyday memory’ protocol, with repeated training across days
602 and weeks, requires the integrity of the septal (dorsal) and/or intermediate
603 hippocampus[45]. Lesions placed at different positions along the longitudinal axis
604 damaging up to 90%+ or less than 20%+ of the hippocampus have indicated that learning
605 is mediated via dorsal and intermediate hippocampus, with intact ventral hippocampal
606 tissue being insufficient for effective day-to-day learning. A key analytic feature of the
607 DMP protocol is that it allows a clean dissociation between memory encoding and
608 memory retrieval. Performance is typically characterised by a long escape latency on trial
609 1 as the animal searches for the platform whose location that day is still unknown,
610 searching initially at the previous session’s location, followed by rapid memory encoding
611 during the 30 s period out of the water followed by relatively direct paths to that day’s
612 location on trials 2–4. Four trials per day are used to ensure that an effective memory is

613 formed of the daily location that can be recalled during trial 1 of the next session. The
614 intervals between trials can be varied systematically, with the trial 1 to trial 2 interval
615 being 20 min in this study, while trial 2 to trial 3, and trial 3 to trial 4 was kept short at
616 no more than 5 s after the 30 s period on the platform. In this way also, the rapidly learned
617 strategy of learning where to go in each session (one session per day) is maintained, even
618 in the face of interventions such as the application of drugs. A large number of different
619 platform locations can be used across sessions, some on an outer virtual ring and the
620 others on the inner ring (Supplementary Figure S1B). This distribution of possible
621 locations (n=24 in this study) encourages widespread searching.

622

623 Memory retrieval is displayed as preferential searching on trial 1 of each session in the
624 location that the escape platform had occupied during the *previous* session. This was
625 quantified by running trial 1 of each session as a rewarded probe test, using an ‘on-
626 demand’ or ‘Atlantis’ platform [8, 46] (Supplementary Figure S1C) which remained
627 inaccessible for 60 s. This consists of a 12 cm diameter escape platform mounted on a
628 stainless-steel spindle, initially at the bottom of the pool and thus unavailable, but which
629 can be computer controlled to rise to within 1.5 cm of the water surface on-demand. By
630 making this platform unavailable for 60 s on trial 1, the trial serves as a memory ‘probe’
631 for the previous session; the platform then rises, the animals find it and then climb onto
632 it in the usual way.

633

634 An overhead camera, associated DVD recorder and on-line analysis software co-
635 developed by Watermaze Software (Edinburgh) and Actimetrics (Evanston, USA) are
636 used to monitor the path taken, and measure latency, path-length etc. During the initial
637 period of 60 s of trial 1, two separate measures of performance were computed:

638

- 639 • **First-crossing latency:** is the time in seconds until the animal first crosses the correct
640 location (12 cm diameter) where the platform will become available. As the platform is
641 not actually available until 60 s has passed, this is not strictly-speaking an ‘escape
642 latency’ (the animal keeps swimming), but it serves as a ‘surrogate’ of escape latency.
643 This measure was also computed for all 4 trials of the session.
- 644 • **Memory search tendency:** The second measure is computed from the time spent
645 swimming in a virtual zone of 40 cm diameter centred on the location of the platform
646 during the previous session. This time is normalised with respect to the full 60 s of the

647 trial, and represented as a percentage relative to the 4% area of the pool that the zone
648 occupies. This 4% level represents 'chance' if the animal were to be swimming around
649 the pool randomly.

650

651 This DMP task offers analytic advantages over the reference memory protocol. First, the
652 learning of several different platform locations can be studied. Second, once the animals
653 have received a number of initial training sessions (8–10 sessions), memory encoding
654 and storage takes place in one session. Third, reversible interventions can be introduced
655 (such as intrahippocampal drug infusions, conducted blind) using a within-subjects,
656 repeated measures design – with all the associated advantages of both reduced variability
657 and reduced use of animals.

