Dequalinium, an SK-channel blocker, dose-dependently enhances CA1 pyramidal neuron excitability and nootropically facilitates spatial learning

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Introduction

Enhanced excitability of CA1 pyramidal neurons plays a significant role in facilitating learning (Disterhoft *et al.*, 1986; Moyer *et al.*, 1996; Oh *et al.*, 2003; Farmer & Thompson, 2011). Small-conductance (SK) Ca^{2+}-activated K^+ channels mediate some of the late components of the post-burst afterhyperpolarizations (AHPs) in these and other pyramidal neurons. A peptide blocker, apamin, binds to SK-channels but does not readily cross the blood-brain barrier (BBB; Baidan & Zholos, 1989). Apamin facilitates learning if infused into the CNS, but is unlikely to be viable as a nootropic compound due to BBB issues as well as potential peptide allergies. Previous work from our lab (Bui *et al.*, 2003) indicates dequalinium, quaternary ammonium compound, crosses the BBB and enhances CA1 neuron excitability when given systemically for two weeks. Dequalinium blocks apamin-sensitive SK channels (Galankis *et al.*, 1996; Gant & Thompson, 2001; Bui *et al.*, 2005), yielding reductions in AHPs and providing a possible mechanism for enhancing learning. The current study assessed dose-dependent nootropic effects of dequalinium , i.e., its ability to facilitate learning in a spatial declarative memory task.

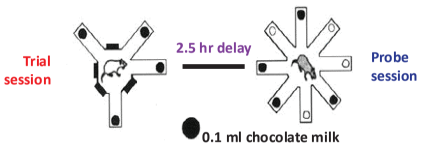

Figure 1. Daily behavioral training and testing was in 2 sessions, a training session and a probe session, with a 2.5 hr delay between sessions. For training trials, 4 of the 8 radial-arms on the maze were baited, and non-baited arms removed. Four trials were allowed to find 0.1 ml chocolate milk rewards, with each arm entry = 1 trial. Correct entries and perseverations (visits to previously visited arms) were recorded. Twenty min after training trials, the young male LE rats received an injection of dequalinium or saline. During probe trials, all 8 arms were on the maze and available for exploration. The 4 arms unbaited during training trials were now baited (win-shift declarative memory task). Again, 4 trials were allowed to find the rewards, and correct entries, perseverations, and errors (visits to arms baited during testing trials) were recorded. A behavioral criterion of 100% correct performance on training trials and 75% correct on probe trials (50% correct was chance performance) for 2 consecutive days was used for successful acquisition, or a maximum of 30 days of training if criterion was not reached.

