Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5

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Recent work has shown that the hippocampus contains a class of receptors for the excitatory amino acid glutamate that are activated by N-methyl-D-aspartate (NMDA) and that exhibit a peculiar dependency on membrane voltage in becoming active only on depolarisation.2,3 Blockade of these sites with the drug aminophosphonovaleric acid (AP5) does not detectably affect synaptic transmission in the hippocampus, but prevents the induction of hippocampal long-term potentiation (LTP) following brief high-frequency stimulation.4,5 We now report that chronic intraventricular infusion of D, L-AP5 causes a selective impairment of place learning, which is highly sensitive to hippocampal damage, without affecting visual discrimination learning, which is not. The L-isomer of AP5 did not produce behavioural effects. AP5 treatment also suppressed LTP in vivo. These results suggest that NMDA receptors are involved in spatial learning, and add support to the hypothesis that LTP is involved in some, but not all, forms of learning.

On day 0 of the place-learning experiment, male Lister rats (n = 36) were implanted with minipumps (Alza, 2002) containing D, L-AP5 (40 mM in 0.5% saline, n = 10); we estimate that the steady-state concentration in cerebrospinal fluid would have risen to 150 μM, L-AP5 (20 mM, n = 6) or served as controls made up of saline minipump (n = 10) and unoperated (n = 10) subgroups. Using stereotaxic techniques, under Avertin anaesthesia, a cannula (25-gauge) was lowered into the right lateral ventricle (Bregma, −0.9 mm). It was attached to a catheter, secured by dental cement, and connected to the subcutaneously implanted minipump. At the completion of testing, a check made that each pump was still connected to its cannula, and the brains removed for histological verification of the cannula locations.

On day 4, the rats were placed into a large pool of opaque water (at 26 ± 1 °C) and trained to find and escape onto a platform which was hidden at the centre of either the SW or NE quadrants of a large 2.14 m diameter pool.6

Training was undertaken on days 4–8. We began by giving a total of 15 trials (3 trials per day at a 4-h inter-trial interval (ITI), randomized starting locations, 5 days). The rats swam until they found the platform (maximum swim time 120 s), with 30 s spent on it before removal. Escape latency was measured with a stopwatch. On day 9, a spatial transfer test was performed and further training was given. The purpose of the transfer test was to find out how much had been learned about the location of the escape platform. It was therefore removed from the pool, and the animals given 60 s to swim freely with no opportunity of escape. Their movements were tracked automatically using an image-analysing device.7,8 Normal rats search near their former location in this kind of test. They were then given eight further training trials in rapid succession (30-s ITI), with the platform back in its original location. In reversal trials, on days 10–13, the rats were trained to learn a new location, in the opposite quadrant from that used in earlier training (only one trial per day).

The control and L-AP5 groups learned to escape relatively rapidly within 15 trials (Fig. 1). The D, L-AP5 group learned more slowly, but their impairment was not striking; considerable

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Unoperated</td>
<td>6</td>
<td>60.8*</td>
<td>41–100</td>
</tr>
<tr>
<td>Saline</td>
<td>8</td>
<td>80.6</td>
<td>50–120*</td>
</tr>
<tr>
<td>D, L-AP5</td>
<td>13</td>
<td>76.8</td>
<td>41–120*</td>
</tr>
<tr>
<td>L-AP5</td>
<td>9</td>
<td>67.9</td>
<td>40–100</td>
</tr>
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* The groups did not differ significantly in rate of learning Kruskal-Wallis H = 7.19, P > 0.05.
† Two saline rats and three D, L-AP5 rats failed to reach criterion before the pumps were exhausted.

‘nonspecific’ instrumental learning occurs early in training (for example, to swim away from the side walls) and this may have masked an impairment in true place-learning. Moreover, some D, L-AP5 animals occasionally fell off the escape platform on the first 2–3 days of training. However, the outcome of the further training on day 9, after the transfer test, showed that the D, L-AP5 rats stabilized their escape latencies at a much higher level, taking longer, more indirect routes to the platform.

