D-Cycloserine Enhances Location Specificity of Hippocampal Place-Cells by Enhancing Theta-Cell and Selectively Suppressing Complex-Spike Cell Firing

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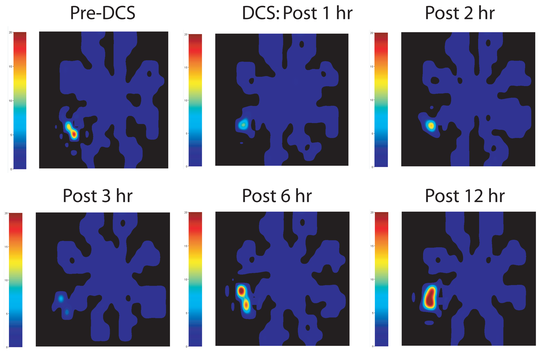
Fig. 1: The location of place-fields was NOT altered by treatment with DCS (6 mg/kg)



Introduction

Hippocampal place-cells exhibit selectivity and stability of neuronal firing patterns (Fig. 1), but are also often considered in the context of neuronal plasticity. Place-cells exhibit reliable increases in firing in specific locations in particular environments; fire at low frequencies in most other locations in those same environments; and exhibit near complete "silence" in other environments (Thompson & Best, 1989).

Hippocampal theta-cells (inhibitory interneurons, Fig. 4) play a crucial role regulating the excitability of place cells, providing feedback- and feedforward-inhibition. *In vitro*, stimulation of interneurons evokes IPSPs in pyramidal neurons, while stimulation of pyramidal neurons evokes EPSPs in interneurons. These local circuit interactions may be affected by pharmacological manipulations altering any or all elements of the circuit.

D-cycloserine (DCS), a glycine-site partial agonist for the NMDA receptor (NR), dose-dependently facilitates both spatial maze learning and rabbit trace eyeblink conditioning (Figs. 2,3; Thompson , 1998; Thompson & Disterhoft, 1997). CA1 pyramidal cells strongly express NRs. Local interneuron NR expression varies, some cells having strong expression, others negligible. The current study assessed whether acute DCS treatment differentially altered theta- and place-cell activity in CA1.

Hypotheses

- DCS will improve place field location-specificity or other aspects of stability
- DCS will inversely affect firing rates of complex-spike vs. theta cells

Fig. 2: DCS facilitation of rat radial-arm maze learning.

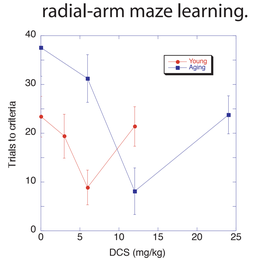


Fig. 3: DCS facilitation of rabbit trace eyeblink conditioning.

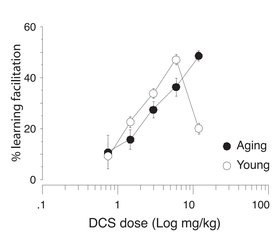


Fig. 4: Theta cells (inhibitory interneurons) exhibit little or no location-specific firing.

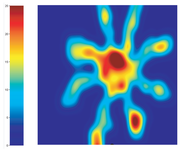


Fig. 5: DCS dose-dependently reduced firing activity of all CA1 pyramidal (complex-spike) cells tested.

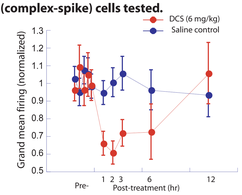
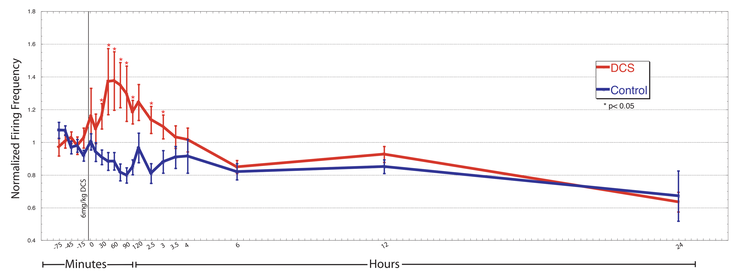


Fig. 6: DCS Transiently Increased Theta Cell Firing Frequency



Methods

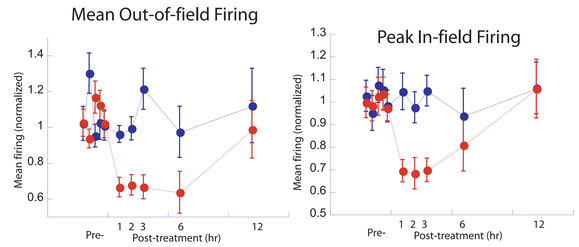
Chronic implants: Two or four tetrodes (2 or 4 twisted bundles of four 25 µm Formvar-insulated nichrome wires) were threaded through a 27 ga. thin wall stainless steel cannula, attached to a vertically drivable connector assembly (adapted from Kubie, 1983), and implanted stereotaxically over the dorsal CA1 field and cemented to skull screws along with a 24 ga. reference wire. Removable red-green LEDs were affixed to a source-follower preamp. head stage for each session to facilitate monitoring of the rats' location. Shielded fine wire cables were connected via a 24-channel commutator to the MAP system (below).

