Nootropic effects of Microhydrin and antioxidants on spatial-learning and psychomotor performance in young and aging rats

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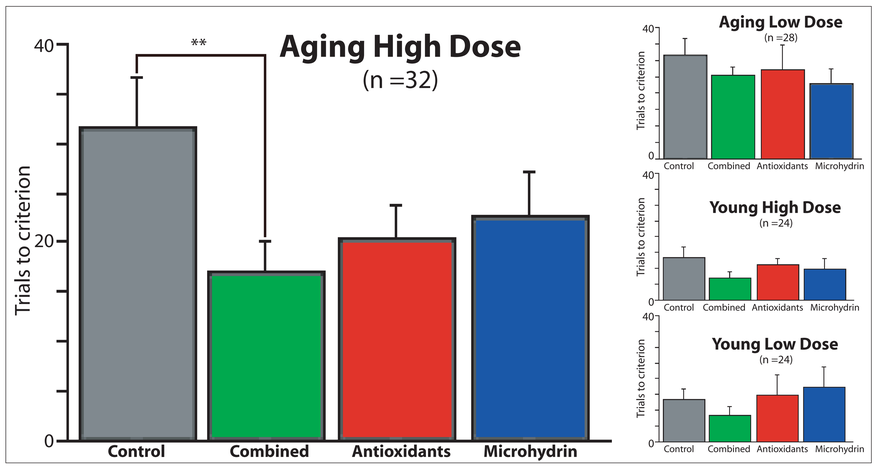

Figure 1. Behavioral facilitation in aging (left). Combined (high dose) treatment significantly reduced trials to criterion (**p<0.01) by about 50% compared to placebo controls. Right: Though no other conditons tested showed a significant reduction in trials to criterion (right), in each case Combined treated rats required fewer trials to reach criterion.

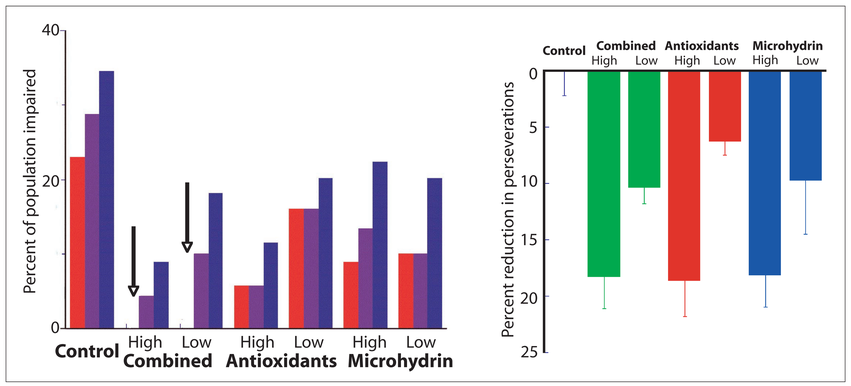

Figure 2. Reduced learning impairment in aging rats. Left: Severe (red), moderate (purple), and mild (blue) impairments in spatial learning was reduced by all treatments, most notably by the Combined treatment. **Note:** The Combined-treatment groups did not exhibit severe impairment (arrows). Right: Perseverative errors were equally dose-dependently reduced by all treatments tested compared to controls.

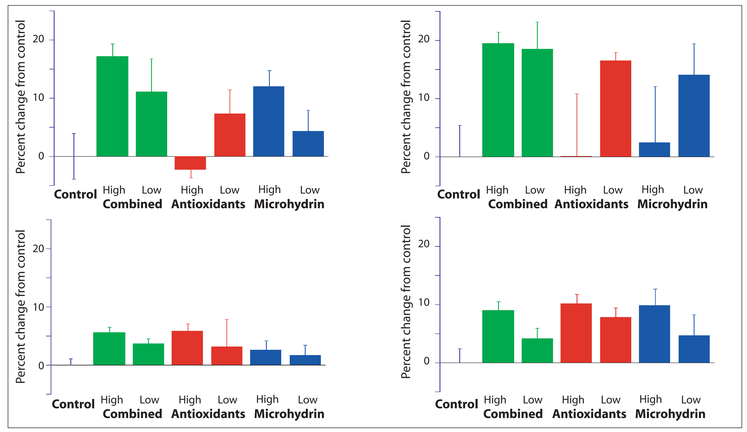

Figure 3. Improvements in psychomotor performance in aging. Combined treatment dose-dependently improved performance on the wire-hang (upper left) and balance-beam tasks (upper right) but had much less effect on the reversal out of a blind alley (lower left) and reversal on the 45° inclined plane tasks (lower right). The other treatments produced variable effects in each task.

Introduction

Nooptropic compounds improve cognitive function, enhancing learning and retention and use (memory) of information. A variety of antioxidants possess nootropic properties, improving performance in many tasks, including spatial learning and memory. *Microhydrin* (see Fig. 4 below) is a potent silicate electron donor compound previously shown to boost antioxidant activity by regenerating activity *in vivo* and *in vitro* (below). A *Combined* blend of *Microhydrin* and 11 antioxidants (see Table 1) is under development, with a recommended daily intake as a human dietary supplement of 500 mg twice daily. Body-mass equivalent daily doses of this *Combined* treatment (*Microhydrin* + antioxidant blend), of *Microhydrin* alone, of the antioxidant blend alone, or of placebo were formulated into 5 g rat food pellets (Bio-Serv, Frenchtown NJ), and potential nootropic effects were tested *in vivo* in young and aging rats, assessing long-delay spatial-maze learning and memory performance on a win-shift radial-arm maze task (see Figure 5), psychomotor effects on a number of other standard tasks (see Figure 6), and effects on longevity in aging rats fed these compounds daily from age 20 mo (see Table 2 below summarizing the compounds tested).