658

659 **Stereotactic surgery**

660 Anaesthesia was induced using isoflurane (induction, 5%; maintenance, 1–2%; air-flow,
661 1 l/min) (Zoetis, USA). The animals were placed in the stereotactic frame (David Kopf
662 Instruments, USA). Infusion guide cannulae (26 gauge, 4.4 mm length, C315, Plastics One,
663 USA) with stylets (33 gauge) that protruded 0.5 mm below the end of the cannula were
664 inserted into the hippocampus bilaterally through small holes drilled into the skull.
665 Cannula implantation coordinate for the hippocampus is as follows: anterior-posterior
666 (AP) from bregma, –4.00 mm; mediolateral (ML), ±3.00 mm; and dorsal-ventral (DV)
667 from the dura, –2.66 mm (Paxinos G, Watson C (2007) *The Rat Brain in Stereotaxic*
668 *Coordinates*, Ed 6. Amsterdam: Academic Press/Elsevier). Carprofen (0.08 ml/kg body
669 weight; Zoetis) was administered by subcutaneous injection at the end of all surgical
670 procedures. Animals recovered on a heating pad until normal behaviour resumed. All rats
671 were allowed a recovery period of at least 7 days for them to regain their pre-surgery
672 weights before behavioural testing.

673

674 **Drugs**

675 With respect to the drugs, phosphate-buffered artificial cerebrospinal fluid (aCSF) (in
676 mM: 150 Na⁺, 3 K⁺, 1.4 Ca²⁺, 0.8 Mg²⁺, 155 Cl⁻, 0.2 H₂PO₄⁻, 0.8 HPO₄²⁻, pH7.2) was used as
677 the infusion vehicle and for control infusions. Drug concentrations for infusions were:

678

- 679 • **0.19 mM of the GABA_A receptor agonist muscimol (C₄H₆N₂O₂; Tocris, UK)**

- 680 • **30 mM of the competitive NMDA receptor antagonist D-AP5 (C₅H₁₂NO₅P; Tocris)**
- 681 • **3 mM of the competitive AMPA/kainate receptor antagonist CNQX (disodium**
- 682 **salt; C₉H₂N₄O₄Na₂·H₂O; Tocris)**

683

684 The pH of the drug solutions was adjusted to 7.2 by addition of 1 M NaOH solution (for D-
685 AP5), or of concentrated phosphoric acid (for CNQX). Both aCSF and drug solutions were
686 prepared in larger quantities, divided into small aliquots, and kept frozen at -20°C until
687 they used. We facilitated the solution of CNQX by sonification. Note higher concentrations
688 of muscimol were used in pilot studies.

689

690 **Behavioural training**

691 We used male Lister Hooded rats (n = 16, 250+ g) for the behavioural aspect of this study.
692 They were stereotaxically implanted with bilateral guide cannulae targeting the dentate
693 gyrus in the dorsal hippocampus. After recovery from surgery, they were trained on the
694 DMP task over 8–10 sessions whereupon they showed the usual striking decline in escape
695 latency between trials 1 and 2 of a session (Data S1) and above chance memory of the
696 previous session's location during trial 1.

697

698 Thereafter, using a counterbalanced Latin-Square design, we examined the impact of
699 aCSF, muscimol, D-AP5 and CNQX on performance in the DMP task. The 16 animals were
700 all used as their own controls (i.e. every animal received each drug condition on different
701 sessions) with ¼ of the group (i.e. 4 animals) being subject to any one drug on each drug
702 session.

703

704 For microinfusion of drugs, the rats were habituated to the experimental procedure of
705 injection for several days before the drug sessions in order to minimise stress. The stylets
706 in the guide cannulae were replaced by two single infusion cannulae (33 gauge, Plastics
707 One) connected to two 10 µl microsyringes (Hamilton, USA) in a microinfusion pump
708 (World Precision, USA) via flexible plastic tubing filled with Fluorinert (3M, USA). The
709 tips of infusion cannulae projected 0.5 mm below the tip of the guide cannulae. The drugs
710 were bilaterally infused, in a volume of 2 µl per hemisphere, over a 4 min period with a 2
711 min period after drug infusion before the infusion cannulae were replaced with stylets.
712 Rats received drug injection 30 min in the case of CNQX and 40 min in the case of

713 muscimol and D-AP5 prior to the start of training, and both 30 and 40 min (for different
714 animals) for aCSF – this interval was based on data from *in vivo* electrophysiological
715 recordings.

