NMDA receptor antagonists sustain LTP and spatial memory: active processes mediate LTP decay

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Although long-term potentiation (LTP) is long-lasting, it is not permanent and decays within weeks after its induction. Little is known about the processes underlying this decay. Here we assessed the contribution of synaptic activity to LTP decay by determining the effect of the competitive NMDA receptor antagonist CPP on the decay of perforant path–dentate LTP. CPP blocked decay over a one-week period when administered daily following the induction of LTP, and blocked decay of the late, protein-synthesis-dependent phase of LTP when administered two days after LTP induction. CPP administered for a five-day period following spatial memory training enhanced subsequent memory retention. These data suggest that LTP is normally a persistent process that is actively reversed by NMDA receptor activation, and that both the early and late phases of LTP are dynamic processes regulated by NMDA receptors. These data also support the view that LTP is involved in maintaining spatial memory.

Long-term synaptic potentiation remains the most studied mechanism of synaptic plasticity thought to reflect the cellular events that underlie memory storage[1,2]. LTP is an activity-dependent synapse-specific increase in synaptic efficacy, and is observed in a variety of brain structures implicated in learning and memory, such as the hippocampal formation[2]. LTP is long-lasting; when observed *in vivo*, LTP persists for days to weeks in freely moving animals, depending on stimulation parameters[3]. Although the processes underlying the induction of NMDA-receptor-dependent LTP have received much attention, the processes underlying the maintenance of LTP, particularly LTP lasting from days to weeks, are poorly understood. Current evidence suggests LTP maintenance comprises at least 2 phases[4–6], an early phase of LTP maintenance (E-LTP) thought to involve activation of protein kinases and the phosphorylation of key substrates, and a later phase of LTP (L-LTP) lasting beyond 24 hours, which is maintained by new protein synthesis[4–7].

Although LTP is persistent and depends on new protein synthesis, it is not permanent, and potentiated responses eventually decay to baseline levels at most synapses in the hippocampal formation. At the medial perforant path–dentate gyrus synapse, LTP typically decays within 3–5 days following a single session of high-frequency stimulation *in vivo*[3]. The mechanisms underlying the decay of LTP are not known, although the gradual decay of LTP observed at most hippocampal synapses *in vivo* could reflect a passive 'rundown' of processes that maintain LTP[8], such as protein turnover or gradual dephosphorylation by basal phosphatase activity of substrates involved in LTP[9]. Alternatively, LTP decay could be mediated by active processes[8,10,11] such as depotentiation or a reversal of LTP by long-term depression (LTD), which are activity-dependent synaptic processes that also require NMDA receptor activation[11–21].

Here we address the possible contribution of synaptic activity and NMDA receptor activation in response to LTP decay by sustained systemic administration of the competitive NMDA receptor antagonist CPP ((*R*,*S*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) following the induction of LTP at the perforant path–dentate gyrus synapse in awake, freely moving animals. Our results indicate that LTP decay is blocked by NMDA receptor antagonists, a finding that suggests that LTP maintenance is a persistent process that is actively reversed by NMDA receptor activation. In addition, sustained NMDA receptor blockade also facilitates the retention of spatial memory, a finding that suggests sustaining LTP also sustains spatial memory, and supports the view that LTP is involved in maintaining spatial memory.

Results

We first determined the efficacy of a single systemic 10 mg/kg dose (intraperitoneal, i.p.) of CPP in blocking NMDA receptors over a 24-hour period. This was estimated by determining the efficacy of CPP in blocking LTP induction at various time points after CPP administration. LTP was induced using a modified theta burst stimulation (TBS) protocol[36] consisting of 3 sets of five 2.3-second trains of 400 Hz (50-ms bursts delivered at 200-ms intervals) delivered 12 or 24 hours following CPP administration. A single 10-mg/kg dose of CPP significantly attenuated potentiation of the synaptic (field EPSP slope, fEPSP) component of LTP when induced 12 or 24 hours after CPP administration (**Fig. 1**; $F_{2,7} = 6.73$, $p < 0.05$), as reported previously[12]. Thus, a single 10-mg/kg dose of CPP afforded a sustained block of NMDA receptors over a 24-hour period. This dose was therefore used in all of our subsequent studies.