Subjects & Behavioral Task: Male Long-Evans rats (350-450 g) were food deprived to 85% of pre-surgical weight over 3 d. Electrodes were implanted, 72 hr allowed for recovery, and unit activity was surveyed. Electrodes were advanced up to 50 µm daily and allowed to settle 4 hr before the next survey. Rats with well isolated single-unit activity were trained on an 8-arm radial-arm maze to visit all arms for drops of chocolate milk at the ends of arms. Each session required a rat to travel to the end of all 8 arms (taking ~2.5-4 min/session).

Pre- and DCS Sessions: Five pre-sessions were recorded to evaluate stability of place-cell and theta cell activity, at intervals >15 min between sessions. Only single-units with stable place-fields [i.e. having stable amplitude and waveform characteristics (physiological stability) and stable location-specificity (field stability) across all pre-treatment sessions] and stable theta cells (having waveform and firing frequency stability) were then used for drug experiments. 1 mL injections of either 6 mg/kg DCS in physiological saline or of 0.9 % saline (control) was administered i.p. The experimenter was blind to the treatment given. Immediately after drug administration, rats were placed on the maze and unit activity was again assessed under identical conditions, at 15 min intervals for the first 2 hr, every 30 min up to the 4th hr, then at the 6th and 12th hour.

Recording & Data Analysis: Amplification and filtering of multi-unit signals was performed with a Multichannel Acquisition Processor (MAP) System (Plexon Inc., Dallas, TX) and spike waveforms recorded and template sorted using Plexon's RASPUTIN and OffLine Sorter software. Spatial location was recorded using Plexon's VideoTracker System. Place-fields were analyzed with NeuroExplorer and NeXScript (Nex Technologies, Littleton, MA). Place-field locations were determined using a threshold of firing > 5 Hz for in-field activity, using NeuroExplorer and NeXScript (Nex Technologies. The mean ± SEM firing activity was calculated for each place-cell and theta cell for each session tested, including overall (grand mean), in-field peak and mean firing, and out-of-field firing. Data for all sessions were normalized to that of the first session for each cell, to allow comparisons to be readily made between cells with different firing rates. Nonparametric Wilcoxin t-tests of two related samples were performed on all data (SPSS, Chicago, IL).

Fig. 7: DCS dose-dependently reduced both in-field and out-of-field firing of place-cells (complex-spike cells)



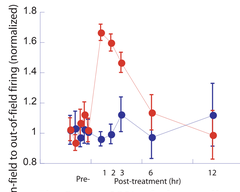


Fig. 8: For 40% of place cells, the suppression of out-of-field firing was greater than that of in-field firing, enhancing the signal-to-noise ratio of location-specific firing.

Results

36 CA1 single-units exhibiting stable place fields were studied from 4 rats (25 units before and after DCS treatment, 11 units in saline-treated controls). A total of 616 individual place-field mapping sessions were assessed and spatially normalized (416 before and after DCS treatment, 200 for controls).

72 theta cells were studied from 9 rats (35 cells before and after DCS treatment, 37 cells in saline-treated controls). A total of 1,432 individual theta cell mapping sessions were assesed and normalized for comparative purposes (538 for DCS, 893 for controls).

All place-fields observed remained spatially stable (i.e. did not change their spatial firing coordinates). Place-cells did exhibit a decrease in overall firing activity, detectable within 10 min of DCS treatment and for up to 6 hr post-injection (Fig. 5).

Despite this decrease in firing, an enhancement in the location-specificity of place-cells (in- vs. out-of-field firing) occurred (Fig. 8). The decrease in either peak or mean within-field firing (Fig. 7) was significantly less than the decrease in overall grand-mean firing or in out-of-field firing, enhancing the signal-to-noise ratio of the location-specific pace-field activity.

Theta cells (inhibitory interneurons with little or no location-specificity) may contribute to this suppression, as they exhibit an increase in overall firing activity, detectable within 15 min of DCS treatment and continuing for up to 4 hr post injection (Fig. 6).

Both place-cells and theta-cells exhibited stability in their grand mean firing frequency in saline controls. All drug effects ended within 12 hr of DCS administration.

Summary

- A dose of **DCS** (6 mg/kg) that facilitates spatial learning (Thompson, 1998) **suppressed firing of** most **CA1 pyramidal neurons** active in awake rats
- For a sizeable percentage of place-cells (40%) **DCS suppressed out-of-field firing** rates **more than in-field firing** rates
- The **net effect of DCS was enhanced location-specific firing** for these 40% of place-cells
- **DCS** (6 mg/kg) **transiently increased firing** rates **of theta cells**
- The time-course of DCS-induced changes in place- and theta-cell activity was similar
- This interaction of place-cells and theta cells mediated by NRs may serve as a local-circuit mechanism for enhancing spatial specificity and spatial learning and memory

Acknowledgements

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