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THE EFFECTS OF AGING AND OXIDATIVE STRESS ON PSYCHOMOTOR AND COGNITIVE BEHAVIOR

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ABSTRACT

Decrements in motor and cognitive function occur in aging, possibly due to oxidative stress-induced damage to the brain. Declines in antioxidant defense mechanisms have been postulated as a causative factor in these age-related decrements, however a clear link between oxidative stress (OS) and behavioral changes in aging has yet to be established. This review shows that age-validated psychomotor and cognitive tests are sensitive to behavioral deficits under different models of OS, including: 1) decreasing OS protection by depleting glutathione and then increasing the OS with dopamine; 2) 100% oxygen exposure; and 3) radiation. Furthermore, interventions that reduce OS result in concurrent improvements in age-associated behavioral deficits. Therefore, age-related changes in behavior may result from an inability to cope with OS that occurs throughout the life-span.

INTRODUCTION

Decrements in motor function and decrements in memory are two main behavioral parameters that are altered in senescence in both humans and animals. These changes occur even in the absence of specific age-related neurodegenerative diseases but these could contribute to the changes. Specifically, aged rats show decrements in performance of behavioral tasks requiring coordinated control of motor and reflexive responses, such as suspension time on a horizontal wire or inclined wire mesh screen and the length of time it takes for the animal to traverse a wooden rod or plank (Dean et al. 1981; Ingram et al. 1994; Joseph et al. 1983; Joseph & Lippa 1986), and on cognitive tasks that require the use of spatial learning and memory, i.e., the ability to acquire a cognitive representation of location in space and the ability to effectively navigate the environment (for reviews see, Barnes 1988; Brandeis et al. 1989; Gallagher & Pelleymounter 1988; Ingram et al. 1994). Age-related deficits in motor performance are thought to be the result of alterations in the striatal dopamine (DA) system as the striatum shows marked neurodegenerative changes with age (Joseph 1992) or in the cerebellum which also shows age-related alterations (Bickford et al. 1992; Bickford 1993). Memory alterations appear to occur primarily in secondary memory systems and are reflected in the storage of newly acquired information (Bartus et al. 1982; Joseph 1992). It is thought that the hippocampus mediates allocentric spatial navigation (i.e., place learning) while the dorsomedial striatum mediates egocentric spatial orientation (i.e., response and cue learning) (Devan et al. 1996; McDonald & White, 1994; Oliveira et al. 1997).

Oxidative stress (OS) is thought to be a contributing factor to the decrements in cognitive and/or motor performance seen in aging. The 'free radical hypothesis of aging' maintains that with age the generation and accumulation of reactive oxygen species (ROS) (such as superoxide and hydroxyl radicals) result in oxidative damage to critical biological molecules, which coupled with the insufficiency of endogenous antioxidant defense mechanisms, contributes to the detrimental effects of aging (Ames et al. 1993; Harman 1992). The brain may be particularly vulnerable to the deleterious effects of oxidative damage because it is relatively deficient in free radical protective antioxidant compounds, utilizes high amounts of oxygen, contains high concentrations of iron and easily peroxidizable fatty acids, and the essentially nonregenerative nature of nervous tissue (Carney et al. 1991; Olanow 1992; Olanow 1993).

The decline in the capacity of normal antioxidant defense mechanisms has been postulated as a causative factor in age-related decrements in the function of biological systems (Halliwell 1994; Harman 1981; Yu 1994; Zhang et al. 1993). However, a clear causative link between oxidative stress and aging has yet to be established. One method to assist in establishing this link is to determine the functional effects of free radical damage, as assessed in different models of OS, on age-sensitive tests.

DISCUSSION

Test Battery Used to Measure Age-Related Behavioral Changes

Psychomotor Testing

There is a battery of tests of psychomotor behavior, and performance on all these tasks has been shown to deteriorate with age (Dean et al. 1981; Ingram 1983; Ingram et al. 1993; Ingram et al. 1994; Joseph 1992; Joseph et al. 1983; Joseph & Lippa 1986). These tests include: 1) Rod walking, which measures psychomotor coordination and the integrity of the vestibular system by

requiring the animal to balance on a stationary, horizontal rod; 2) Wire suspension/wire hanging, which measures muscle strength and the prehensile reflex, an animal's ability to grasp a horizontal wire with its forepaws and to remain suspended; 3) Plank walking, which measures balance and coordination by exposing the rats to different sizes of horizontal planks; 4) Inclined screen, which measures muscle tone, strength, stamina, and balance by placing the animal on a wire mesh screen that is tilted 60°-90° to the horizontal plane of the floor; and 5) Accelerating rotarod, which measures fine motor coordination, balance, and resistance to fatigue by measuring the amount of time that a rat can remain standing/walking on a rotating, slowly accelerating rod. With age, performance on all these tests deteriorates, so that old animals cannot remain on the rods, wire, planks, or screen for as long as young animals.

Cognitive testing

The Morris water maze (MWM) is a known, conventional cognitive test that requires an animal to use spatial learning and memory to find a hidden platform just below the surface of a circular pool of water, and to remember its location from the previous trial (Morris 1981; Morris 1984). Spatial memory performance is the ability to acquire a cognitive representation of location in space and the ability to effectively navigate the environment; therefore, the animal must use distal cues to effectively locate the hidden platform. Accurate navigation is rewarded by escape from the pool. Performance on the maze has been shown to deteriorate with aging (Barnes 1988; Brandeis et al. 1989; Brandeis et al. 1990; Gage et al. 1984; Gallagher & Pelleymounter 1988; Ingram et al. 1994; Rapp et al. 1987; Van der Staay & de Jonge 1993), due to a specific deficit in the ability of aged animals to utilize spatial information (Rapp et al. 1987).

