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Aged SOD Overexpressing Mice Exhibit Enhanced Spatial Memory While Lacking Hippocampal Neurogenesis

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ABSTRACT

The recent finding that hippocampal slices from aged mice overexpressing the gene for superoxide dismutase (SOD) exhibit long-term potentiation (LTP) of reactivity to afferent stimulation that is significantly larger than that produced in aged wild-type (wt) mice has encouraged the exploration of the effects of reactive oxygen species (ROS) on learning in aged mice. In addition, young-adult and aged wt and SOD transgenic mice were used in an attempt to correlate adult neurogenesis with spatial learning. Aged wt and SOD mice exhibited a 90% reduction in doublecortin-labeled new dentate gyrus neurons as compared to young mice, with no significant difference between genotypes. In addition, aged SOD mice exhibited better performance than wt controls in both reference and working memory tasks in a water maze. These findings provide a behavioral measure to demonstrate that excessive production of H_2O_2 is beneficial in aged mice. *Antioxid Redox Signal* 9, 181–189.

INTRODUCTION

Reactive oxygen species (ROS) have been hypothesized to be a major contributor to cognitive decline in aged individuals (40). We have shown recently (21) that adding 50 μ*M* H_2O_2 to the perfusion medium could restore long-term potentiation (LTP) of responses to afferent stimulation in hippocampal slices taken from aged (2-year-old) mice. Interestingly, hippocampal slices from aged mice overexpressing the H_2O_2 producing enzyme Cu/Zn superoxide dismutase (SOD) expressed a large LTP that was similar to that induced in young wild-type (wt) slices. This was the reverse result to that found in slices from young adult SOD and wt mice where SOD slices were impaired in LTP in a way that could be restored by extrinsic addition of 50 μ*M* H_2O_2. In contrast, the wt expressed an impaired LTP by H_2O_2. We hypothesized that hippocampal synaptic plasticity results from synaptic events that induce a transient H_2O_2 flux that is necessary for long-term changes in the synapses. According to this hypothesis, the hippocampal slices taken from the aged SOD mice showed LTP that was similar to that seen in aged wt slices when exogenous H_2O_2 was added due to an intrinsic increase in H_2O_2 that was necessary for LTP. We therefore predicted that aged SOD mice will perform better than wt in hippocampus dependent tasks.

The intrinsic differences in hippocampal function between SOD and wt mice, particularly in aged individuals, provides an opportunity to monitor the contribution of another unique hippocampal quality, neurogenesis, to the performance of hippocampus-dependent tasks.

The dentate gyrus (DG) of the mammalian hippocampus is one of the few brain regions to undergo neurogenesis in the adult (2, 18). The abundance of knowledge available in regard to hippocampal structure and function has prompted studies aimed at understanding the possible relationship between the newborn neurons and functions ascribed to the hippocampus. Whereas many studies show little connection between neurogenesis and hippocampus-dependent learning, it is still debated whether neurogenesis in the DG is in fact necessary for successful completion of hippocampus dependent memory tasks (13, 30, 38, 42, 43). It has been further argued that electrical stimulation of developing progenitor cells in the DG determines a neuronal outcome with functional significance for hippocampal learning (12).

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Aging in mammals is accompanied by a decline in mental capacities such as learning and memory (4). There is also evidence of specific hippocampal degeneration that occurs with age. Several studies have shown that the rate of DG neurogenesis is dramatically slowed in senescence (5, 9), offering a possible underlying cause for a decline in hippocampus-dependent task performance. It is therefore of great interest to study whether the degree of decline in the rate of neurogenesis in aged individuals correlates with the level of decline in performance of hippocampus dependent tasks. Drapeau *et al*. (13) found that water maze performance in aged rats was indeed correlated with the amount of cells labeled by BrdU in the DG. In contrast, Merrill *et al*. (32) showed that hippocampal neurogenesis does not correlate with spatial learning ability in aged rats. Interestingly, it has been shown in several studies that neurogenesis correlates with success in hippocampus dependent tasks but not with spatial navigation memory of a water maze (30, 42), while others did show such a correlation (3, 13, 23, 29, 34, 38, 43, 46). Although these studies and many recent others (summarized in Table 1) find correlations between neurogenesis and hippocampus-dependent memory function, none of them has been successful in establishing a causal relationship. Furthermore, there are more parsimonious explanations explaining the correlation in most cases.

We therefore studied the effect of altered ROS homeostasis affected by aging and transgenically expressed SOD on spatial learning and utilized this model to further examine the relationship between new DG neurons and the completion of these tasks.

