D-cycloserine (DCS) enhances excitability of hippocampal pyramidal neurons in a novel manner: Evidence for glycine-site saturation

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Fig. 1. DCS, APV or APV+DCS dose-dependently reduced both post-synaptically- and synaptically-evoked AHPs.

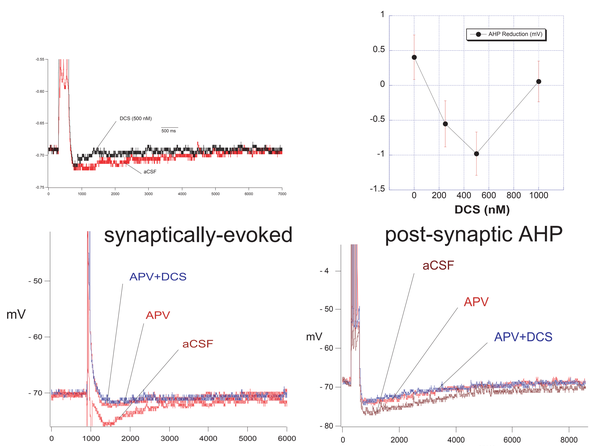


Fig. 2. DCS, APV and APV+DCS dose-dependently reduced spike frequency adaptation

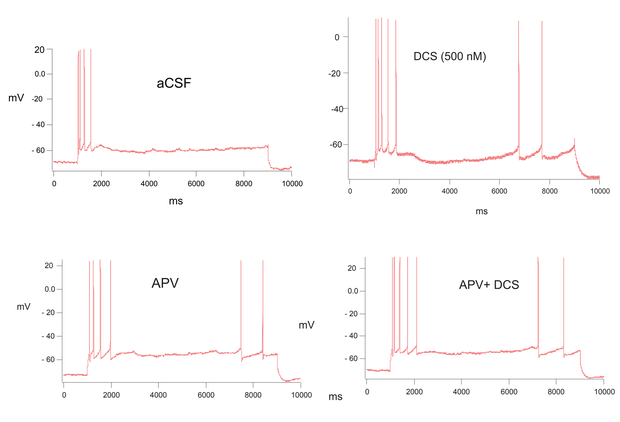


Fig. 3. Kynurenic acid and kynurenic acid+DCS dose-dependently reduced post-synaptically- and synaptically-evoked AHPs

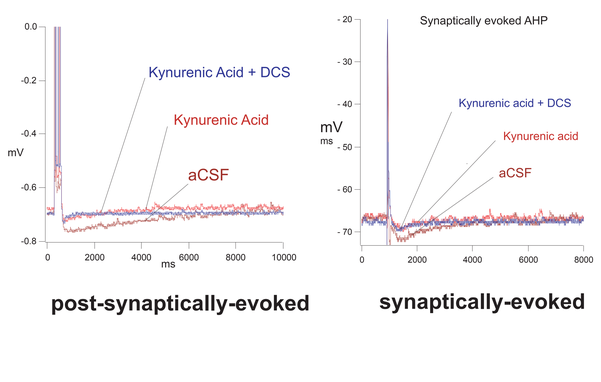
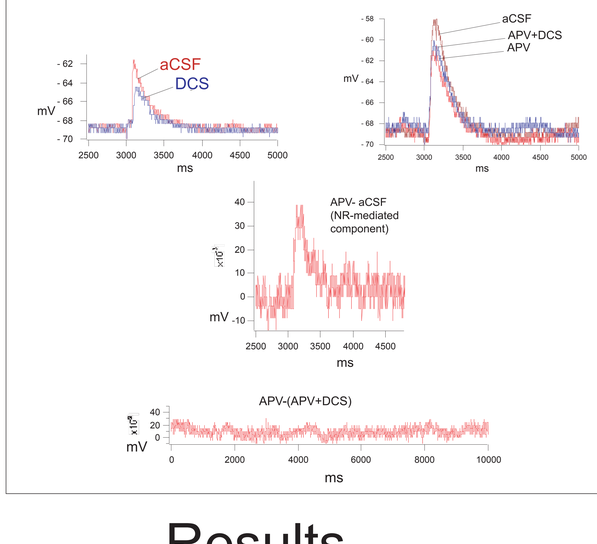


Fig. 4. DCS, APV and APV+DCS reduced synaptically evoked EPSP amplitudes

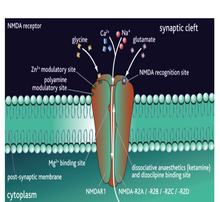


Introduction

The contributions of NR-mediated calcium signaling to postsynaptic afterhyperpolarization (AHP) plasticity were examined in CA1 pyramidal neurons. Prior work shows that successful activation of NMDA receptors (NRs) is necessary for learning many hippocampal-dependent tasks. Neural plasticity at hippocampal CA1 Schaffer collateral synapses often requires NR mediated transmission, with the transient influx of Ca_{2+} ions transiently increasing intracellular calcium.

Research also demonstrates that the postburst AHP strongly regulates CA1 excitability and contributes to learning-related plasticity observed in CA1 pyramidal neurons. Late AHP currents are generated by calcium-activated K_+ currents. Activation of NRs actually increases intracellular Ca_{2+}, potentially **enhancing** the AHP. A necessary prerequisite for NR activation is glycine-site occupancy.