Performance in the MWM can be videotaped and analyzed with image tracking software. Several dependent measures are used to estimate the efficiency of the rat's performance: directionality, latency of finding the hidden platform on each trial, length of swim path as well as the distribution of swimming time and swim path in the four quadrants of the pool during transfer or probe trials (in which the platform is removed from the pool) or during reversal trials (in which the platform is moved to another quadrant of the pool), number of crossings and latency to cross a previous platform location, and swim speed (Brandeis et al. 1989; Morris 1984). Several experimental procedures have been developed to measure different aspects of cognitive performance, including reference (long-term) and working (short-term) memory; these procedures include: on demand; acquisition and extinction; working memory and matching-to-sample; discrimination; non-mapping strategies; and measurement of sensory variables (Brandeis et al. 1989).

Aging Studies with Fischer 344 Rats

Our laboratory has validated the age sensitivity of these motor and cognitive tasks; we conducted studies to assess complex motor behavior and spatial learning and memory as a function of age in male Fischer 344 rats (Shukitt-Hale et al. 1998). In one of our studies, a cross-sectional age analysis was performed using rats that were 6, 12, 15, 18, and 22 months of age, to determine at what age these behaviors begin to deteriorate. It was found that complex motor behaviors, as measured by rod walk, wire suspension, plank walk, inclined screen, and accelerating rotarod performance, declined steadily with age, with most tests being adversely affected as early as 12 to 15 months of age, as shown in Figure 1.

Spatial learning and memory performance was measured by the working memory version of the Morris water maze (MWM), in which each session consisted of two trial swims, with a 10 minute intertrial interval between the two trials. At the beginning of each trial, the rat was gently immersed in the water at one of four randomized start locations (located 90° apart on the perimeter of the pool). Each rat was allowed 120 seconds to escape onto the platform, which was submerged 2 cm below the surface of the water in the center of one of the quadrants. The platform location was changed to a different quadrant for each session of testing, but it was always located the same distance away from the wall of the pool. Once the rat reached the platform, it remained there for 15 s (Trial 1; reference memory or acquisition trial), was returned to its home cage, and 10 minutes elapsed before the next trial (Trial 2; working memory or retrieval trial), which used the same platform location and start position as Trial 1. MWM testing was performed daily for four consecutive days, with a morning and an afternoon session and two trials each session. As shown in Figure 2, spatial memory showed decrements at 18 and 22 months of age (higher latencies on the working memory trial), with some change noticeable as early as 12-15 months of age (no improvement on the second trial following a 10 min retention interval); these differences were not due to swim speed. Therefore, complex motor and spatial memory behaviors show noticeable declines early in the lifespan of the male Fisher 344 rat.

For the five motor tests examined in this study, performance decrements were seen on 100% of them for both the 18 and 22 month old rats, 80% for the 15 month old group, and 60% for the 12 month old group. Therefore, these tests are sensitive enough to measure age-related deficits in motor behavior, which are evident as early as middle-age (12 to 15 months) in male, F344 rats. For the MWM, decrements in spatial working memory were visible at 18 and 22 months of age, with some change noticeable as early as 12-15 months of age (i.e., no difference in latency between Trials 1 and 2); therefore, the MWM paradigm used in this study is sensitive enough to test the nature of cognitive dysfunction in senescence. These results are in agreement with those from other cross-sectional studies that determined age-related changes in motor (Spangler et al. 1994; Wallace et al. 1980b) and spatial learning and memory performance (Frick et al. 1995; Wallace et al. 1980a; Walovitch et al. 1987).