MATERIALS AND METHODS

Subjects

Homozygous SOD mice overexpress the entire native human Cu/Zn superoxide dismutase (SOD) gene with its own promoter (15). SOD mice were developed from outbred mating (F1) between CBYB/6 [(Balb/C x C57BI/6j) F1] and B6D/2 [(C57BI/6J × DBA) F1]. Mice with the same background but lacking the transgene were inbred for use as wild type (wt). The enzymatic activity of the transgene in these mice represents a sixfold increase over the level of endoge-

TABLE 1. CORRELATION OF HIPPOCAMPAL NEUROGENESIS AND BEHAVIOR IN RODENTS

Reference	*Biological model*	*NG*	*Memory task*	*Result*
47	PS1 mutation	D	Contextual fear conditioning	Less freezing
49	Irradiation	D	DMTS	Increased errors after 240 sec
			Contextual fear	Less freezing
48	Aging	D	Fear conditioning	Less freezing
14	Aging	D	MWM	Less memory of new location
11	D-Galactose exposed mice	D	MWM	Shorter latency to platform
36	Irradiation	D	MWM	N.D.
			Barnes maze	Longer latency to tunnel
38	Irradiation	D	MWM	Less time in target quadrant
43	Irradiation	D	MWM	Less time in target quadrant
				At 2–4 week probe test
39	Nicotine administration	D	MWM	Less time in target quadrant
41	NT-3 conditional ko	D	MWM	Slower learning
				Less time in target quadrant
1	Pup food deprivation	D	MWM	Less time in target quadrant
44	Serotonin depletion	D	MWM	N.D.
20	Olfactory bulbectomy	D	Passive avoidance	Less avoidance
			Contextual fear	Less freezing
24	Prenatal music	U	8 arm radial maze	Faster completion of task
				Less errors
35	Brief neonatal hypoxia	U	Multiple T maze	Better retention at 40 days
34	Enriched environment	U	MWM	Shorter latency to platform
28	Erythropoietin after TBI	U	MWM	Improved learning
25	S100B after TBI	U	MWM	Improved cognitive performance
50	T cell treatment in Immunodeficient mice	U	MWM	Shorter latency to plateform
7	Enriched environment	U	Object recognition	More time in safe chamber
	+ MAM	D		Less time on new object
10	VEGF gene transfection	U	Passive avoidance	More time in safe chamber
			MWM	More time in target quadrant
26	Maternal swimming During pregnancy	U	Step down avoidance	More avoidance

NG, neurogenesis; D, down; U, up; DMTS, delayed match to sample; MWM, Morris Water Maze; N.D., no difference; TBI, traumatic brain injury.

nous CuZnSOD in the brain of control mice. During the establishment of the colony, the presence of the transgene was followed by SOD gel activity (showing mouse and human proteins in brain extracts), as well as PCR from tail genomic DNA. The homozygocity for SOD genes was predicted by quantitative PCR and confirmed by backcrossing tg-mice with control animals. No noticeable phenotype was observed concerning young or adult mice, except that young SOD animals were somewhat smaller than controls. The mice were kept in two groups of young adult (4-month-old) SOD and wt mice and two groups of aged (22- to 24-months-old) SOD and wt. A week prior to behavioral testing, the mice were handled and weighed by the examiner and divided into randomized cages in mixed groups. The average weights of the mice were 34 ± 1, 32 ± 2, 33.2 ± 1.8, and 31.7 ± 1.6 g for aged wt, aged SOD, young wt, and young SOD, respectively.

Behavior

Locomotor activity in open field. Mice were tested for locomotor activity in 1 x 1 m open field in one trial of 5 min. The arena was divided into 16 squares 25 cm x 25 cm and the total number of line crossing was counted. A small percentage of the mice (1/6 of the old wt and 3/8 of the young wt) remained immobile for the whole trial and they were not added to the data shown in Table 1.

Measurement of spatial learning in the water maze. The water maze consisted of a round tank, 1.25 m in diameter, filled with water. Water temperature was adjusted to 28°C for the aged mice to avoid hypothermia due to deficits in thermoregulation. Prior to the training of the young mice, a pilot study was carried out with three mice that were not included in the study. These young mice did not prefer staying on the platform to swimming in the water at 28°C, therefore water temperature for the young mice was adjusted to 25°C. In the spatial memory experiment, mice were trained to find the location of a hidden platform (12 cm in diameter), submerged 1 cm below the water surface, and visual cues were placed around the maze. Training consisted of four trials per day for aged mice, and five trials per day for young mice over 3 consecutive days, the entrance point to the maze was varied across trials. On the 4th day after the second trial, a "probe trial" was performed: the platform was removed and the swimming trajectory of the mice was manually recorded. The drawn traces of the mice swim patterns were scanned to a computer and the density of pixels in each of the four quadrants of the maze was measured digitally (Adobe Photoshop).

After the probe trial the platform was placed in a new location and the mice were trained for two trials. The latency of finding the platform in the new location on the second trial was considered as a measure of working memory.