716

717 Drug sessions only occurred every 4th session as the test sessions were conducted as
718 follows (Figure 1A). First, each set of sessions consisted of three successive sessions
719 (called 3 linked sessions) that are referred to as session 1, session 2 and session 3 (s1, s2
720 and s3, respectively). The nomenclature is potentially confusing, because these 3 linked
721 sessions were then repeated according to requirements of the Latin-Square design. Each
722 repetitive 3 linked sessions always consisted of s1, s2 and s3 and the resulting scores
723 were concatenated in Excel file until we had tested all 16 animals in each condition.

724

725 Second, in each 3 linked sessions, the drugs were only administered on s2. In this way,
726 we could examine the following:

727

- 728 • **Impact of the presence of a drug (within the hippocampus) on memory retrieval**
729 **of the location of the platform on the previous session (i.e. memory for s1).**
- 730 • **Impact of the drug on new learning (i.e. delivered during s2, but measured on s3**
731 **in the then drug-free state).**
- 732 • **Impact of the drug on ‘updating’ by comparing, on s3, the relative memory for**
733 **s2 (the immediately preceding session) and that of s1 (two sessions before that).**

734

735 Third, these 3 linked sessions were separated by 1+ interleaved training session. The
736 object of these additional training sessions was to maintain stability of the strategy of
737 memory retrieval followed by new encoding, this being monitored by checking that the
738 first-crossing latency remained stable throughout the experiment (which it did). The data
739 plotted in Figure 1E shows first crossing latencies across the 4 trials of each interleaved
740 training session; the longest time was spent searching on trial 1 with rapid escape to the
741 newly learned location on trials 2–4. Representative search paths on critical probe trials
742 are shown in Figure 3A, with the group behavioural data for the critical probe sessions
743 shown in **Data S3**.

744

745 In earlier pilot work (behavioural and electrophysiological), we examined the impact of
746 higher concentrations of muscimol (0.5 mM and 1.3 mM) and different time-periods after
747 infusion for testing. In these cases, encoding during s2 and long-term potentiation (LTP)
748 induction were each impaired. The dissociation between impaired retrieval and intact
749 encoding only emerges at the low-dose of muscimol (0.19 mM).

750

751 **Main Electrophysiology Studies**

752 The aim of the electrophysiology was to provide data to guide the choice of drug doses
753 for muscimol, D-AP5 and CNQX, and to examine excitatory synaptic transmission, cell-
754 firing and LTP induction. We sought concentrations that definitively blocked LTP
755 induction with D-AP5, but paradoxically spared LTP induction with muscimol.

756

757 Separate animals (male Lister-Hooded rats, weighing 250+ g, n=5 per drug condition)
758 were used in the non-recovery electrophysiology studies. These animals were prepared
759 for acute surgery in a stereotaxic apparatus (David Kopf Instruments) under non-
760 recovery urethane anaesthesia (1.3 g/kg body weight; Sigma-Aldrich, USA), with the first
761 intraperitoneal injection given during brief isoflurane anaesthesia (4% isoflurane in 0.8
762 l/min O₂). The electrophysiology studies typically lasted 6–8 hr, with the initial 2 hr being
763 spent securing accurate placement of the stimulating and recording electrodes and
764 cannula, and the subsequent 4 hr monitoring field-potential baseline and the impact of
765 intrahippocampal drug infusions.