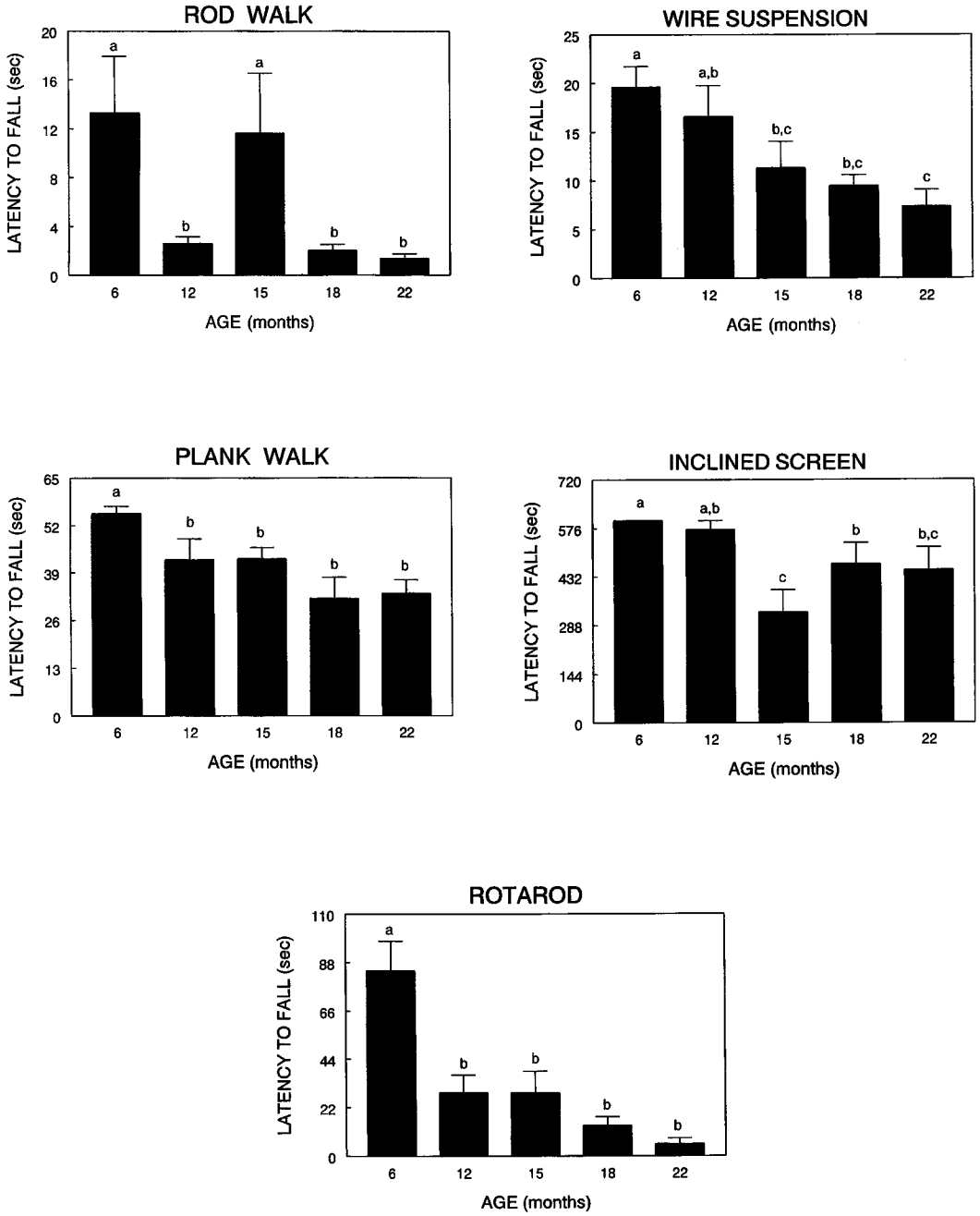

Figure 1. Latency (mean + SEM: secs) to fall in the rod walk, wire suspension, plank walk, inclined screen, and rotarod tests for five different age groups (6, 12, 15, 18, and 22 months). Means not sharing a common letter are significantly different from each other ($p < .05$, Fischer's LSD).

Oxidative Stress Models to Study Age-Related Changes in Behavior

Given that the test battery described above is sensitive to age-related changes in motor and cognitive function, it is possible to employ this same test battery to determine behavioral deficits under different models of OS. If there is a similarity in behavioral deficits between aged rats and animals in an OS model, then it could be that age-related changes in behavior result from an inability to cope with OS that occurs throughout the life-span. If an appropriate model of OS is found that mimics the effects of aging, this model can then be used with young animals, which would eliminate the potentially serious confounding effects of deteriorating visual ability on the attempts to assess cognitive functioning of aged rats on tasks requiring utilization of visual cues, such as the Morris water maze (Spencer et al. 1995).

Buthionine Sulfoximine Plus Dopamine Studies

One simple and effective method that our laboratory developed to produce OS similar to that seen in aged

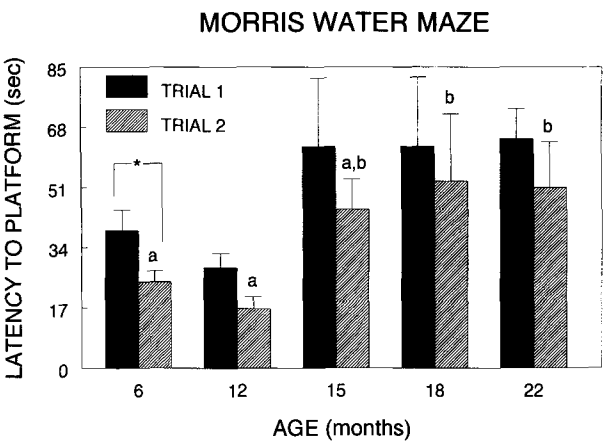

Figure 2. Latency (mean + SEM: secs) to find the hidden platform in the Morris Water Maze for five different age groups (6, 12, 15, 18, and 22 months). Trial 1 (solid bars) and Trial 2 (hatched bars) were separated by a 10 minute intertrial interval; bars represent an average of 8 different trials (two sessions per day for four days). Means not sharing a common letter for Trial 2 are significantly different from each other ($p < .05$, Fischer's LSD). An asterisk (*) denotes a significant ($p < .05$) difference between Trial 1 and Trial 2 within an age group.

rats involves decreasing OS protection by administering a glutathione-depleting drug, buthionine sulfoximine (BSO), and then increasing the OS with an injection of dopamine (DA). The rationale behind this model was as follows: in aging there are indications that ROS accumulate (Ames et al. 1993; Harman 1992), which coupled with declines in antioxidant defenses (Mo et al. 1995), result in oxidative damage to critical biological molecules.

Glutathione (GSH) is an endogenous protective agent found in the brain and other tissues that plays a critical role in intracellular antioxidant defense by scavenging and neutralizing free radicals generated by OS (Pileblad & Magnusson 1990; Vanella et al. 1993). Depletion of GSH in the brain may result in less OS protection and subsequent free-radical induced neuronal damage by causing an excess of H_2O_2 and alkyl peroxides, which can cause oxidative damage to DNA, proteins, and other macromolecules (Ames et al. 1993). Because brain GSH may be decreased in aging (Chen et al. 1989; Mo et al. 1995), endogenous protection against free-radicals may also be decreased with age, which could be a contributing factor to the behavioral decrements seen in aging.