Immunohistochemistry

After behavioral testing, the mice were anesthetized with ketamine (100 mg/Kg) and fixed by intracardiac perfusion with 4% paraformaldehyde. Floating 16 μm (cryotome) sections were cut at the level of the dorsal hippocampus, and were immunostained with specific antibodies raised against DCX (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or the neuronal marker NeuN (Chemicon, Temecula, CA). Immunohistochemistry was performed on sections of wt and SOD mice in parallel. The staining was revealed by an avidin-biotin peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) reacting with diaminobenzidine/H_2O_2 and observed by light microscopy. For monoclonal antibodies, the protocol provided by the M.O.M kit detection (Vector Laboratories, CA) was followed.

Quantification of DCX positive cells

Four coronal sections from the dorsal hippocampus of each of 4 young (total of 16 sections) and 6 old mice (total of 24 sections) of both SOD and wt genotypes were immunostained for DCX. The DCX positive cells found at the granular layer of the DG on both hemispheres were counted.

Statistical analysis

Two-way analysis of variance (ANOVA) followed by Scheffe' post hoc multiple comparison tests were used as indicated. For comparisons between two groups, an independent *t*-test was used. Finally, to characterize the relations between different measures, a Pearson's correlation coefficient was calculated.

All tests were two-tailed. The results are presented as means ± S.E.M.

RESULTS

Aged SOD mice exhibit better spatial memory than aged wt.

Open field behavior. First we examined the motor ability of the mice in an open field (Table 2) to determine whether there were significant motor deficiencies that might affect the performance in the water maze task. Analysis of total line crossings showed no effect for age [$F(1,37)<1$] but it did reveal a genotype specific difference [$F(1,37) = 13.43$, $p < 0.001$] with the SOD mice exhibiting a higher degree of locomotor activity as was shown previously for these mice (17). There was no significant interaction between the factors of age and genotype [$F(1,37) = 1.878$, $p > 0.179$)].

Spatial learning in a water maze. Next we tested the mice performance in a water maze (33). The young mice

TABLE 2. OPEN FIELD BEHAVIOR IN MICE

Mouse	*Line crossings*	*Center crossings*
Young wt	50 ± 34	5 ± 2
Young SOD	105 ± 25	10 ± 2
Old wt	56 ± 41	5 ± 2
Old SOD	82 ± 33	5 ± 2

A summary of the average number of times the mice crossed lines in a 1 m × 1 m arena divided into 16 squares 25 cm × 25 cm during a 5-min trial. Also shown are the number of times the mice crossed the center squares.