766

767 Stimulating and recording electrode positions are shown in Figure S2A. The stimulating
768 electrode was a twisted bipolar Teflon-coated platinum-iridium electrode (20 µm
769 diameter, 400 µm coated diameter for each of the two single strands) aimed at the
770 angular bundle of the perforant path (AP 0.0 mm from lambda; ML 4.2 mm; DV 2.15 mm
771 from dura). The recording electrode was a single Teflon coated platinum-iridium wire
772 targeting the hilus of the dentate gyrus (AP 4.08 mm from bregma; ML 2.5 mm; DV 3.5
773 mm). The drug cannula was a 28 gauge stainless steel tube whose tip was, for the data
774 reported in Figure 2, stereotaxically located at least 0.5 to 1.0 mm (\pm 0.3) mm away from
775 the recording electrode (AP 3.6 mm from bregma; ML 2.6 mm; DV 3.5 mm).

776

777 Conventional field-potential recordings were made, with stimulation every 20 s, and
778 these monitored and calculated on-line using EPS software [47]. In response to biphasic
779 200 μ s stimulus pulses of circa 600–800 μ A, we measured both the early-rising slope of
780 the evoked potential by linear regression over several points, and the amplitude of the
781 evoked dentate population spike. The stimulus intensity was adjusted to secure initial
782 population spike amplitudes of circa 3–6 mV. LTP induction was attempted in some
783 studies using a high-frequency stimulation protocol. This tetanic stimulation consisted of
784 trains of 50 pulses (200 Hz), and repeated 3 times at an interval of 5 min [47].

785

786 Once acquired using suitable electrode placements, potentials typically remained
787 relatively stable over periods of up to 3–4 hr, with a small upward drift of the population
788 spike (but not fEPSP) that rarely exceeded 15% over this long period. Animals for which
789 the potentials were unstable were discarded. The same long time-period stability was
790 observed when aCSF was infused into the dorsal hippocampal formation at a depth
791 targeting a region encompassing the stratum moleculare of area CA1. A volume of 2 μ l
792 was infused (0.5 μ l/min) that, on the basis of previous autoradiographic data [24, 48]
793 would be expected to diffuse throughout the entire CA1, CA3 and dentate gyrus regions
794 of the septal (dorsal) hippocampus.

795

796 We then examined the impact of varying doses of drugs. We examined muscimol, D-AP5
797 and CNQX. Intrahippocampal infusions (2 μ l) of artificial cerebrospinal fluid (aCSF, as
798 vehicle), muscimol (0.19 mM), D-AP5 (30 mM) or CNQX (6-cyano-7-nitroquinoxaline-2,3-
799 dione, 3 mM) were made into the dorsal hippocampus of male, Lister-hooded rats (n = 20,
800 n = 5 per drug condition, aCSF at pH7.2; experimenter blind to drug administered;
801 urethane anaesthesia; perforant path stimulation, recording electrode in the hilus of the
802 dentate gyrus, drug cannula 1.0 mm distance, Figures 2G and S2A; [Data S2](#)). The infusion
803 volume and doses were varied in pilot work, settling on a protocol of 2 μ l for all three
804 drugs that would, on the basis of autoradiographic data[13, 15, 48], likely affect the entire
805 dorsal (septal) hippocampal formation, and extend to the intermediate region (to
806 minimise the chances of a false negative in the behavioural study[45, 49]). While 2 μ l is
807 high, such a volume should still display minimal spread beyond hippocampus. The aim
808 was to achieve:

809

- 810 • **Shutting down cell-firing but with minimal effect on fast synaptic transmission**
811 **and LTP induction (with low-dose muscimol – 0.38 nanomoles)**
- 812 • **Blocking the induction of LTP with minimal effect on fast synaptic transmission**
813 **or cell-firing (D-AP5 – 60 nanomoles).**
- 814 • **Shutting down both fast synaptic transmission and cell-firing (CNQX – 6**
815 **nanomoles)**

816

817 Effective doses for D-AP5 and CNQX were known from previous work[9, 10], but checked
818 to realize internal consistency. Concentrations of 1.3, 0.5 and eventually 0.19 mM of
819 muscimol were examined until, at the lowest dose, the dissociation we were seeking was
820 secured with an infusion volume of 2 μ l. Figure 2 shows the primary results (lowest dose
821 of muscimol – 0.38 nanomoles).