Administration of buthionine sulfoximine (BSO) decreases GSH levels since it is a selective and potent inhibitor of GSH synthesis (Griffith 1982). Therefore, treatment with BSO may lower the protective antioxidant capabilities of the organism to make it more susceptible to neuronal damage by OS, similar to that seen in aging. In addition, administration of the oxidative stressor, dopamine (DA), when GSH levels are compromised has been found to increase OS, because DA rapidly oxidizes to form ROS (Hastings et al. 1996; Rabinovic et al. 1994). With age, brain DA undergoes increasing oxidation (Carlsson & Winblad 1976; Fornstedt et al. 1990), which in turn leads to the formation of free radicals (Obata & Yamanaka 1995). Conditions that increase concentration and/or turnover of DA should increase the potential for the formation of reactive metabolites, especially under conditions in which the ratio of available DA to antioxidant capacity is high (Hastings & Zigmond 1994).

While Hastings and colleagues (Hastings 1995; Hastings & Zigmond 1994; Hastings et al. 1996; Rabinovic et al. 1994) have characterized the ROS formed by DA oxidation, there is little information on how these compounds may alter motor and cognitive behavior under decreased GSH conditions, such as those seen in aging. Thus, our laboratory investigated the behavioral implications of modifications of vulnerability to OS by examining motor and cognitive behavioral performance in rats treated with BSO and DA (Shukitt-Hale et al. 1997; Shukitt-Hale et al. 1998). We hypothesized that injections of BSO plus DA would be a simple and effective method to lower protection, produce OS, and alter behavioral performance in rats, similar to that seen in aging.

Therefore, BSO (3.2 mg in 30µl), followed by 6µl Ringers solution, was administered to young male Fischer rats every 48h via a cannula implanted in the right lateral ventricle (since BSO does not readily pass the BBB). DA (15µl of 500µM) was administered (icv) every 24h, either 1h after BSO (BSO + DA group) or 1h before BSO (DA + BSO group), when given on the same day as BSO. Psychomotor behavior (rod walking, wire suspension, and plank walking) (Shukitt-Hale et al. 1997) and spatial learning and memory (Morris Water Maze, six trials/day) (Shukitt-Hale et al. 1998) were assessed.

BSO given prior to DA administration selectively impaired psychomotor and cognitive behavior; however, in the reverse condition (DA + BSO), no decrements in performance were observed relative to vehicle administration (Shukitt-Hale et al. 1997; Shukitt-Hale et al. 1998). Additionally, neither BSO alone or DA alone had decremental effects on behavior. Specifically, BSO + DA administration impaired motor performance by decreasing latency to fall in the rod, as seen in Figure 3A, and plank walk, as seen in Figure 3B, compared to a vehicle group (Shukitt-Hale et al. 1997). BSO + DA rats demonstrated cognitive impairment compared to a vehicle group as evidenced by increased latencies to find the hidden platform, particularly on the first trial each day. Also, the BSO + DA group utilized non-spatial strategies during the probe trials (swim with no platform): i.e., less time spent in the platform quadrant, as seen in Figure 4A; fewer crossings, as seen in Figure 4B, and longer latencies, as seen in Figure 4C, to the previous platform location; and more time spent around the edge of the pool rather than in the platform zone (Shukitt-Hale et al. 1998).

Thus, injections of BSO plus DA produced OS similar to that seen in aged rats, because decreasing OS protection with BSO and then increasing the OS with DA

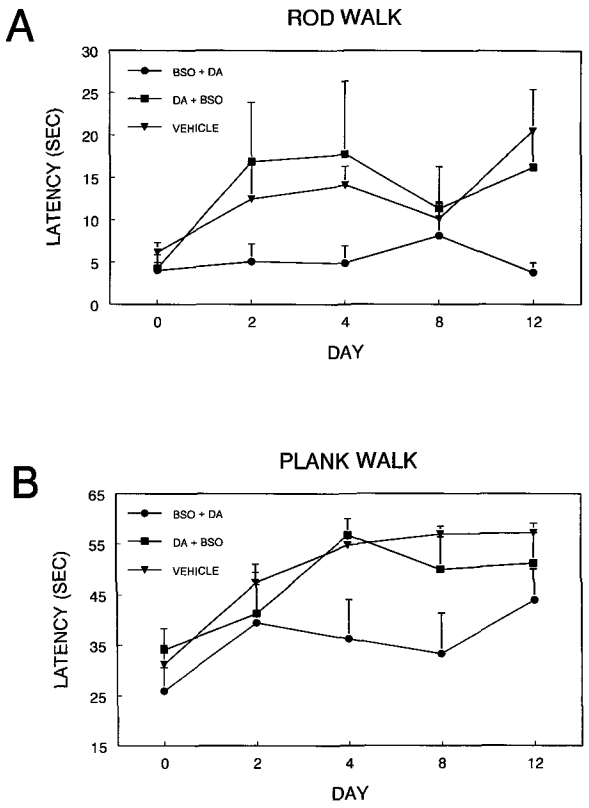

Figure 3. Latency to fall (mean + SEM: secs) in the rod walk (A) and plank walk (B) tests at five intervals throughout the study for the BSO + DA, DA + BSO, and vehicle groups. Latency to fall was significantly shorter ($p < .05$) for the BSO + DA group compared to the vehicle group; the DA + BSO group was not different from vehicle.

