# Molecular Mechansims of DCS Induced Changes in Hippocampal Neuron Excitability

E. Donzis, L. Wilmott, & L. T. Thompson
Aging & Memory Research Lab, School of Behavioral & Brain Sciences, Univ. of Texas at Dallas, Richardson, TX

Fig. 1: DCS reduced both in-field and out-of-field firing activity of complex-spike cells tested.

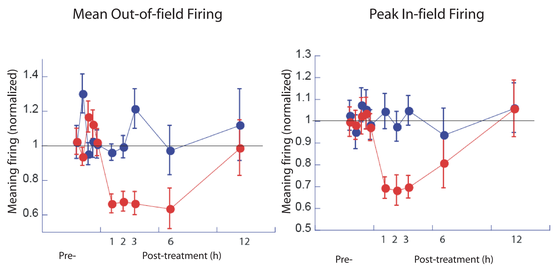



Fig. 2: Acute DCS injections reduced arc protein expression but not pAkt in the hippocampus.

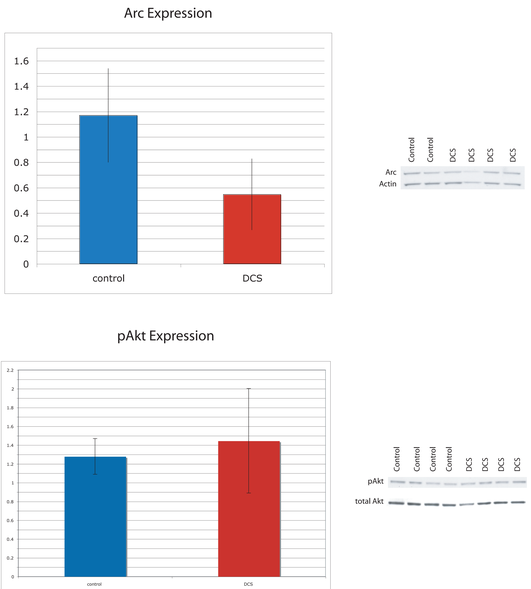
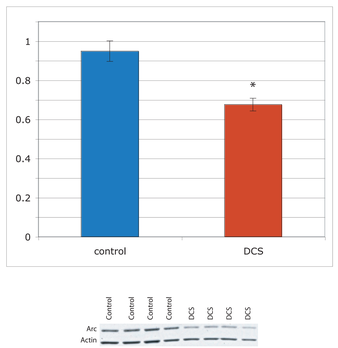



Fig. 3: Chronic DCS injections reduced arc protein expression in the hippocampus (* p<0.05).




## Background

Arc gene expression has been used as a marker for neuronal activity (Tzingounis, 2006; Guzowski, 1999). Specifically, arc gene expression has been correlated with the activity of hippocampal place cells (Guzowski, 1999). Although D-cycloserine (DCS) is known to be a partial NR1 agonist (Hood, 1989; McBain, 1989; Priestley, 1994), our lab has shown *in vitro* that DCS functionally acts as an NR1 antagonist (Seif & Thompson, 2007), decreasing Schaffer collateral-evoked EPSPs in CA1, by competing for binding with the endogenous full agonists serine and glycine, which enhance EPSP amplitudes. Since increased NMDAR activity is correlated with increased place cell activity (Kentros, 1998) as well as arc expression (Bloomer, 2008), then administration of DCS acting as a partial antagonist should decrease place cell activity as well as decrease arc expression.