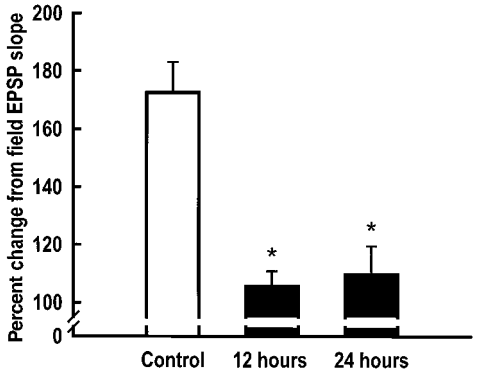

Fig. 1. Efficacy of a 10-mg/kg dose of the NMDA receptor antagonist CPP on LTP induced 12 and 24 h following administration. CPP significantly reduced the magnitude of medial perforant path–dentate LTP induced 12 and 24 h following CPP administration as compared to the magnitude of LTP in vehicle-treated controls (*$p < 0.05$; control, $n = 4$; 12 h, $n = 4$; 24 h, $n = 4$).

We next assessed the effect of NMDA receptor blockade on the decay of LTP. LTP was induced using the modified TBS protocol as described. The magnitude of LTP as reflected in measures of the field EPSP slopes one hour after TBS stimulation was equivalent in animals treated subsequently with either CPP or the water vehicle (mean change in field EPSP slopes 1 h after tetanization, 167 ± 19% and 152 ± 5% for animals treated subsequently with either vehicle or CPP, respectively; $p > 0.05$, **Fig. 2**). We gave animals either CPP (10 mg/kg i.p.) or an equivalent volume of the water vehicle 1 hour following LTP induction and every 24 hours thereafter for a 6-day period. Measures of LTP magnitude over this six-day period revealed that control animals receiving the vehicle displayed decremental LTP typical of medial perforant path–dentate synapses, with response magnitudes decreasing significantly by day 2 as compared to the magnitude of LTP observed 1 hour after tetanus. In contrast, in animals that received CPP after LTP induction, response magnitudes remained potentiated over the seven-day period of CPP administration and did not differ significantly from the initial magnitude of potentiation observed one hour following LTP induction (**Fig. 2**; $F_{\text{interaction}1,10} = 2.44$, $p < 0.05$). LTP decay was evident following cessation of CPP administration by day 8. CPP administered to untetanized animals was without effect on unpotentiated perforant path responses throughout the time course. Thus, administration of a competitive NMDA receptor antagonist beginning one hour after LTP induction blocked LTP decay over the seven-day period of administration.

LTP maintenance is thought to involve at least two phases, an early (E-LTP) phase thought to involve kinase activation and subsequent phosphorylation of various substrates, and a late phase (L-LTP) lasting more than 24 hours and thought to be mediated by *de novo* protein synthesis[4–7]. Both the early and the protein-synthesis-dependent late phases of LTP are observed at the medial perforant path–dentate gyrus synapse *in vitro* and *in vivo*[4–6]. In the previous study, CPP was administered one hour following LTP induction, a time point during the early phase of LTP thought to be maintained by phosphorylated substrates and reversed by calcium-dependent phosphatases[9,10]. It therefore is possible that a blockade of NMDA receptors shortly after LTP induction may have prevented LTP decay simply by preventing activation of calcium-dependent phosphatases, thereby prolonging LTP by sustaining the early phase of LTP. Because the late phase of LTP at perforant path–dentate synapses is established by 24 hours[4–7], we determined if administration of CPP at a time point after the decay of E-LTP and the establishment of L-LTP could 'rescue' established L-LTP. This was tested by administering CPP 2 days after LTP induction. As before, LTP was induced using a modified TBS protocol. The magnitude of LTP measured one hour post-tetanus did not differ among the groups that were treated with either CPP or vehicle (mean change in responses one hour after tetanization, 167 ± 19% and 156 ± 9% for animals treated subsequently with either vehicle or CPP, respectively, $p > 0.05$, **Fig. 3**). Following LTP induction, the water vehicle was administered to all animals at 1 and 24 hours following LTP induction. Forty-eight hours after LTP induction, either CPP (10 mg/kg) or equivalent volumes of the water vehicle was then administered to animals immediately following collection of daily responses, and daily thereafter for an additional five days. As observed in animals given CPP 1 hour following LTP induction, CPP administered to animals 2 days following LTP induction also prevented the decay of LTP over the 5-day period of drug administration, with response magnitudes over the 3–7-day period not differing significantly from the magnitude of LTP observed 2 days after tetanus (**Fig. 3**). In contrast, vehicle-treated animals displayed a significant decrement in response magnitudes over the 3–7-day period (**Fig. 3**; $F_{\text{interaction}1,4} = 2.79$, $p < 0.05$). Thus, decaying LTP can be rescued by NMDA receptor antagonists, even after establishment of the late phase of LTP requiring *de novo* protein synthesis.