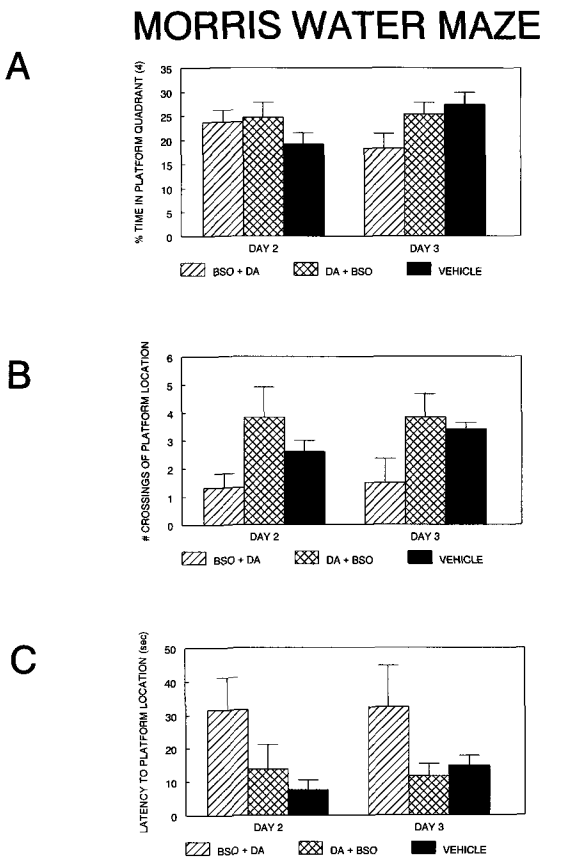

Figure 4. Percent time (A; mean + SEM) spent in quadrant 4 (the quadrant that had previously contained the platform), as well as number of crossings (B; mean + SEM) and latency to cross (C; mean + SEM) the previous location of the hidden platform, during the probe trials on testing days 2 and 3 in the Morris water maze for the BSO + DA, DA + BSO, and vehicle groups. Performance for the BSO + DA group was significantly impaired ($p < .05$) when compared to the vehicle group.

resulted in impaired psychomotor and cognitive performance. The similarity in behavior deficits between aged rats and the BSO + DA groups in these studies suggests that the oxidation of DA, coupled with a reduced capacity to respond to oxidative stress, via GSH alterations, may be responsible for induction of age-related behavioral deficits.

Oxygen Exposure Studies

Another model used to produce oxidative stress, and then to assess changes in behavior for their similarity to those seen in aging, involves exposing young rats to a normobaric hyperoxia environment of 100% oxygen (O_2) at 760 mm Hg (sea level pressure). Producing oxidative stress in rats with normobaric hyperoxia has been shown to lead to an accumulation of protein carbonyls and alterations in glutamine synthetase and glucose-6-phosphate dehydrogenase (Starke-Reed & Oliver 1989); this treatment also leads to an increase in ROS in the brain (Carney & Carney 1994; Hensley et al. 1995). We have recently shown that exposing young rats (6-8 months) to 100% O_2 for 48 hours produces changes on three parameters of CNS function that resemble those seen in aging: 1) loss of striatal muscarinic receptor sensitivity to oxotremorine-enhanced stimulation; 2) decreases in nerve growth factor (NGF); and 3) decreases in the efficacy of norepinephrine (NE) on cerebellar Purkinje cell responsiveness (Bickford et al. 1997). Additionally, we have assessed changes in motor function following 48 hours of 100% O_2.

Rats were tested on a series of psychomotor tasks (accelerating rotarod, wire suspension, small rod walk, large rod walk) one week prior to hyperoxia (baseline) and one hour following 48h hyperoxia exposure. The difference between these two scores was calculated for each rat as change from baseline. As can be seen from Figure 5, performance on all motor tasks was significantly impaired following 48h 100% O_2 compared to animals not exposed to hyperoxia. These changes are similar to those seen in aging.

Radiation

Radiation is another method used to examine the damaging effects of free radicals on central nervous system function, and the effects of radiation appear comparable to those seen in aging. Joseph and colleagues (Joseph et al. 1992; Joseph et al. 1993) have shown that exposure to whole-body-irradiation with high-energy iron