## Methods

**Drug Treatment**: 3 – 5 mo male Long Evans rats were given a single injection or 21 daily injections of either saline vehicle (0.9% NaCl) or of D-cycloserine (6 mg/kg, i.p., 0.5 mL/250 mg, pH 7.4). The rats were sacrificed and brain tissue collected 1 h post-injection for Western blotting.
Place-cell activity (cf. Thompson *et al*., 2006) was recorded at intervals indicated post-injection.
**Place-Cell Recording and Data Analysis**: Rats explored a radial-arm maze for 5 baseline (pre-DCS) and then for multiple post-DCS recording sessions during which hippocampal activity and spatial location were monitored. Unit signals were amplified and filtered using a MAP System (Plexon Inc., Dallas, TX). Complex-spike (pyramidal neuron) extracellular waveforms were recorded and template sorted using Plexon's RASPUTIN and OfflineSorter software, and analyzed by spatial location using NeuroExplorer and NexScript (Nex Technologies, Littleton, MA). Place-fields were defined as a set of at least 5 contiguous pixels with a firing rate more than 2 standard deviations above the grand mean (i.e., the average firing rate for the session) of that single unit. Pixel to pixel comparisons were performed in MATLAB (Mathworks, Natick, MA) and used to calculate plasticity in place cell firing rate between 5 min duration sessions.

ANOVA statistical comparisons were performed with StatView (SAS Institute Inc., Cary, NC) to assess differences between sessions. ANOVA was performed on in-field and out-of-field firing rates, grand-mean firing rates, and place-field stability measures.

**Western Blot Analysis**: Each brain was flash frozen by a 2 min submersion in 2-methylbutane cooled by a dry ice/ethanol bath to ~ -80°C. 500 µm thick sections were taken with a cryostat at the level of the dorsal hippocampus (-2.3 to -3.3 mm posterior from Bregma) and 1.22 mm diameter punches were taken with a tissue punch. These punches were collected in a buffer containing 0.1 M phosphate buffer (10% glycerol, 20% protease inhibitor cocktail, and 10% protease inhibitor cocktail II; pH 7.4; room temperature) and then sonicated for 1-3 s. Protein concentrations were determined using a Qubit fluorometer and Qubit protein assay kit (Invitrogen, Carlsbad, CA). Approximately 15 µg of tissue was heated to 70°C in a sample buffer with a reducing agent and then run on a 4-12% Bis-Tris MIDI gel (Invitrogen). The gel was electroblotted to a nitrocellulose membrane using an iBlot dry blotting system (Invitrogen). Membranes were washed in Tris-buffered saline (150 mM NaCl/100 mM Tris base; pH 7.5; room temperature) and probed with arc, pAkt, and total Akt primary antibodies diluted in blocking solution (5% BSA with 1M NaF and 0.5 M NaOrthovanidate) overnight at 4°C. The membranes were incubated in a secondary HRP-linked antibody for 1 hr. Immunoreactivity was detected using chemiluminescence (ECL Western Blot Kit). Actin was used as a loading control. Lysate from each brain was run in its own lane, and the protein levels were quantified individually by densitometry analysis with ImageJ Software from NIH.

## Results

Figure 1. Stable place-field activity was obtained from five or more baseline sessions from 63 CA1 complex-spiking place-cells from 7 rats. Post-DCS, place-cell firing rates were significantly reduced ($p < 0.003$) for periods > 6 hr post injection, then returned to basal rates within 12 hr. Both out-of-field (non-specific) and in-field (location-specific) firing rates were reduced (blue-saline injected; red-DCS injected). Signal-to-noise ratios (i.e. ratios of in- to out-of-field firing) were actually enhanced by DCS (data not shown).
Figure 2. Tissue obtained from 4 DCS treated and 2 control rats showed that a single DCS-treatment reduced arc expression (by more than 50%), but had no effect on pAkt expression.
Figure 3. Tissue obtained from 4 DCS treated and 4 control rats showed that chronic treatement (21 daily injections) with DCS also significantly reduced arc expression (p<0.05).

## Discussion

- DCS administration transiently decreases the firing activity of complex-spike place cells.
- Both chronic and acute administration of DCS decrease arc protein expression, but do not alter pAkt or total Akt expression.
- DCS *in vivo* functionally acts as a NMDAR antagonist, competing for binding to NR1 subunits.
- The nootropic effects of DCS *in vivo* are consistent with a moderate and transient reduction in NR-mediated firing activity by CA1 hippocampal pyramidal cells, NOT with increased activity as many models assume. Other work has shown a similar time course of increased inhibition in CA1 of hippocampus, yielding a net improvement in signal-to-noise ratio of CA1 place cell firing.

## Acknowledgements

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