If LTP is a cellular mechanism that contributes to memory storage and NMDA antagonists can prolong the duration of LTP, then

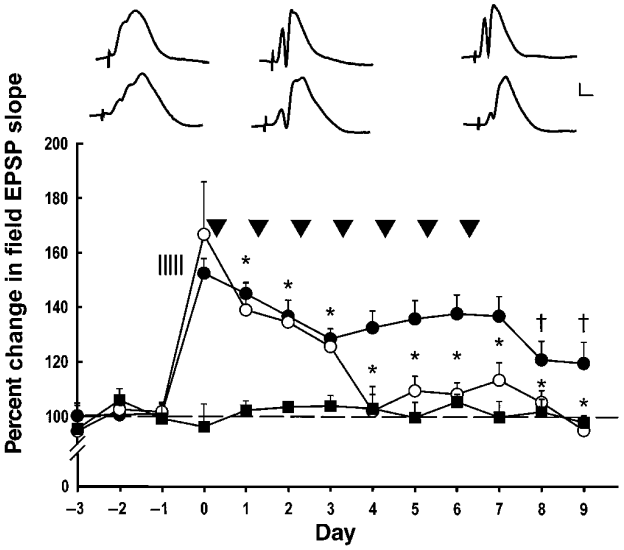

Fig. 2. Administration of CPP blocks LTP decay. Effect of CPP or the water vehicle on LTP decay when administered 1 h following LTP induction (IIIII). CPP (black circles, $n = 5$) or the water vehicle (white circles, $n = 6$, pooled control data) were administered 1 h after LTP induction (day 0), and daily thereafter for 6 days (black triangles). Decay was observed in vehicle-treated animals over the 6-day period when compared with the magnitude of LTP 1 h after tetanus (*$p < 0.05$). In CPP-treated animals, the magnitude of response over the 7-day period of CPP administration did not differ significantly from LTP measured at 1 h after tetanus. Decay became evident after day 7 (crosses, $p < 0.05$). CPP was without effect on unpotentiated perforant path responses (black squares, $n = 3$). Insets show representative traces of baseline response (left), and responses 1 h (middle) and 7 days (right) following LTP induction. Scale bar, 5 ms, 0.5 mV.

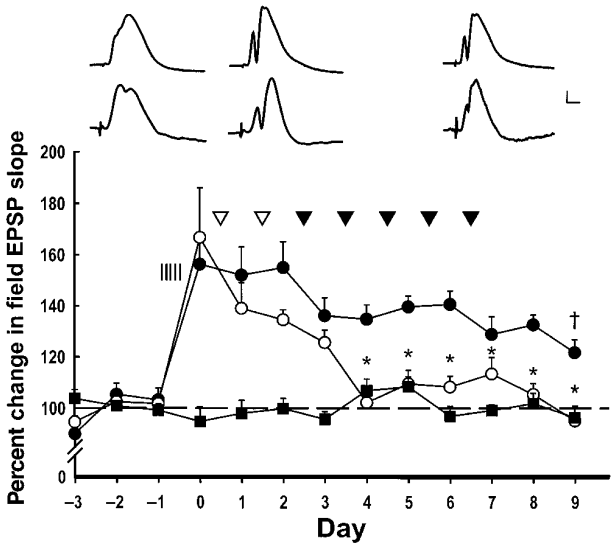

Fig. 3. CPP blocks the decay of late-phase LTP. Effect of CPP or vehicle on LTP decay when administered 2 days after LTP induction (IIIII). Water vehicle was administered to all animals 1 h and 24 h following LTP induction (white triangles). Forty-eight hours after LTP induction, CPP (black circles, n = 4) or the water vehicle (white circles, n = 6, pooled control data as in **Fig. 2**) was administered daily for an additional 5 days (black triangles). Decay was observed in vehicle-treated animals as compared with the magnitude of LTP on day 2 (*p < 0.05). In CPP-treated animals, the magnitude of potentiated responses over the 3–7-day period of CPP administration did not differ significantly from the magnitude of LTP on day 2 (cross, p < 0.05). CPP was without effect on unpotentiated perforant path responses (black squares, n = 3). Insets show representative traces of perforant path–dentate baseline responses (left) and responses collected 1 h (middle) and 7 days (right) following LTP induction for vehicle and CPP-treated animals. Scale bar, 5 ms, 0.5 mV.

