Acute versus chronic NMDA receptor blockade and synaptic AMPA receptor delivery

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Published online: 22 April 2002, DOI: 10.1038/nn850

Anatomical and electrophysiological experiments[1–6] show that central excitatory synapses initially display NMDA (*N*-methyl-D-aspartate) receptors (NMDARs) and subsequently mature by acquiring AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs). NMDAR activation can lead to rapid synaptic delivery of AMPARs ('AMPAfication')[7,8], but the view that AMPAfication during development requires NMDAR activation has been challenged by studies showing that chronic removal of NMDAR function (either genetically[9,10] or pharmacologically[11–14]) has no apparent effect on acquisition of AMPAR-mediated synaptic transmission. Here we show that NMDARs are crucial in the developmental acquisition of AMPAR-mediated synaptic transmission, and that chronic disabling of NMDAR function triggers compensatory mechanisms for NMDAR-independent AMPAfication.

We monitored AMPAfication during development by measuring the ratio of synaptic AMPAR responses to synaptic NMDAR responses (A/N). As previously shown in hippocampus[1,15] and other brain regions[2,3], A/N increases rapidly during the first postnatal week (**Fig. 1a** and **b**), with little change during the second week. To determine the role of NMDAR activity, we prepared hippocampal slices from rats of different ages—postnatal day 3 (P3), P6, P9 and P12—and incubated them for 20–24 hours in culture medium containing the NMDAR antagonist AP5 (200 μM DL-2-amino-5-phosphonovaleric acid). In slices treated with AP5 (200 μM), A/N was significantly smaller ($P < 0.05$) compared to untreated slices (**Fig. 1a** and **b**), but only at ages where A/N was increasing. Thus, NMDAR blockade prevented the developmental increase in A/N, supporting the view that AMPAfication during development requires NMDAR function.

To examine the effects of chronic NMDAR blockade, we prepared slices from P6 rats and maintained them in culture medium, either with or without 200 μM AP5. The A/N ratio was calculated at 1–2 day intervals for 12 days; AP5 was refreshed along with media every 48 hours (**Fig. 1c** and **d**). As expected, A/N of neurons in AP5-treated slices was significantly smaller