![Fig. 1 Mean latency of escape(s) for each of the 10 days of behavioural testing. Days 4–8, mean of the 3 daily trials; day 9, subdividing into 4 blocks of 2 trials; days 10–13, the single reversal trial of each day. The transfer test on day 9 immediately preceded the 8 further training trials. To avoid problems of heterogeneity of variance, the successive phases of the experiment were analysed separately. Training (days 4–8): unweighted means (unequal n) analysis of variance revealed significant effects of group (F = 7.11, d.f. 2/33, P < 0.005), and days (F = 101.6, P < 0.0001). Subsequent orthogonal comparisons showed that the escape latency impairment was restricted to the D, L-AP5 group (Control versus L-AP5, P > 0.10; D, L-AP5 versus control, P < 0.01). Further training (day 9): the groups differed significantly (F = 7.87, d.f. 2/33, P < 0.002) with the impairment again restricted to the D, L-AP5 group (D, L-AP5 versus control, P < 0.0005). Reversal (days 10–13): all groups showed a marked increase in latency on the first trial of reversal, the increases being 75.3 and 53.4 s for the control and L-AP5 groups, but only 34.9 s for the D, L-AP5 group, but this trend did not differ between groups. Over the reversal trials, the groups differed significantly in escape latency (F = 7.68, d.f. 2/33, P < 0.005) with the impairment in learning to approach the new platform position specific to the D, L-AP5 group. On day 13 group D, L-AP5 escaped more slowly than the L-AP5 and control groups combined (F = 4.31, P < 0.05). ○, Control; ○, D, L-AP5; □, L-AP5.
L-AP5 and control rats learned to escape with minimum latencies, using direct paths to the platform. During the four reversal trials of days 10–13, the D₅-L-AP5 group failed to learn the new platform location, while L-AP5 and control animals showed a significant decline in latency across trials.

Analysis of the paths taken during the transfer test on day 9 provided the most convincing demonstration that the D₅-L-AP5 rats had learned little about the platform location. Figure 2 shows the paths swum by a typical rat of each group, and the mean distribution of time spent in the four quadrants. The D₅-L-AP5 group showed little spatial bias to the training quadrant.

These data imply that chronic infusion of an NMDA antagonist causes a marked impairment in place learning, similar to that caused by hippocampal lesions. However, we could not distinguish between a primary effect on spatial learning per se, and a secondary effect upon sensorimotor or motivational processes. In a second behavioural study, we used the same apparatus, and thus the same motivation to escape from water, but a learning task which did not require spatial learning.

To assess visual discrimination, two discrimimable platforms (grey, and black-and-white stripes) were placed into the pool with their top surfaces 2 cm above the water surface. One was rigid and provided escape; the other was floating and sank under the water whenever the rat attempted to climb on. Black curtains surrounded the perimeter of the pool obscuring extra-maze cues, and the platforms were moved between several locations over successive trials throughout training. The rats' task was to learn to approach the rigid platform irrespective of location. A total of 36 male Lister rats were trained (D₅-L-AP5, 40 mM, minipumps, n = 13; L-AP5, 20 mM, n = 9; saline, n = 8, unoperated n = 6). They were given 10 trials per day (30 s ITI), to a criterion of nine successive correct trials on one day (P < 0.0005), starting on day 3 after surgery and continuing until the pumps were exhausted on day 14 (maximum, 120 trials).

The results (Table 1) showed that there was a nonsignificant trend towards faster learning by unoperated control rats but, overall, the groups did not differ. These findings imply that a secondary sensorimotor or motivational impairment is unlikely to be the cause of the place-learning deficit described earlier.

Finally, to determine the effect of AP5 on induction of LTP in vivo, we examined LTP using a third set of male Lister rats (n = 22), also prepared with intraventricular cannulae and minipumps (D₅-L-AP5, 40 mM, n = 9; L-AP5, 20 mM, n = 6; control n = 7 made up from saline, n = 3, and unoperated n = 4). Between 6 and 12 days later, each rat was anaesthetized with urethane (1.5 g per kg, interperitoneally, i.p.) and placed into a Kopf stereotaxic instrument. A bipolar stimulating electrode (110 μm diameter) was lowered into the perforant path (action potential 7.6 mV, length 4.0 mm); and a bipolar, staggered tip, recording electrode (110 μm diameter) placed in the dentate gyrus to record both hilus and molecular layer field potentials. After stable potentials had been recorded for at least 30 min, low frequency test pulses (100 μs, 7 V, 0-5 Hz) were applied for 100 min. Two brief bursts of high-frequency activation (100 μs, 7 V, 400 Hz, 250 ms) were applied exactly 20 and 40 min into the experiment. We measured the maximum voltage, and the slope of the early rising phase of the extracellular field potential (1.8-2.5 ms) using linear regression, sampling the waveform at 10 kHz.

D₅-L-AP5 infusion over 6 to 12 days caused no obvious effects on potentials evoked at low-frequency (pre-tetanus maximum amplitudes were: D₅-L-AP5 = 7.0 ± 1.4 mV; L-AP5 = 7.7 ± 1.7 mV; control = 5.5 ± 1.2 mV; P > 0.10). Other strictly qualita-
tive observations indicated that paired-pulse facilitation and recurrent inhibition were also normal (not shown). However, D,L-AP5 caused a total blockade of the induction of LTP in vivo (Fig. 3). Infusion of L-AP5 brought about a faster decay of LTP than that in control rats but did not block its induction. We have shown that AP5 impairs place but not visual discrimination learning—a profile similar, although not as severe, as that caused by hippocampal lesions (unpublished data and refs 7, 8). As place learning is relatively resistant to other selective disturbances of the brain including amygdala, thalamic and parietal lesions 1-3, to depletion of noradrenaline and striatal dopamine 4, and as anticholinergic drugs impair both the spatial and visual tasks used here 5, the profile of impairment with AP5 points to a mechanism involving interruption of hippocampal NMDA receptors. The mechanism remains a matter of speculation but could involve the voltage dependency of the ionic channels associated with NMDA receptors 6,7. Depolarization by afferent input would allow a second input to cause an influx of Na+ and/or Ca2+ ions through the activated NMDA receptors, triggering long-term changes in synaptic efficacy, and giving rise to an association between the two simultaneous inputs. Blockade of hippocampal NMDA receptors could, therefore, impair those types of learning mediated by hippocampal circuitry.