administration of an NMDA receptor antagonist following learning would also be expected to prolong retention of recently acquired information. We therefore assessed the effect of post-training administration of CPP on the retention of a spatial memory task known to require the hippocampus and dentate gyrus, the eight-arm radial maze[37]. Animals food-deprived to 80% of normal body weight were trained daily over a 2-week period to retrieve food from 4 baited arms of an 8-arm radial maze. After reaching criteria (1 or 0 working or reference errors over 2 consecutive days), each animal was returned to its home cage, and randomly assigned to groups that received either CPP (10 mg/kg) or equivalent volumes of the water vehicle. CPP or the vehicle was administered 24 hours following the last training session and daily thereafter for 4 days without any further exposure to the maze. Twenty-four hours following the last dose of CPP or vehicle (6 days following the last training session), the animals were reintroduced to the maze and tested for memory retention. Analysis of spatial memory errors over the training period 5 days before reaching criterion and before administration of either CPP or vehicle revealed no differences between groups in acquiring the task (**Fig. 4**; $F_{1,10} = 1.78$, $p > 0.05$). However, upon reintroduction to the maze six days after the last training session, within-groups analysis revealed that animals receiving the vehicle displayed significantly more arm entry errors in the retention test than observed on the last day of training (**Fig. 4**), suggesting normal memory loss over the 5-day delay period ($F_{1,10} = 5.29$, $p < 0.05$). An analysis of arm entry errors revealed significant increases in both reference ($F_{1,10} = 5.60$, $p < 0.05$) and working memory errors ($F_{1,10} = 6.43$, $p < 0.05$; **Fig. 4**). By contrast, no significant differences were observed in retention test entry errors in CPP-treated animals when compared to the number of errors observed on the last day of training ($F_{1,10} = 1.62$, $p > 0.05$; **Fig. 4**). Comparisons of memory error type (working memory (WM) versus reference memory (RM)) also indicate no differences in either WM ($p > 0.05$) or RM errors ($p > 0.05$) in the retention test as compared to the last day of training. These differences are unlikely due to increased motivation resulting from a CPP-mediated reduction in appetite and subsequent weight loss, for both groups were food-deprived for this appetitive task, and CPP- and vehicle-treated groups did not differ significantly in weight as measured on the first day of retention testing (mean weight of animals treated with CPP, 330 ± 10 g versus 322 ± 14 g for vehicle-treated controls; $F_{1,10} = 0.24$, $p > 0.05$). These differences also are unlikely due to effects of CPP on locomotor activity, as analysis of half of the animals randomly selected from both groups indicated no significant differences in running speed (CPP-treated animals, 38 ± 5 cm/s; vehicle-treated animals, 46 ± 8 cm/s; $F_{1,19} = 0.74$, $p > 0.05$). These data indicate daily systemic administration of CPP enhanced both working and reference spatial memory in an eight-arm radial maze task. Thus, administration of CPP in doses effective in blocking LTP decay also enhanced spatial memory.

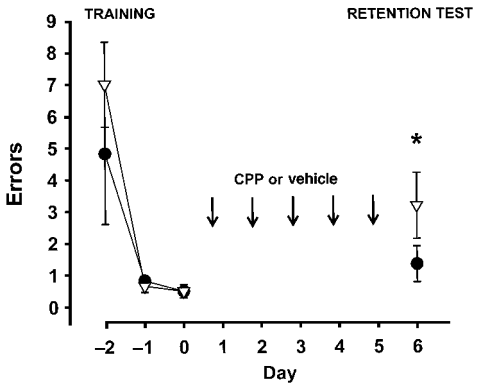

Fig. 4. CPP enhances retention of spatial memory. Shown are the number of entry errors (working and reference memory errors) 3 days before and 24 h after daily administration of CPP (black circles, n = 6) or vehicle (white triangles, n = 6) over a 5-day period (arrows). When tested for memory retention (day 6), vehicle-treated animals displayed significantly more entry errors in the retention test as compared to the last day of training (*p < 0.05). In contrast, the number of entry errors in the retention test did not differ significantly from the number of errors on the last day of training in CPP-treated animals.