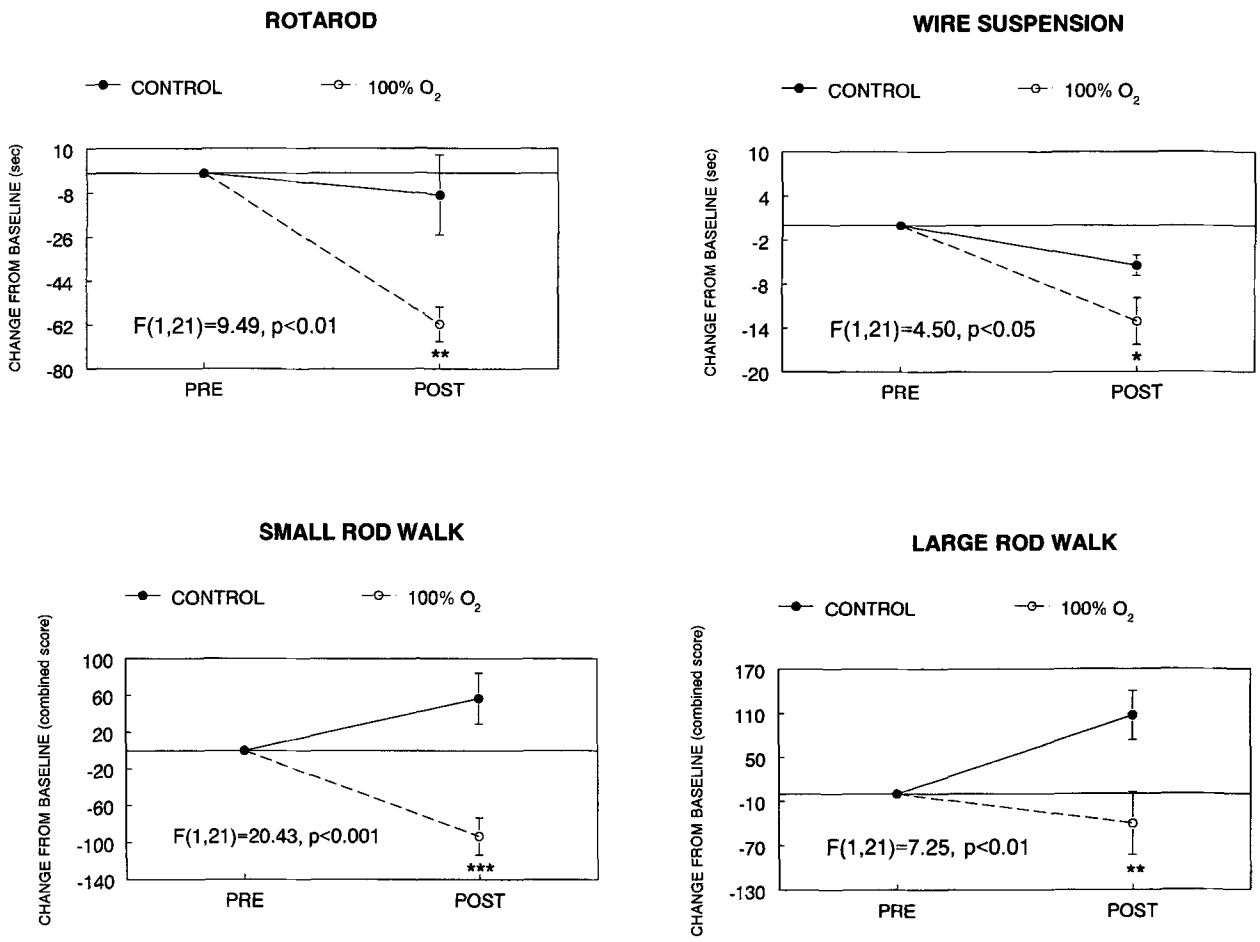

Figure 5. Latency (mean ± SEM: secs; change from baseline, pre-exposure to post-exposure) to fall in the rotarod, wire suspension, and small and large rod walks for control animals and animals exposed to a normobaric hyperoxia environment of 100% oxygen (O_2) for 48h. Asterisks indicate significant differences from control (*= $p < .05$, **= $p < .01$, ***= $p < .001$).

(^{56}Fe) particles (600 MeV/amu) adversely affects motor behavior and the sensitivity of muscarinic receptors to stimulation as assessed by oxotremorine-enhanced K^+-evoked release of dopamine from striatal slices. Motor behavior was assessed by the wire suspension test in various groups of rats at 12h to 14d after irradiation. Irradiated (0.10 Gy - 1.0 Gy) rats remained suspended on the wire for a shorter time than sham-irradiated rats at 12 hours and 3, 8, and 14 days postirradiation. Additionally, radiation-induced deficits in reducing K^+-evoked release of dopamine to agonist stimulation lasted as long as 180 days post-radiation (Joseph et al. 1992). Therefore, changes induced by radiation are similar to those that occur during aging, are associated with free-radical damage, and support the hypothesis that these changes may share a common chemical/biological mechanism (Joseph et al. 1992).

Other evidence

Other studies have also shown that oxidative stress may be a causal factor in age-related deficits, due to a recent finding that implicates protein oxidative damage as a determinant of individual variation in the age-related decline of specific brain functions related to cognitive and motor capacity (Forster et al. 1996). More evidence for this hypothesis comes from studies which have shown that reduction in the level of intracellular oxidized protein in old gerbils resulted in cognitive improvement, as evidenced by decreased errors in a radial arm maze after treatment with a free radical spin trapping compound, phenyl-α-*tert*-butyl nitrone (PBN) (Carney et al. 1991). Additionally, aged rats treated for 2 months with antioxidants (PBN, α-tocopherol, and ascorbate) had a greater rate of acquisition and exhibited greater memory retention than vehicle-treated rats in MWM testing, suggesting a link between oxidative damage and decreased cognitive function in aging (Socci et al. 1995). In another study, long-term treatment with PBN alone resulted in improved cognitive performance, greater survival, and decreased oxidative damage within brain areas important for cognitive function (Sack et al. 1996). Therefore, experimental interventions that reduce levels of protein oxidative damage result in concurrent improvement in age-associated behavioral deficits.

CONCLUSIONS

It appears that oxidative stress is a contributing factor to the behavioral decrements seen in aging. Age-validated psychomotor and cognitive test batteries were sensitive to different models of OS, which also showed behavioral deficits. Therefore, age-related changes in behavior may result from an inability to cope with OS that occurs throughout the life-span. When appropriate models of OS are found that mimic the effects of aging, these models can then be used to test the effects of antioxidants (e.g., beta-carotene, ascorbate), phytochemicals (e.g., flavonoids), or nitrone trapping agents (e.g., PBN) in young animals, either via diet or acute administration, for their efficacy in preventing or restoring decrements in behavior due to age or OS.

REFERENCES

Ames, BN, Shigenaga, MK, and Hagen, TM: Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA, 90:7915-7922, 1993.

Barnes, CA: Aging and the physiology of spatial memory. Neurobiol. Aging, 9:563-568, 1988.

Bartus, RT, Dean, RL, Beer, B, and Lippa, AS: The cholinergic hypothesis of geriatric memory dysfunction. Science, 217:408-417, 1982.

Bickford, P: Motor learning deficits in aged rats are correlated with loss of cerebellar noradrenergic function. Brain Res., 620:133-138, 1993.