822

823 **Diffusion of muscimol**

824 Critically, we sought to measure the diffusion of low-dose of muscimol along the
825 longitudinal axis of the hippocampal formation in two ways: electrophysiology and
826 fluorescent imaging. As outlined in the main text, we wondered if "silent learning" in the
827 presence of muscimol could be an artefact of minimal drug diffusion from the site of
828 dorsal infusion to the intermediate region of the hippocampal formation. This could leave
829 intact hippocampal tissue to mediate learning. Accordingly, further electrophysiological
830 experiments were conducted with the same low-dose of muscimol (0.38 nanomoles), but
831 with the recording electrode location in the intermediate hippocampus (AP, -5.52 mm;
832 ML, 3.8 mm; DV; 4.1 mm) - a distance of 2.0 to 2.5 mm from the infusion cannula (Figure
833 S2B). Drug concentration and volume remained unchanged. Note that cell-firing
834 remained substantially inhibited in this intermediate zone of the hippocampus (Figures
835 S2C-S2E).

836

837 While these data reflect the impact of the drug, we also sought direct evidence of drug
838 diffusion. This is tricky to do with some studies conducting radiography using tritiated
839 (C^{14}) drugs, others using fluorescently labelled compounds. Muscimol is available as a
840 fluorescently labelled compound that can be visualised microscopically, but it suffers
841 from the difficulty that the molecular weight is much higher (607 instead of 114) and may
842 be more "sticky" with respect to diffusion in aCSF. Accordingly, it is likely a conservative

843 estimate of diffusion but is at least a direct measure. Fluorescently-labeled muscimol-
844 bodipy dissolved in aCSF (0.19 mM; Hello Bio, UK) was used to analyze the distribution
845 profile of fluorescent muscimol 40 min after its bilaterally infusion into the dorsal
846 hippocampus. Cannulae positions and the drug infusion procedure were same as in the
847 main behavioural experiment. Brains were removed and shock-frozen on powdered dry
848 ice, and 50- μ m-thick coronal sections were acquired with a cryostat (CM1900, Leica
849 Biosystems, Germany), and mounted on Silane-coated glass slides. Bright field and
850 fluorescent images of serial sections equally spaced 100 μ m were taken with a BZ-X700
851 Microscope (Keyence, Japan). Fluorescent images in grayscale were shown as arbitrarily
852 assigned color display mode (pseudocolor) according to their gray levels within a range
853 of 0–90[arbitrary units (AU)] and a 3D image showing the distribution of fluorescent
854 signal was reconstructed from serial sections using a Metamorph software (Molecular
855 Devices, USA). Overlaid images of bright-field and pseudocolor images were made using
856 a Photoshop (Adobe, USA).

857

858 **The problem of population spike potentiation**

859 A separate complication with low-dose muscimol was that not only was it permissive for
860 the induction of LTP measured using the early rising slope of the fEPSP, it was also
861 permissive for spike potentiation. Thus, while minimal cell-firing was observed *before*
862 LTP, some cell-firing under low-dose muscimol was observed *after* LTP induction (Figure
863 S3). This was modest and so, rather than plot normalised data to a near-zero pre-tetanus
864 baseline, absolute data are plotted. This raises the possibility that, in the behavioural
865 study, it may have been possible for the animals to retrieve information about a previous
866 session (s1) under low-dose muscimol after they had started encoding new information
867 about platform location in the current session (s2) (because cell-firing might then be
868 possible). In practice, we suspect such cell-firing is very unlikely in the behavioural
869 situation. This is because LTP induction using 3 trains of 50 pulses at 250 Hz is an
870 artificial tetanisation protocol designed to investigate activity-dependent synaptic
871 plasticity *in vivo* but a firing pattern that does not occur during normal behavioural
872 learning. Activity-dependent synaptic potentiation *in vivo* depends on more subtle
873 patterns of neuronal activation, such as spike-timing dependent plasticity in a subset of
874 neurons which is unlikely to cause much post-LTP cell-firing.