Discussion

Here we demonstrate that daily administration of the competitive NMDA receptor antagonist CPP beginning 1 or 48 hours after LTP induction blocks the subsequent decay of LTP. This indicates that LTP decay, rather than reflecting a passive 'run-down' of mechanisms that maintain LTP, seems to be an active process mediated by activation of NMDA receptors[8,10,11]. Thus, NMDA receptors not only mediate the induction of LTP, but also its eventual decay. Because voltage-dependent NMDA receptors are localized postsynaptically at glutamatergic synapses in

the hippocampal formation, the maintenance and decay of LTP is likely regulated by postsynaptic activation of NMDA receptors, as is the induction of LTP. In addition, CPP blocks LTP decay when administered after establishment of the late phase of LTP maintenance requiring *de novo* protein synthesis[4–7]. Thus, the maintenance of the protein-synthesis-dependent late phase of LTP, rather than establishing stable or irreversible changes[7], seems to be a labile process[16,18,22] dynamically regulated by NMDA receptor activation. Together, these data suggest that LTP maintenance normally is a persistent process, and its eventual decay is an active process mediated by synaptic activity and NMDA receptor activation.

Whether NMDA-receptor-dependent LTP is a mechanism of synaptic plasticity that normally contributes to learning and memory remains debated[23,24]. Given that NMDA antagonists block LTP decay, if LTP is involved in memory storage, then it would be predicted that blocking the decay of LTP would enhance spatial memory. Previous correlational studies support this view, and indicate that posttraining administration of NMDA antagonists facilitate both spatial memory retention and LTP longevity[25,26]. Our data also support this view, and indicate that doses of CPP that enhance LTP longevity also enhance retention of spatial memory.

The mechanism by which NMDA receptor activation mediates LTP decay remains to be determined. Among the possibilities are mechanisms that serve to decrease synaptic strength, such as depotentiation (reversal of established LTP by subsequent activity in the same afferents[18–20]) or LTD (both reviewed in ref. 13), processes that are observed at perforant path–dentate synapses and that require the activation of NMDA receptors[10,13–17]. However, depotentiation at perforant path–dentate synapses is limited to brief periods (minutes) following LTP induction[18,19]. Heterosynaptic LTD is more likely to mediate LTP decay[13–17,20,21]; this process is observed at inactive synapses in response to LTP induction or synaptic activity at other, neighboring synapses. The induction of heterosynaptic LTD can reverse medial perforant path–dentate LTP several days after its induction[21]. Thus, previously established LTP normally may be 'erased' by heterosynaptic LTD induced by a subsequent induction of LTP at neighboring synapses, possibly as a result of subsequent learning[16,20,22]. If LTP indeed reflects a cellular mechanism of information storage, extrapolation of these data to learning theory seems to support the view that interference[27] resulting from subsequent learning is an important process underlying memory loss. However, the induction of heterosynaptic LTD does not require LTP induction at neighboring synapses[13,17,29]. Furthermore, NMDA receptors can be activated by tonic granule cell activity[30]. Thus, background synaptic activity unrelated to information storage also could mediate LTP decay. Such a process would be more in line with memory loss resulting from non-specific decay[28]. Taken together, these data suggest that not only may both interference and decay contribute to forgetting, but also that these processes are both active and involve identical cellular mechanisms, specifically, the activation of NMDA receptors.

The present study demonstrates that LTP decay, rather than reflecting a passive 'rundown' of processes that serve to maintain LTP, reflects an active process mediated by the activation of NMDA receptors. Furthermore, the maintenance of both the early and late protein-synthesis-dependent phases of LTP maintenance are dynamic processes regulated by NMDA receptor activation. Whether LTP reflects a mechanism of synaptic plasticity that normally contributes to learning and memory remains debated[23,24]. The present study demonstrating that sustained NMDA receptor blockade enhances both LTP longevity and spatial memory retention adds to the accumulating convergent evidence[31–34] that LTP is a mechanism of synaptic plasticity involved in maintaining spatial memory.

Methods

All experiments were performed under NIH guidelines for the care and use of animals in research and were approved by the Institutional Animal Care and Use Committee of the University of Texas at San Antonio. Medial perforant path responses were recorded in the dentate gyrus of the hippocampal formation in awake, freely moving animals with permanently implanted electrodes. Procedures for surgery and electrode implantation were similar to those described previously[3]. Adult male Sprague–Dawley rats (300–350 g, Charles River, Raleigh, North Carolina) were anesthetized with pentobarbital sodium. Medial perforant path responses were evoked by stimulation of the extreme dorsomedial aspect of the angular bundle (AP, –8.1; ML, +4.1; DV, –2.3 mm, relative to bregma[35]) using a bipolar electrode constructed from twisted Teflon-coated stainless steel wire (0.008-inch diameter, A-M Systems, Carlsborg, Washington). Medial perforant path responses were recorded in the hilus of the ventral dentate gyrus using a single Teflon-coated stainless steel wire (AP –3.5, ML +2.0, DV –3.3 mm, ref. 35). Following surgery, animals were given a single dose of the antibiotic Pen BP-48 (100,000 units/kg i.m., Pfizer Pharmaceuticals, Lee's Summit, Missouri, New Jersey) and ibuprofen (1 mg/ml *ad lib* for 3 days), and were allowed to recover for a 2-week period.