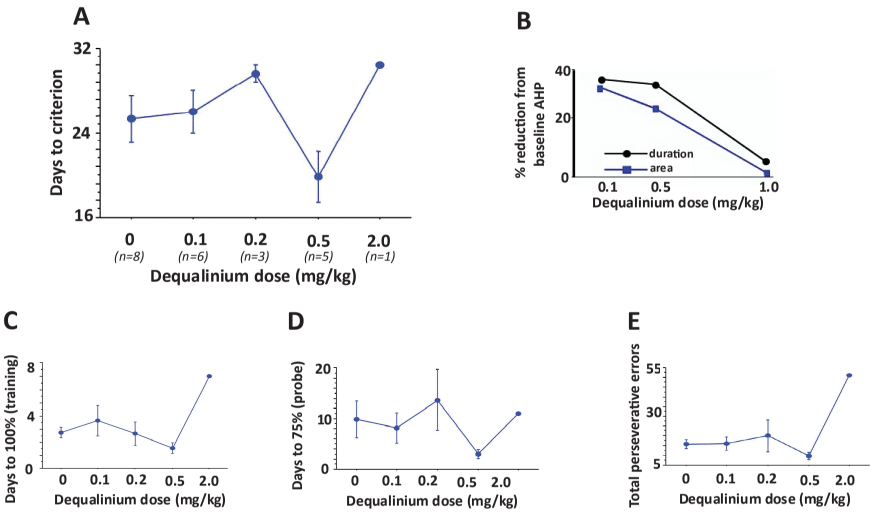

Figure 2. Dequalinium dose-dependently facilitated spatial learning and reduced AHP peak amplitudes. Additional doses are being tested, with an n = 10 per group planned. **A.** Trials to criterion were significantly reduced by daily treatment with 0.5 mg/kg of dequalinium. **B.** AHP recorded *in vitro* were significantly reduced by daily treatment with both 0.1 and 0.5 mg/kg dequalinium. **C.** While days to 100% correct performance during training trails were reduced by daily treatment with 0.5 mg/kg dequalinium, treatment with 2.0 mg/kg increased this measure. **D.** Days to reach 75% correct performance during probe trials was reduced by daily treatment with 0.5 mg/kg dequalinium. **E.** While perseverative errors were reduced by daily treatment with 0.5 mg/kg dequalinium, treatment with 2.0 mg/kg increased this measure.

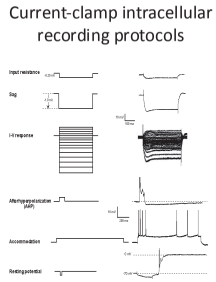

Figure 3. CA1 pyramidal neurons were recorded from rats post-acquisition. Measures of excitability (including AHP peak amplitude and duration, and of accommodation), input resistance, and sag were assessed from neurons held at -67±3 mV.

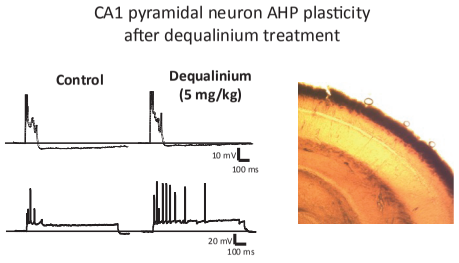

Figure 4. Left. Reduced post-burst AHP and accommodation from a CA1 neuron after treatment with 0.5 mg/kg daily (post-training) for 15 days compared to CA1 neuron from control. Scale bar 100 ms, 10 mV. **Right.** Biocytin stained dorsal CA1 hippocampal pyramidal neuron. CA1 AHP plasticity occurs after eyeblink conditioning, water maze or long-delay win-shift spatial learning, and after inhibitory avoidance or fear

Results

Dose-dependent increases in postsynaptic excitability were noted in CA1 pyramidal neurons from trained animals 24 hr post-training, specifically reductions in AHPs (Figure 2B) and decreased accommodation, corroborating previous data (Bui *et al.*, 2003) showing enhanced excitability of CA1 pyramidal neurons following 15 days of daily dequalinium treatment. It is also consistent with previous studies of the AHP and accommodation reductions *in vitro* with bath application of dequalinium (Gant & Thompson, 2001).

Rats receiving 0.5 mg/kg of dequalinium demonstrated more rapid acquisition of the spatial task than controls or those receiving other doses, suggesting this may be an optimal dose for learning enhancement. Rats receiving this dose required fewer trials to criterion (Figure 2A). Rats treated with this dose also reached 100% correct trials during learning sessions in fewer days (Figure 2C), reached 75% or better accuracy during probe trials faster than other groups (Figure 2D) and demonstrated fewer perseverative errors (Figure 2E). Interestingly, rats treated with the highest dose (2.0 mg/kg) exhibited significantly more perseverations, consistent with a U-shaped dose-response curve.

Summary

Nootropic effects were assessed for treatment with different daily doses of dequalinium, and physiological plasticity post-training was also assessed in neurons from a small subset of trained rats.

- Both behavioral and postsynaptic plasticity were observed when rats receiving dequalinium injections were compared to those serving as saline controls.
- 0.5 mg/kg of dequalinium enhanced learning rate and task accuracy on a long (2.5 hr) delay win-shift hippocampal-dependent spatial learning task (~ 30% facilitation).
- CA1 pyramidal neurons from rats receiving this dose exhibited enhanced excitability, i.e. decreased AHPs and accommodation.
- This correlation between CA1 pyramidal neuron excitability enhancement and nootropic facilitation of acquisition in a declarative memory task is consistent with a hypothesis that transient AHP plasticity is a conserved mechanism utilized for learning and memory.

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