875

876 **QUANTIFICATION AND STATISTICAL ANALYSIS**

877

878 **Data Analysis**

879

880 ***Behavioural Data Analysis***

881 The measures of performance used were (1) first crossing latency (s) during training and
882 (2) percent time spent swimming in a target zone during DMP probe tests. The retrieval
883 tests were always on the first trial of each session and, as described above, involved the
884 hidden platform being raised to within 1.5 cm of the water surface only after 60 s had
885 passed.

886 Repeated-measures analysis of variance (ANOVA) was used to examine the impact of
887 within subjects variables on behavioral measures with within subject factors session and
888 condition. Orthogonal comparisons were used to further examine main effects of the
889 ANOVA. Two-tailed one-sample t tests were used to compare search preference measures
890 to the value expected by chance (4%). The level of significance was set $P < 0.05$. Data are
891 presented as mean \pm SEM. The statistical analysis was made using IBM SPSS Statistics
892 (IBM, USA).

893

894 ***Electrophysiological Data Analysis***

895 The analyses were done using routines implemented in Spike2, version 6.03 (Cambridge
896 Electronic Design, UK). Quantitative measurements reflecting the fEPSP and the
897 population spike (PS) activity were done following standard criteria. The PS recorded in
898 the Dentate Gyrus is measured as the difference between the maximal negativity of the
899 spike and the maximal point of the precedent positive crest. The fEPSP is measured as the
900 steepest slope in the negative going potential in mV/ms.

901

902 All statistics were performed and plotted using GraphPad Prism 5.04 software (GraphPad
903 Software, USA). For any statistical analysis shown, two-tailed repeated-measures t test
904 (for two groups, or one group vs. a fixed number) or 1-way ANOVA (for more than two
905 groups) for a significance level of $P < 0.05$ was used. When 2 factors concur, 2-way ANOVA
906 is utilized with the same significance threshold. For repeated measures experiments,
907 repeated-measures ANOVA are utilized. Post-hoc Bonferroni multiple-comparisons test
908 is used to describe the origin of significance. All graphs represent Mean \pm SEM.

909

910 ***Histological Data Analysis***

911 For analysis of diffusion for fluorescent muscimol, areas in the dorsal/intermediate
912 hippocampus, retrosplenial cortex and parietal association/secondary visual cortices
913 were equally divided into grid squares (200 × 200 μm) and the averaged fluorescent
914 intensity was measured. Also, we divided the entire cortical area at the injection point
915 (i.e., -4.00 mm from the Bregma) into ten equal regions, calculated the averaged
916 fluorescent intensity, and set the threshold as the mean + 2SD. The measurements of
917 fluorescent intensity and area was made using a MetaMorph software.

918

919 **DATA AND SOFTWARE AVAILABILITY**

920 Electrophysiological and behavioural data are available upon request by contacting the
921 Corresponding Authors, Richard Morris (r.g.morris@ed.ac.uk) and on-line.

922

923 **SUPPLEMENTAL INFORMATION**

924 **Data S1:** First-crossing latency for behavioural task during initial training displaying
925 within day learning to approach the correct escape location across each daily set of 4
926 trials. Related to Figure 1E.

927

928 **Data S2:** Full electrophysiology data for Figure 2.

929

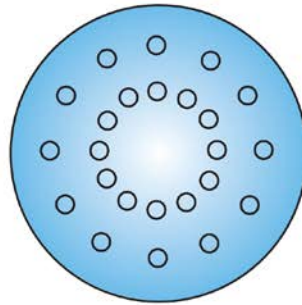
930 **Data S3:** Probe test data showing patterns of recall and new learning across different
931 drug conditions.