Following recovery, input/output (I/O) curves reflecting evoked field EPSP slopes as a function of current intensity (50–600 μA) were collected for each animal. Current intensities that elicited field EPSP slopes that were 50% of the asymptotic amplitude were used for all stimulation, including tetanic stimulation used to induce LTP. Measurements of the magnitude of dentate field EPSPs were confined to the initial slope (dV/dt) of the field EPSP measured over the 2–4-ms period after response onset. Measures of daily response magnitudes used measures of the slope of the average of 10 evoked responses collected at 20-s intervals. Only animals in which perforant path–dentate responses appeared stable for at least 1 week were used for these studies. LTP was induced by high-frequency stimulation of the medial perforant path using a modified theta burst[36] stimulation (TBS) paradigm (3 sets of five 2.3-s trains consisting of ten 50-ms 400-Hz bursts delivered at 200-ms intervals, with each set delivered at 20–300-s intervals.) Both daily recordings and high-frequency trains were delivered in the animal's home cage when the animal was alert. Animals that did not display LTP (less than a 20% increase over baseline responses 1 h following LTP induction) were omitted from these studies. Responses were collected daily for at least 1 week following LTP induction. Changes in synaptic response magnitudes are presented as the percent change from the average of baseline response amplitudes collected over the 5-day period before tetanus.

CPP (Tocris, Ballwin, Missouri) is a competitive and selective NMDA receptor antagonist. In experiments assessing the effect of CPP on LTP induction, CPP was administered 12 and 24 h before TBS stimulation. LTP magnitude was assessed 50–60 min post-tetanus as compared with pre-tetanus baselines. In experiments assessing the effect of CPP on LTP induced 1 h previously, either CPP or equivalent volumes of the water vehicle were administered intraperitoneally 1 h following LTP induction, and every 24 h thereafter for a 6-day period immediately following collection of daily evoked responses. In experiments assessing the effect of CPP on LTP established 2 days previously, the water vehicle was administered to all animals 1 and 24 hours following LTP induction. Animals were then randomly assigned to 2 groups that subsequently received either CPP or vehicle over the 2–6-day period following LTP induction. Statistical comparisons of LTP magnitude and decay were assessed using a 2-way repeated measures ANOVA, and a Newman–Keuls test for *post hoc* pair-wise comparisons of the magnitude of LTP observed 1 h after tetanus with responses observed over the 1–9 day period after tetanus and comparisons of the magnitude of LTP observed 2 days after induction with response magnitudes observed over the 3–9 day period of drug or vehicle administration.

For behavioral studies, spatial memory was assessed using an 8-arm radial maze (Lafayette Instruments, Indiana) using a standard training protocol[37]. Animals were food-deprived and maintained at 80% of the weight of aged-matched cohorts. Animals were then allowed to habituate to the maze daily for a 5-min interval over a 7-day period. Training was initiated 24 h following the 7-day habituation period. Animals were allowed to explore the maze each day with 4 of the 8 arms baited until either all baited arms were visited, or until a maximum time period of 10 min was reached. Animals were trained over a maximum period of 2 weeks. Once an animal reached criterion (1 or 0 working or reference memory errors over 2 consecutive days within the 2-week training period), it was returned to its home cage and arbitrarily assigned to 1 of 2 groups that were given i.p. injections of either CPP (10 mg/kg i.p. dissolved in water) or the water vehicle alone 24 h after training, and daily thereafter for a 5-day period. Tests for memory retention were conducted 6 days after the last day of training (24 hours after the last dose of CPP or vehicle). For the retention test, animals were placed in the maze for a 10-min period. Both reference memory (RM) and working memory (WM) errors (entry into unbaited arms or re-entry into baited or unbaited arms[37]) were recorded with the experimenter blind to the drug conditions. Measure of spatial memory was operationally defined by combining both reference and working memory 'entry errors.' Both entry errors and WM and RM errors were evaluated statistically within groups using repeated measures ANOVA and a *post hoc* Newman–Keuls test for planned pair-wise comparisons.

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Competing interests statement

The authors declare that they have no competing financial interests.

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