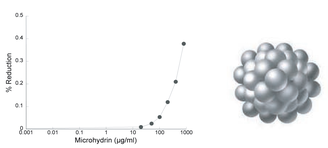

Figure 4. Microhydrin. Above left: Reduction of of NAD+ to NADH by Microhydrin *in vitro* (McCord, 1998). Above right: Microhydrin silicate complex.

Table 1: Antioxidants

N-Acetyl Cysteine
Bacopa monniera
Citrus Bioflavonoids
CoQ-10
Curcumin (tumeric)
Ginkgo biloba
Phosphatydyl choline
Phosphatydyl serine
Quercitin
Resveratrol
Rhodiola rosea

Table 2: Treatments and doses tested

TREATMENT (mg/kg/daily)	Low dose	High dose
Placebo	0	
Combined (Microhydrin + Antioxidants)	3.96	7.92
Microhydrin	3.16	6.32
Antioxidants	0.8	1.6

Methods

All testing was carried out in a blind fashion, and data analysed before groups were decoded. Mildly food-deprived young (2-3 mo) or aging (20+ mo) Fisher 344 x Norway (FN) hybrid male rats were shaped to explore an 8-arm radial-maze for small (~ 0.1 ml) chocolate milk rewards. Cohorts groups of aging and young rats were daily fed high or low doses of the Combined, Microhydrin, antioxidant or placebo rat food and tested, first on a spatial task (up to 40 d), then on psychomotor tests. Longevity was assessed by continuing to treat rats daily until they died of old age.

Figure 5 illustrates the long-delay spatial maze task used for nootropic testing. Briefly, each morning 4 of the maze arms were randomly removed, and the remaining arms baited. Rats were then allowed 4 trials each (entry onto and leaving an arm constituted a trial) to locate the rewards. After a 2.5 hr delay (during which they returned to their home cages), all 8 maze arms were replaced, and rats were tested on a win-shift task with a total of 4 trials allowed. Testing continued until rats reached a criterion of 75% correct choices on this win-shift task for 2 successive days of testing. Performance was assessed as days required to reach criterion, with perseverative errors also scored.

After nootropic testing had been completed, rat psychomotor function was assessed using latency measures (see Figure 6). Strength/endurance was tested with a wire hang task (rat was suspended grasping a wire 40 cm above a soft foam cushion, recording latency to drop). Balance and agility was tested on a balance beam test (rat was placed midway on a 1.2 m long 25 cm wide bridge and latency to cross to a platform at either end was recorded). Latency to escape from a blind alley assessed additional contextual processing abilities. Vestibular processing was assessed using a 45° inclined plane task (rat was placed face downward, and latency to turn face upward was recorded). Data are reported as means ± SEM or as percent changes.

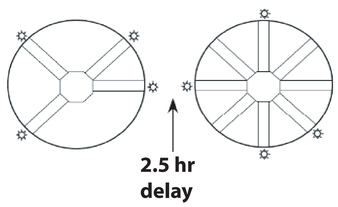

Figure 5. Spatial learning. A long-delay win-shift radial-arm maze task was used for testing.

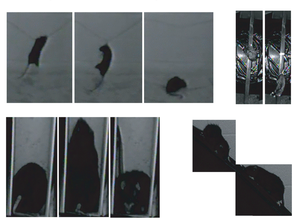

Figure 6. Psychomotor testing of strength/endurance, balance, contextual processing and vestibular processing.

Results

As has been reported in numerous previous studies, aging rats were impaired compared to young rats in the acquisition of the win-shift long-delay spatial task (see Figure 1), requiring more trials to learn the task and showing greater individual variability. Treatment with the higher Combined dose (the combination of Microhydrin and 11 antioxidants) significantly reduced the trials to criterion required for aging subjects to acquire the task, and put their learning rate much closer to the normal range of young rather than aging subjects. Neither Microhydrin alone nor the antioxidants alone significantly facilitated learning, although both tended to reduce trials to criterion. In young rats, none of the treatments tested significantly reduced trials to criterion, although Combined treated rats again learned faster than all other groups tested. This lack of significant effects may be due to the relatively small group sizes and also to floor effects from the relatively fast learning rates and low variance among young rats.

To further characterize this faciliation of learning, the performance of aging rats was compared to that typical of young rats. Each aging subject's performance was ranked as mildly impaired (between one standard deviation (SD) and 2 SD slower than the mean performance of young subjects), moderately impaired (between 2 - 3 SD slower), or severely impaired (more than 3 SD slower than the young mean). As seen in the left panel of Figure 2, Combined treatment eliminated the incidence of severe impairment at both doses tested, while all treatments reduced impairments compared to control aging rats. The facilitation in learning produced by Combined treatment was not, however, due to a specific reduction in perseverative errors (repeated entries to previously visited arms of the maze; Figure 2, right). Perseverative errors were relatively rare for all rats tested (less than 12% of all trials for aging rats), but all treatments tested equivalently and dose-dependently reduced these errors compared to controls.

Psychomotor effects of the different treatments were less pronounced. Combined treatment increased performance on the wire hang and balance beam tasks, but had little effect in the blind-alley reversal and inclined plane reversal tasks. Results with Microhydrin or the antioxidants were less robust and more variable. The pattern and magnitude of the psychomotor task improvements did not strongly correlate with the enhancements observed on the spatial-learning task. Although psychomotor facilitation may have contributed to learning improvement, it could not account for all of the nootropic effects observed.

In conclusion, daily higher dose Combined treatment demonstrably improved performance of aging rats on a task often considered to assess both working and declarative memory, and may improve other age-associated deficits.

Acknowledgements

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