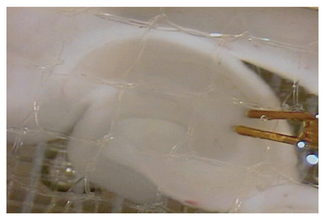
The effects of D-cycloserine (DCS), a partial agonist of the glycine site of the NR with well demonstrated nootropic effects, were examined in CA1 pyramidal neurons from hippocampal slices of young rats using current-clamp intracellular recording techniques. Synaptic and postsynaptic effects of DCS were examined, with particular emphasis on interactions between synaptically-evoked and intrinsic excitability measures. It is postulated that the facilitating effect of DCS on spatial learning and memory are achieved by increasing CA1 pyramidal neuron excitability by modulating NR-evoked Ca_{2+} influx. Although characterized as a **partial agonist** at non-saturated NR glycine-sites, DCS may effectively act as a **competitive partial antagonist** if the sites prevalent *in vitro* are saturated, and thus enhance AHP-mediated plasticity in a novel manner.



Hypotheses

- DCS will reduce CA1 excitability (AHP, accommodation) if glycine sites non-saturated
- DCS will ehance CA1 excitability (AHP, accommodation) if glycine sites are saturated
- DCS will reduce synaptic excitability [acting as an antagonist] only during saturation
- The effects of DCS are mediated via NRs, and therefore blocked by AP5
- The effects of DCS are mediated via glycine-sites of NRs, and therefore blocked by kynurenic acid.

Methods



Subjects: Young (2-4 mo) male Long-Evans rats were housed in a controlled facility with a 12 h light/dark cycle. Rats were housed socially with *ad libitum* access to food pellets and water.
Technique: Experiments were performed using current-clamp intracellular recording.

Slice preparation: Rats were anesthetized with isoflurane and decapitated. The brain was quickly hemisected and immersed in cooled s-aCSF [in mM: 124 sucrose; 3 KCl; 1.3$MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose, pH 7.4]. After the brain was chilled for 3-4 min, it was blocked and 400 µm slices were cut using vibratomes and placed in room temperature (25°C) aCSF [in mM, 124 NaCl; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose,pH 7.4]. Both CSF solutions were continuously oxygenated (95% O_2;5% CO_2).

Slices were equilibrated for > 1 hr before recording. For recording, slices were individually transferred to a submersion chamber and continuously perfused (1.5 ml/min) with oxygenated aCSF at 31°C.

Sharp micropipettes were pulled from borocillate glass (35-80 MΩ, 3 M KCl) and used for intracellular recordings from pyramidal cells in the CA1 region.

Criteria: All cells had input resistances > 35 MΩ, overshooting action potentials > 80 mV and resting potentials -68 ± 3 mV. If necessary, < 0.2 nA of current was injected to clamp the potential at -68 mV for testing.

EPSPs: Synaptic potentials were evoked by Schaffer collateral stimulation via 75-µm twisted stainless steel bipolar electrodes aligned with the end of the dentate gyrus. After cell acquisition, 100-µs current pulses to the Schaffer collaterals were applied. The stimulus strength was adjusted to approximately one-third of the threshold needed to evoke an action potential, yielding EPSPs with amplitudes of 5-15 mV.

Summating EPSPs: brief (100 µs) single Schaffer collateral pulses were used to determine the current required to evoke a single 2-4 mV amplitude EPSP. Trains of 10 of these pulses were given with an interpulse interval of 10 ms.
AHPs were evoked by 100 ms depolarizing current injections sufficient to elicit 4 APs. Accommodation was tested using 800 ms depolarizing current injections sufficient to elicit 4 APs within the first 100 ms. Hyperpolarizing sag was determined by subtracting the maximum voltage deflection from baseline to steady-state voltage after a -1.0 nA 100 ms step.

Data were digitally acquired using National Instruments hardware and Lab View Software (National Instrument, Austin, TX). Data analysis was performed in IGOR (Waremetrics) software. Data were analyzed with ANOVAs in StatView, using Shceffe post-hoc tests.

Results

APV blocked the effects of DCS:

- The effects of DCS on subthreshold EPSPs were recorded before and after bath application of APV. DCS reduced EPSP amplitudes. Blocking NRs with APV (50 µM) reduced EPSP amplitudes. Application of APV (50 µM) + DCS (500 nM) blocked the effects of DCS on EPSPs.

- The effects of DCS on post-synaptically- and synaptically-evoked AHPs were recorded pre- and post-application of APV. DCS dose-dependently reduced AHP amplitudes. Application of APV (50 µM) reduced both postsynaptically- and synaptically-evoked AHPs and spike frequency adaptation. APV+DCS did not further change AHP amplitude or spike frequency adaptation.

Kynurenic acid blocked the effects of DCS:

- Kynurenic acid (100 µM) was applied to block the glycine site of the NMDA receptor. Application of kynurenic acid reduced both post-synaptically and synaptically evoked AHP amplitudes. Addition of DCS to the bath subsequently did not alter excitability.

Non-specific passive membrane properties and action potential characteristics were unaffected by treatment with DCS, APV, kynurenic acid or combinations of DCS/APV or DCS/kynurenic acid.

Summary

- DCS facilitates learning & memory *in vivo* (Thompson et al., 1992; Thompson & Disterhoft, 1997).
- DCS appears to act both *in vitro* and *in vivo* to reduce NR-mediated responses
- Under saturated glycine-site conditions, DCS, a partial agonist, competes with the endogenous (full agonist) glycine and results in less NR-mediated Ca_{2+} influx, functioning as an NR antagonist.
- Within a narrow range of concentrations, NR antagonism briefly enhances hippocampal excitability and facilitates learning and memory dependent upon this transient shift in excitability.

Acknowledgements

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