932

A Photograph of 2 metre watermaze



B Possible platform locations



C Atlantis Platform

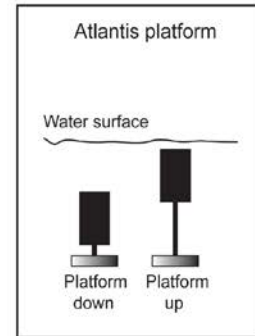


Figure S1. The Watermaze and Atlantis Platform. Related to Figure 1.

(A) Photograph of the pool and associated 3D extramaze cues in the laboratory in Edinburgh. (B) The possible platform locations ($n=24$), with only one used per session, located on virtual outer and inner rings as viewed by the overhead camera. (C) The 'on-demand' or 'Atlantis Platform' is unavailable at the bottom of the pool until it rises on a spindle until its top surface is 1.5 cm below the water surface, and thus available for escape.

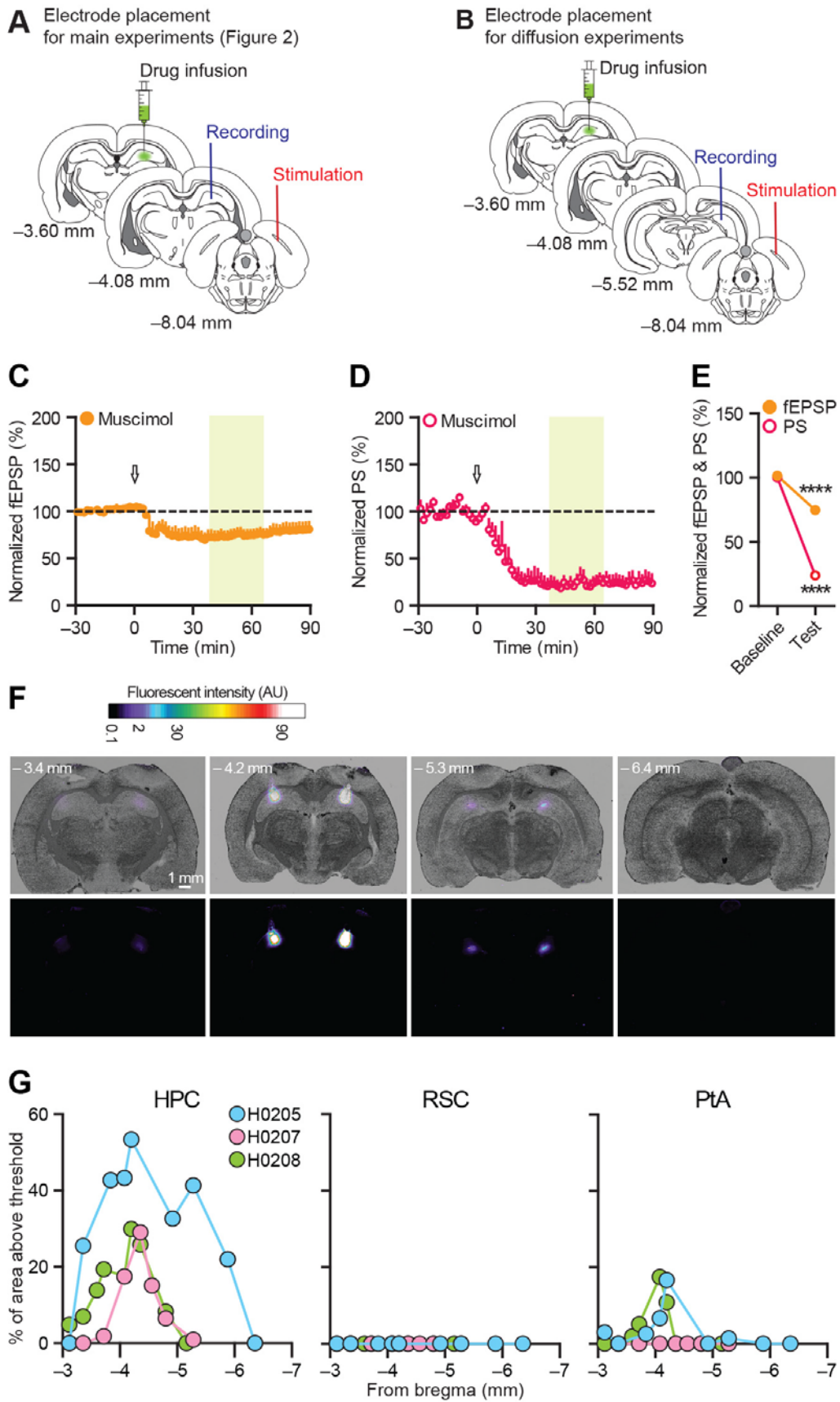


Figure S2. Electrophysiological analysis of effect drug diffusion through longitudinal axis of hippocampus, and of diffusion of a fluorescent labelled muscimol. Related to Figure 2. (A) Electrode

placements for the main study in which the recording electrode was within 0.5 mm from the AP location of the 2 ul drug infusion (i.e. region of maximal effect). Data shown in main Figure 2. (B) Electrode placements for the subsidiary study in which the recording electrode was 2 mm from the AP location of the 2 ul drug infusion. (C, D, E) fEPSP, Population Spike and averaged data (n=5) of the animals in the