Bickford, PC, Chadman, K, Taglialatela, G, Shukitt-Hale, B, Prior, RL, Cao, G, and Joseph JA: Dietary strawberry supplementation protects against the age-accelerated CNS effects of oxidative stress. FASEB J., 11:A176, 1997.

Bickford, P, Heron, C, Young, DA, Gerhardt, GA, and de la Garza, R : Impaired acquisition of novel locomotor tasks in aged and norepinephrine-depleted F344 rats. Neurobiol. Aging, 13:475-481, 1992.

Brandeis, R, Brandys, Y, and Yehuda, S: The use of the Morris water maze in the study of memory and learning. Intern. J. Neurosci., 48:29-69, 1989.

Brandeis, R, Dachir, S, Sapir, M, Levy, A, and Fisher, A: Reversal of age-related cognitive impairments by an M1 cholinergic agonist, AF102B. Pharmacol. Biochem. Behav., 36:89-95, 1990.

Carlsson, A, and Winblad, B: Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. J. Neural Trans., 38:271-301, 1976.

Carney, JM, and Carney, AM: Role of protein oxidation in aging and in age-associated neurodegenerative diseases. Life Sci., 55:2097-2103, 1994.

Carney, JM, Starke-Reed, PE, Oliver, CN, Landum, RW, Cheng, MS, Wu, JF, and Floyd, RA: Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-*tert*-butyl-α-phenylnitrone. Proc. Natl. Acad. Sci. USA, 88:3633-3636, 1991.

Chen, TS, Richie, JP, and Lang, CA: The effect of aging on glutathione and cysteine levels in different regions of the mouse brain. Proc. Soc. Exp. Biol. Med., 190:399-402, 1989.

Dean, RL, Scozzafava, J, Goas, JA, Regan, B, Beer, B, and Bartus, RT: Age-related differences in behavior across the life span of the C57BL/6J mouse. Exp. Aging Res., 7:427-451, 1981.

Devan, BD, Goad, EH, and Petri, HL: Dissociation of hippocampal and striatal contributions to spatial navigation in the water maze. Neurobiol. Learn. Memory, 66:305-323, 1996.

Fornstedt, B, Pileblad, E, and Carlsson, A: In vivo autoxidation of dopamine in guinea pig striatum increases with age. J. Neurochem., 55:655-659, 1990.

Forster, MJ, Dubey, A, Dawson, KM, Stutts, WA, Lal, H, and Sohal, RS: Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc. Natl. Acad. Sci. USA, 93:4765-4769, 1996.

Frick, KM, Baxter, MG, Markowska, AL, Olton, DS, and Price, DL: Age-related spatial reference and working memory deficits assessed in the water maze. Neurobiol. Aging, 16:149-160, 1995.

Gage, FH, Dunnett, SB, and Bjorklund, A: Spatial learning and motor deficits in aged rats. Neurobiol. Aging, 5:43-48, 1984.

Gallagher, M, and Pelleymounter, MA: Spatial learning deficits in old rats: A model for memory decline in the aged. Neurobiol. Aging, 9:549-556, 1988.

Griffith, OW: Mechanism of action, metabolism, and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J. Biol. Chem., 257:13704-13712, 1982.

Halliwell, B: Free radicals and antioxidants: A personal view. Nutr. Rev., 52:253-265, 1994.

Harman, D: The aging process. Proc. Natl. Acad. Sci. USA, 78:7124-7128, 1981.

Harman, D: Role of free radicals in aging and disease. Ann. N.Y Acad. Sci., 673:126-141, 1992.

Hastings, TG: Enzymatic oxidation of dopamine: The role of prostaglandin H synthase. J. Neurochem. 64:919-924, 1995.

Hastings, TG, Lewis, DA, and Zigmond, MJ: Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. Proc. Natl. Acad. Sci. USA, 93:1956-1961, 1996.

Hastings, TG, and Zigmond, MJ: Identification of catechol-protein conjugates in neostriatal slices incubated with [^{3}H] dopamine: Impact of ascorbic acid and glutathione. J. Neurochem., 63:1126-1132, 1994.

Hensley, K, Howard, BJ, Carney, JM, and Butterfield, DA: Membrane protein alterations in rodent erythrocytes and synaptosomes due to aging and hyperoxia. Biochem. Biophys. Acta, 1270:203-206, 1995.

Ingram, DK: Toward the behavioral assessment of biological aging in the laboratory mouse: Concepts, terminology, and objectives. Exp. Aging Res., 9:225-238, 1983.

Ingram, DK, Jucker, M, and Spangler, EL: Behavioral manifestations of aging, in: Pathobiology of the Aging Rat, Vol. 2, edited by Mohr, U, Cungworth, DL, and Capen, CC, Washington, D.C., ILSI Press, 1994, pp. 149-170.

Ingram, DK, Wiener, HL, Chachich, ME, Long, JM, Hengemihle, J, and Gupta, M: Chronic treatment of aged mice with L-deprenyl produces marked striatal MAO-B inhibition but no beneficial effects on survival, motor performance, or nigral lipofuscin accumulation. Neurobiol. Aging, 14:431-440, 1993.

Joseph, JA: The putative role of free radicals in the loss of neuronal functioning in senescence. Integ. Physiol. and Behav. Sci., 27:216-227, 1992.

Joseph, JA, Bartus, RT, Clody, D, Morgan, D, Finch, C, Beer, B, and Sesack, S: Psychomotor performance in the senescent rodent: Reduction of deficits via striatal dopamine receptor up-regulation. Neurobiol. Aging, 4:313-319, 1983.