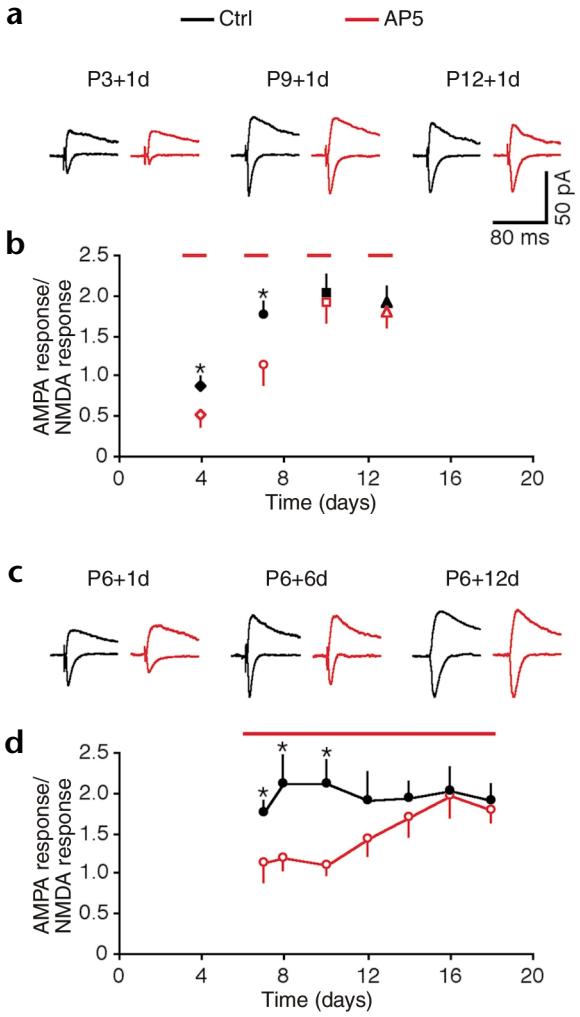

Fig. 1. Effects of acute and chronic blockade of NMDA receptors on glutamatergic synapses in hippocampus. (**a**) Evoked AMPAR-mediated (–60 mV, measured at the peak) and NMDAR-mediated (+40 mV, measured at 75 ms after stimulation) synaptic responses in hippocampal CA1 neurons recorded in physiological solution. Slices were prepared from rats of different ages (P3, P9, P12) and maintained[6] in culture medium with (AP5, red traces) or without (Ctrl, black traces) AP5 for one day (1d). Animals were treated in a manner consistent with Cold Spring Harbor Laboratory Animal Care and Utilization Review Board. (**b**) Plot of the ratio of AMPA response to NMDA response (A/N) versus time (age of animal + culture time). Horizontal red bars indicate period of AP5 treatment. For P3+1d cells, control (black diamond) values were 0.86 ± 0.09, n = 14; and AP5 (red diamond), 0.50 ± 0.13, n = 16 ($P < 0.05$). For P6+1d cells, control (black circle) values were 1.75 ± 0.15, n = 12; and AP5 (red circle) values were 1.12 ± 0.24, n = 12 ($P < 0.05$). For P9+1d cells, control (black square) values were 2.01 ± 0.26, n = 16; and AP5 (red square) values were 1.92 ± 0.26, n = 16 ($P = 0.80$). For P12+1d cells, control (black triangle) values were 1.92 ± 0.20, n = 16; and AP5 (red triangle) values were 1.80 ± 0.18, n = 15 ($P = 0.67$). (**c**) Evoked AMPAR- and NMDAR-mediated synaptic responses from CA1 neurons. Slices were prepared from P6 animals and maintained in culture medium (with or without AP5) for 1–12 d. Scale bar as in (**a**). (**d**) Plot of A/N against time (age of animal + culture time). Values for P6+1d cells are the same as in (**b**). Values for P6+2d cells: Ctrl, 2.12 ± 0.34, n = 16; AP5, 1.20 ± 0.15, n = 16 ($P < 0.05$); P6+4d cells: Ctrl, 2.13 ± 0.29, n = 12; AP5, 1.10 ± 0.13, n = 15 ($P < 0.005$); P6+6d cells: Ctrl, 1.90 ± 0.37, n = 16; AP5, 1.43 ± 0.22, n = 16 ($P = 0.29$); P6+8d cells: Ctrl, 1.94 ± 0.22, n = 16; AP5, 1.79 ± 0.23, n = 16 ($P = 0.45$); P6+10d cells: Ctrl, 2.02 ± 0.32, n = 16; AP5, 1.96 ± 0.26, n = 16 ($P = 0.90$); P6+12d cells: Ctrl, 1.92 ± 0.19, n = 16; AP5, 1.80 ± 0.17, n = 16 ($P = 0.65$). AP5 was refreshed with culture medium every 48 h. Note that inclusion of AP5 (200 μM) in the medium, which has been shown to completely block NMDA-R-mediated responses[11–13], impaired AMPAfication for the first 4 d. Asterisks indicate significant difference between AP5-treated and untreated cells ($P < 0.05$, t-test).

($P < 0.05$) than in control slices after 1–3 days of treatment, indicating that AP5 blocked AMPAfication. However, A/N began to increase after 4 days of treatment with AP5. Slices treated with AP5 for 8 or more days had significantly higher A/N ratios than did slices treated for 4 days or less ($P < 0.05$). After 8 days of AP5 treatment, slices became indistinguishable from untreated slices. The appearance of AMPAR responses after chronic NMDAR blockade is consistent with previous pharmacological[11–14] and genetic[9,10] experiments. Together, these findings indicate that additional NMDAR-independent mechanisms, recruited by chronic blockade of NMDAR activity, may be capable of driving AMPAfication.