subsidiary study. The drug continues to have a substantial impact at 2 mm distance, suggesting a likely spread over 4 mm in the AP direction of muscimol. (F, G) Image and quantitation of diffusion of fluorescent muscimol bodipy from an infusion site at AP 4.0. One animal (H0205) showed diffusion of muscimol bodipy over approximately 3.0 mm, the other 2 animals showed less diffusion. There is no diffusion in the retrosplenial cortex, but some is detected in the parietal area immediately above the tip of infusion cannula reflecting "leakage" up the cannula track.

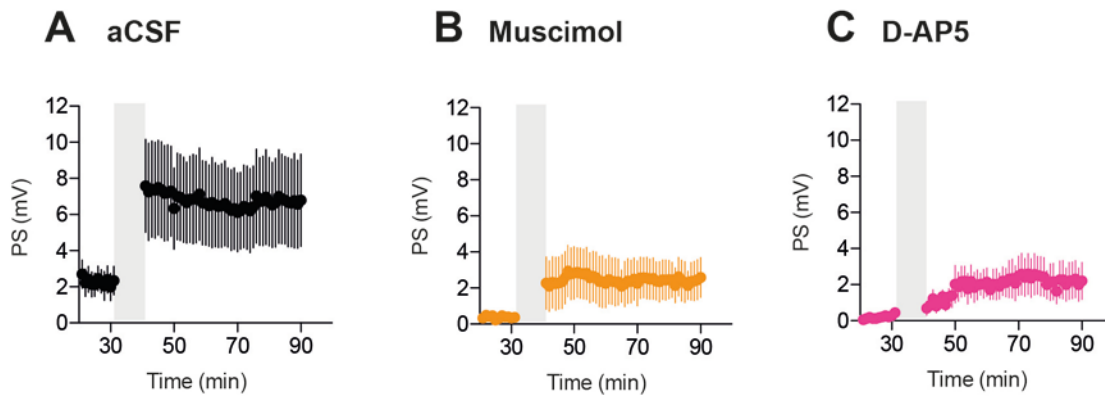


Figure S3. Population spike LTP shown in mV. The usual way in which LTP data is plotted is normalised to a pre-tetanus baseline. However, this is not sensible with a near zero baseline for the muscimol treated animals. Related to Figure 2.

(A) Clear potentiation of the population spike (PS) occurred after aCSF infusion, with the mean population spike reaching circa 8 mV. (B) Potentiation of the spike also occurred with low-dose muscimol (0.38 nanomoles), but the absolute magnitude was very small and a mean pop spike of circa 2 mV. (C) D-AP5 blocked spike potentiation, when judged against the steadily rising control condition (see Figure 2). However, some spiking is observed as with muscimol.