Joseph, JA, Hunt, WA, Rabin, BM, and Dalton, TK: Possible "accelerated striatal aging" induced by ^{56}Fe heavy-particle irradiation: Implications for manned space flights. Radiat. Res., 130:88-93, 1992.

Joseph, JA, Hunt, WA, Rabin, BM, Dalton, TK, and Harris, A.H. Deficits in the sensitivity of striatal muscarinic receptors induced by ^{56}Fe heavy-particle irradiation: Further "age-radiation" parallels. Radiat. Res., 135:257-261, 1993.

Joseph, JA, and Lippa, AS: Reduction of motor behavioral deficits in senescent animals via chronic prolactin administration - II. Non-stereotypic behaviors. Neurobiol. Aging, 7:37-40, 1986.

McDonald, RJ, and White, NM: Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. Behav. Neural Biol., 61:260-270, 1994.

Mo, JQ, Hom, DG, and Andersen, JK: Decreases in protective enzymes correlates with increased oxidative damage in the aging mouse brain. Mech. Ageing Dev., 81:73-82, 1995.

Morris, RGM: Spatial localization does not require the presence of local cues. Learning Motiv., 12:239-261, 1981.

Morris, R: Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci. Meth., 11:47-60, 1984.

Obata, T, and Yamanaka, Y: Intracranial microdialysis of salicylic acid to detect hydroxyl radical generation by monoamine oxidase inhibitor in the rat. Neurosci. Lett., 188:13-16, 1995.

Olanow, CW: An introduction to the free radical hypothesis in Parkinson's disease. Ann. Neurol., 32:S2-S9, 1992.

Olanow, CW: An radical hypothesis for neurodegeneration. TINS, 16:439-444, 1993.

Oliveira, MGM, Bueno, OFA, Pomarico, AC, and Gugliano, EB: Strategies used by hippocampal- and caudate-putamen-lesioned rats in a learning task. Neurobiol. Learn. Memory, 68:32-41, 1997.

Pileblad, E, and Magnusson, T: Effective depletion of glutathione in rat striatum and substantia nigra by L-buthionine sulfoximine in combination with 2-Cyclohexene-1-one. Life Sci., 47:2333-2342, 1990.

Rabinovic, AD, Zigmond, MJ, and Hastings, TG: Intrastriatal dopamine oxidizes to quinones: Role of endogenous glutathione. Soc. Neurosci. Abstracts, 20:413, 1994.

Rapp, PR, Rosenberg, RA, and Gallagher, M. An evaluation of spatial information processing in aged rats. Behav. Neurosci., 101:3-12, 1987.

Sack, CA, Socci, DJ, Crandall, BM, and Arendash, GW: Antioxidant treatment with phenyl-á-*tert*-butyl nitrone (PBN) improves the cognitive performance and survival of aging rats. Neurosci. Lett., 205:181-184, 1996.

Shukitt-Hale, B, Denisova, NA, Strain, JG, and Joseph JA: Psychomotor effects of dopamine infusion under decreased glutathione conditions. Free Radic. Biol. Med., 23:412-418, 1997.

Shukitt-Hale, B, Erat, SA, and Joseph JA: Spatial learning and memory deficits induced by dopamine administration with decreased glutathione. Free Radic. Biol. Med., 24:1149-1158, 1998.

Shukitt-Hale, B, Mouzakis, G, and Joseph JA: Psychomotor and spatial memory performance in aging male Fischer 344 rats. Exp. Gerontol., 33: 615-624, 1998.

Socci, DJ, Crandall, BM, and Arendash, GW: Chronic antioxidant treatment improves the cognitive performance of aged rats. Brain Res., 693:88-94, 1995.

Spangler, EL, Waggie, KS, Hengemihle, J, Roberts, D, Hess, B, and Ingram, DK: Behavioral assessment of aging in male Fischer 344 and Brown Norway rat strains and their F1 hybrid. Neurobiol. Aging, 15:319-328, 1994.

Spencer, RL, O'Steen, WK, and McEwen, BS: Water maze performance of aged Sprague-Dawley rats in relation to retinal morphologic measures. Behav. Brain Res., 68:139-150, 1995.

Starke-Reed, PE, and Oliver, CN: Protein oxidation and proteolysis during aging and oxidative stress. Arch. Biochem. Biophys., 275:559-567, 1989.

Van der Staay, FJ, and de Jonge, M: Effects of age on water escape behavior and on repeated acquisition in rats. Behav. Neural Biol., 60:33-41, 1993.

Vanella, A, Di Giacomo, C, Sorrenti V, Russo, A, Castorina, C, Campisi, A, Renis, M, and Perez-Polo, JR: Free radical scavenger depletion in post-ischemic reperfusion brain damage. Neurochem. Res., 18:1337-1340, 1993.

Wallace, JE, Krauter, EE, and Campbell, BA: Animal models of declining memory in the aged: Short-term and spatial memory in the aged rat. J. Gerontol., 35:355-363, 1980a.

Wallace, JE, Krauter, EE, and Campbell, BA: Motor and reflexive behavior in the aging rat. J. Gerontol., 35:364-370, 1980b.

Walovitch, RC, Ingram, DK, Spangler, EL, and London, ED: Co-dergocrine, cerebral glucose utilization and maze performance in middle-aged rats. Pharmacol. Biochem. Behav., 26:95-101, 1987.

Yu, BP: Cellular defenses against damage from reactive oxygen species. Physiol. Rev., 74:139-162, 1994.

Zhang, JR, Andrus, PK, and Hall, ED: Age-related regional changes in hydroxyl radical stress and antioxidant in gerbil brain. J. Neurochem., 61:1640-1647, 1993.