We conclude that blockade of NMDAR activity can impair AMPAfication of glutamatergic synapses during development. However, our finding that AMPAfication of synaptic responses resumed after 4 days of chronic blockade of NMDAR function indicates that other mechanisms are involved. Although we cannot eliminate the possibility of an incomplete blockade by 200 μM AP5, this is unlikely given that similar results were obtained in animals completely lacking functional NMDARs[9]. It will be interesting to determine the nature of these mechanisms and whether other NMDAR-dependent processes, such as long-term potentiation and long-term depression, become NMDAR-independent after chronic NMDAR blockade. Lastly, this study shows that multiple 'reserve mechanisms' may exist for certain cellular processes, and that they may be recruited only under special conditions. This could explain why chronically disabling a gene or suppressing a protein's function often shows no obvious effects or an unexpected phenotype.

Acknowledgments

We thank N. Dawkins-Pisani for technical assistance, and H. Cline and members of the Malinow laboratory for helpful comments and discussions. This study was supported by the National Institutes of Health (R.M.), the Alle Davis and Maxine Harrison Endowment (R.M.), the Alzheimer's Association (J.J.Z.) and the Fraxa Medical Research Foundation (J.J.Z.). J.J.Z. is a Naples Investigator of the NARSAD (National Alliance for Research on Schizophrenia and Depression) Foundation.

Competing interests statement

The authors declare that they have no competing financial interests.

RECEIVED 23 JANUARY; ACCEPTED 26 MARCH 2002

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Sniffing neuropeptides: a transnasal approach to the human brain

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Published online: 29 April 2002, DOI: 10.1038/nn849

Neuropeptides act as neuronal messengers in the brain, influencing many neurobehavioral functions[1]. Their experimental and therapeutic use in humans has been hampered because, when administered systemically, these compounds do not readily pass the blood–brain barrier, and they evoke potent hormone-like side effects when circulating in the blood[2,3]. We administered three peptides, melanocortin(4–10) (MSH/ACTH(4–10)), vasopressin and insulin, intranasally and found that they achieved direct access to the cerebrospinal fluid (CSF) within 30 minutes, bypassing the bloodstream.

We selected the three peptides for their well-documented effects on brain functions including learning, memory, and body-weight regulation[1,4,5]. We administered the peptides intranasally to healthy humans (9 female, 27 male, 25–41 years of age), and the concentration of each peptide was measured within 80 minutes after administration in samples of CSF and systemic blood obtained through intraspinal (between L4 and L5) and intravenous (forearm) catheters. Catheterization was done two hours before the sampling period began.

Intranasal administration of each peptide resulted in an elevation of its concentration in the CSF (**Fig. 1**). We saw statistically significant peptide accumulation in the CSF within 80 minutes after administration with the higher dose of MSH/ACTH(4–10) (10 mg), with the higher and lower doses of vasopressin (80 and 40 IU) and with insulin (40 IU), as compared to pre-administration baseline concentrations and to concentrations in subjects administered sterile water as a placebo (**Table 1**). Also, a marginally significant ($P = 0.05$) increase in CSF concentration occurred between 60 and 80 minutes after administration of the lower dose of MSH/ACTH(4–10) (5 mg). Increases in the CSF concentration of each peptide varied considerably among subjects. For all three peptides, however, mean CSF concentrations began to rise within 10 minutes of intranasal administration. For MSH/ACTH(4–10) and insulin, peak levels were attained within 30 minutes after administration; for vasopressin, CSF concentrations continued to increase for up to 80 minutes after administration. For each peptide, concentrations did not return to baseline before the end of the 80-minute sampling period. More prolonged sampling in a subgroup of subjects receiving the higher doses of MSH/ACTH(4–10) and vasopressin showed that concentrations of peptides in the CSF levels were still above those in placebo-treated subjects 100–120 minutes after administration ($P < 0.03$ for MSH/ACTH(4–10), $P < 0.009$ for vasopressin).