However, the demonstration that blockade of LTP by D,L-AP5 is associated with an AP5-induced learning impairment does not prove that these phenomena are causally related. Further experiments are required. However, we do now know that two very different antecedent treatments which block LTP—pharmacological receptor blockade and high-frequency stimulation saturating synaptic enhancements— are both sufficient to cause an anterograde spatial amnesia in the rat.

Finally, NMDA antagonists are known to be anticonvulsants and, subject to the development of similar compounds with adequate penetration of the brain 8,9, may soon be subject to clinical trials. It would be valuable to include thorough neuropsychological testing of effects on memory within the design of these trials.

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Elevation of intracellular calcium reduces voltage-dependent potassium conductance in human T cells

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Both voltage-activated potassium channels 1,2 and the concentration of free intracellular calcium 3,4 have been implicated in the activation of T lymphocytes. Using the patch-clamp technique 5, we now show an unexpected relationship between the level of intracellular calcium [Ca2+]i in human lymphocytes and the amplitude of a voltage-dependent current: the elevation of [Ca2+]i decreases the potassium conductance. This is in contrast to other systems where [Ca2+]i activates K+ channels. Our results suggest that the level of intracellular calcium regulates the effective number of K+ channels capable of activating.

We used the whole-cell configuration as the main mode of recording in our experiments and, on the basis of the observations of Marty and Neher 6, we assume that, at least in steady-state conditions, the calcium concentration in the cell, [Ca2+]i, corresponds to the concentration of free Ca2+ in the pipette. The voltage steps (given from a holding potential of −80 mV to a potential of more than −30 mV) produced outward currents that had kinetics and amplitudes similar to those described previously 2,7 (Fig. 1a). These currents flow through K+-selective channels 2,7. They were blocked by 100 mM tetraethylammonium, 0.1 mM quinine and 5 mM cadmium. The threshold for the cells investigated was between −40 and −30 mV, and the conductance was maximal at around +40 mV. The recovery from inactivation was very slow, and so we had to apply the pulses at 1-min intervals.

The peak amplitude of the K+ current was strongly dependent on [Ca2+]i (Fig. 1). For example, at a membrane potential of +20 mV, with [Ca2+]i = 0.01 μM, the peak amplitude was 330 ± 224 pA and at [Ca2+]i = 1 μM it was 72 ± 33 pA (mean ± s.d. for 10 or 11 cells). When the pipette was filled with solution containing 0.01 μM Ca2+, the amplitude of the outward currents usually increased during the first 5–10 min after the establishment of the whole-cell configuration, whereas when the pipette contained 1 μM Ca2+, the currents recorded decreased during the first 5–10 min after cell penetration.

The effect of [Ca2+]i on the potassium conductance was confirmed in experiments with the Ca2+-ionophore A23187 (ref. 10), which is known to stimulate lymphocyte activation 11. As Fig. 2 shows, when 1 μM A23187 was added, the K+ inactivation was accelerated and the current amplitude decreased continuously. Ten minutes after A23187 application, the conductance was inhibited on average by 56% (4 cells). The presence of Ca2+-EGTA buffer inside the pipette might have been expected to prevent these [Ca2+]i-induced changes. However, in similar conditions of low Ca2+ buffering, the application of the ionophore leads to the activation of Ca2+-dependent K+ channels in the lacrimal gland 12. Thus, it is possible that in our experiments the reduced K+ conductance following the addition of A23187 is due to the elevation of [Ca2+]i in the cell.

There are three main ways in which [Ca2+]i could affect the potassium conductance: an alteration in single-channel conductance, an alteration in the kinetics of the channels or a change in the number of channels capable of being activated. To evaluate these possibilities, we examined the single-channel currents at different levels of [Ca2+]i.

When the membrane potential is held at a depolarized level, the outward current is almost completely inactivated 5 and the current can then be recorded through single channels in the whole-cell configuration (Fig. 3a). Figure 3b shows the voltage dependence of single-channel currents for several cells at two different [Ca2+]i levels. The data have been averaged for four or five cells at each [Ca2+]i concentration. The slope conductance is −14 pS when [Ca2+]i is 0.01 μM and −12 pS when [Ca2+]i is 1 μM.