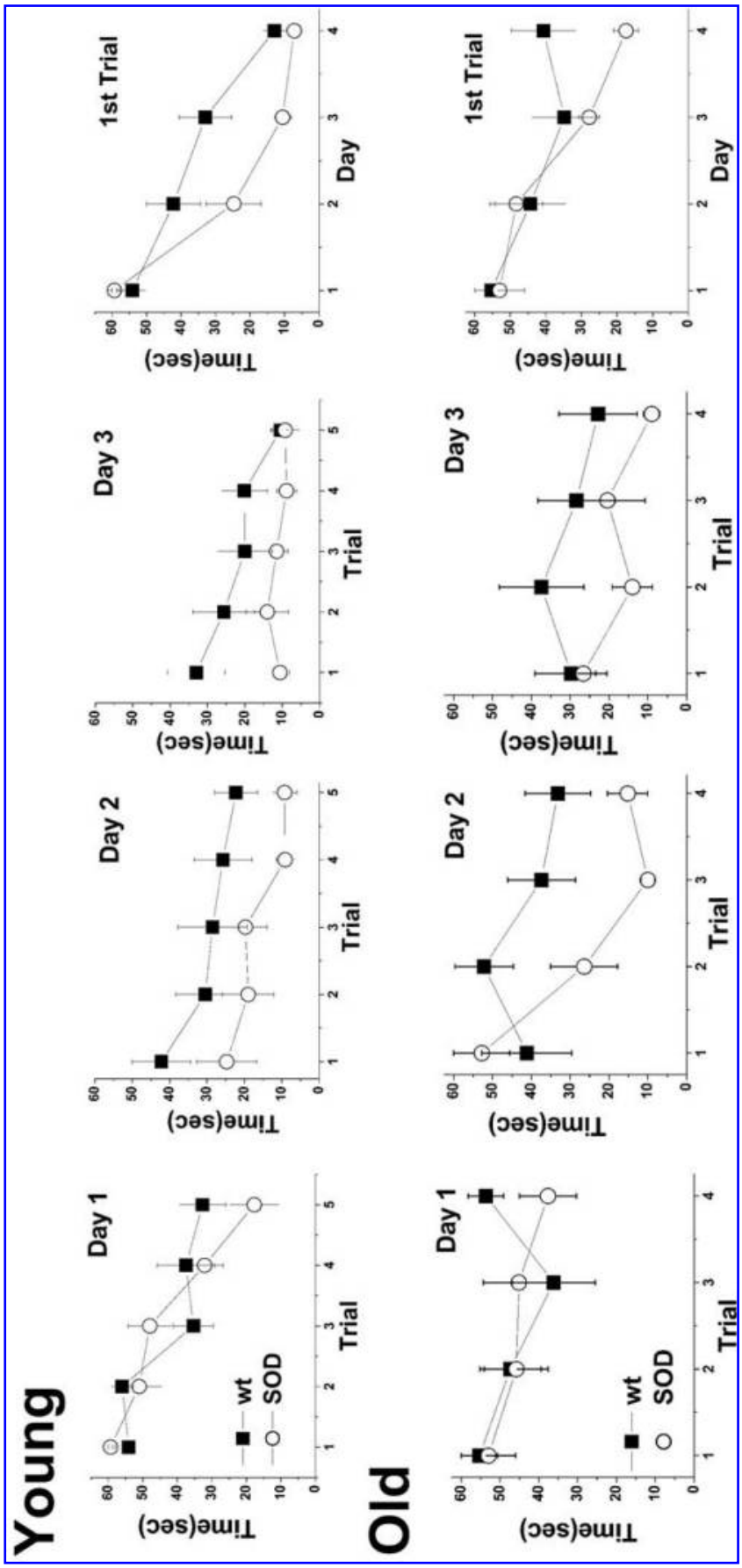

FIG. 1. Water maze training in wt and SOD mice. Results of water maze training are expressed as the latency to find a hidden platform. The *top panels* present results obtained for young-adult mice (4 months old). The *three left panels* present different trials conducted on single days. The *right panel* presents results obtained for the first trial of each of 4 days of training. The *bottom panels* present similar results obtained for old mice (2 years old).

showed a marked improvement between trials on day 1, two way ANOVA with genotype as a between-subjects factor and trial as a within-subjects factor revealed a significant main effect of trial [$F(4,24) = 10.81$, $p < 0.0001$] indicating that both groups acquired the task. In fact, contrary to our previous findings, the SOD mice did not acquire the task significantly slower than the wt as seen by the lack of interaction between genotype and trial $F(4,24) = 1.124$, $p > 0.369$]. We did not find a significant difference between the two genotypes [$F(1,27) < 1$].

On day 2 the rate of improvement was slower but still significant [$F(4,24) = 3.099$, $p < 0.034$]. There was no significant difference in performance between the groups [$F(1,27) = 1.547$, $p > 0.224$], and the interaction between trial and genotype was also insignificant [$F(4,24) < 1$].

The performance on day 3 showed similar tendency {effect of trial [$F(4,24) = 4.841$, $p < 0.005$], genotype [$F(1,27) = 1.29$, $p > 0.266$], interaction [$F(4,24) = 1.725$, $p > 0.177$]}.

The first trial on the fourth day of training shows a similar degree of learning for both genotypes [$t(24) = 1.972$, $p > 0.06$].

To examine the consolidation of learning, we analyzed the performance on the first trial of each day, across all 4 days. There was clear improvement over days [$F(3,25) = 87.037$, $p < 0.0001$]. However, there was no significant effect for genotype [$F(1,25) = 1.561$, $p > 0.222$] nor for the interaction between them [$F(3,25) = 1.614$, $p > 0.211$]. This indicates that the young SOD and their counterpart wt controls showed similar rate of consolidation of the spatial task.

In the old mice group, the SOD mice acquired the task faster and reached a significantly lower score than the wt. Two way ANOVA of the latencies to reach the platform on the first day revealed no significant effect for trial [$F(3,8) = 2.08$, $p > 0.181$], genotype [$F(1,10) < 1$], or the interaction between them [$F(3,8) < 1$].

On the second day there was an improvement across trials [$F(3,8) = 4.786$, $p < 0.034$], with a clear difference between SOD and wt [$F(1,10) = 6.247$, $p < 0.031$], but with insignificant interaction [$F(3,8) = 1.022$, $p > 0.433$].

On day 3 the improvement across trials was smaller and not significant [$F(3,8) < 1$], as well as the difference between genotypes [$F(1,10) = 3.85$, $p > 0.078$], and the interaction between the two factors [$F(3,8) = 1.64$, $p > 0.256$]. However, the first trial of the fourth day showed a marginally significant improvement of the SOD mice compared with the wt [$t(6) = 2.387$, $p < 0.051$]. Analysis of performance on the first trial over the four days of training showed a significant improvement over days [$F(3,8) = 6.457$, $p < 0.016$], with no

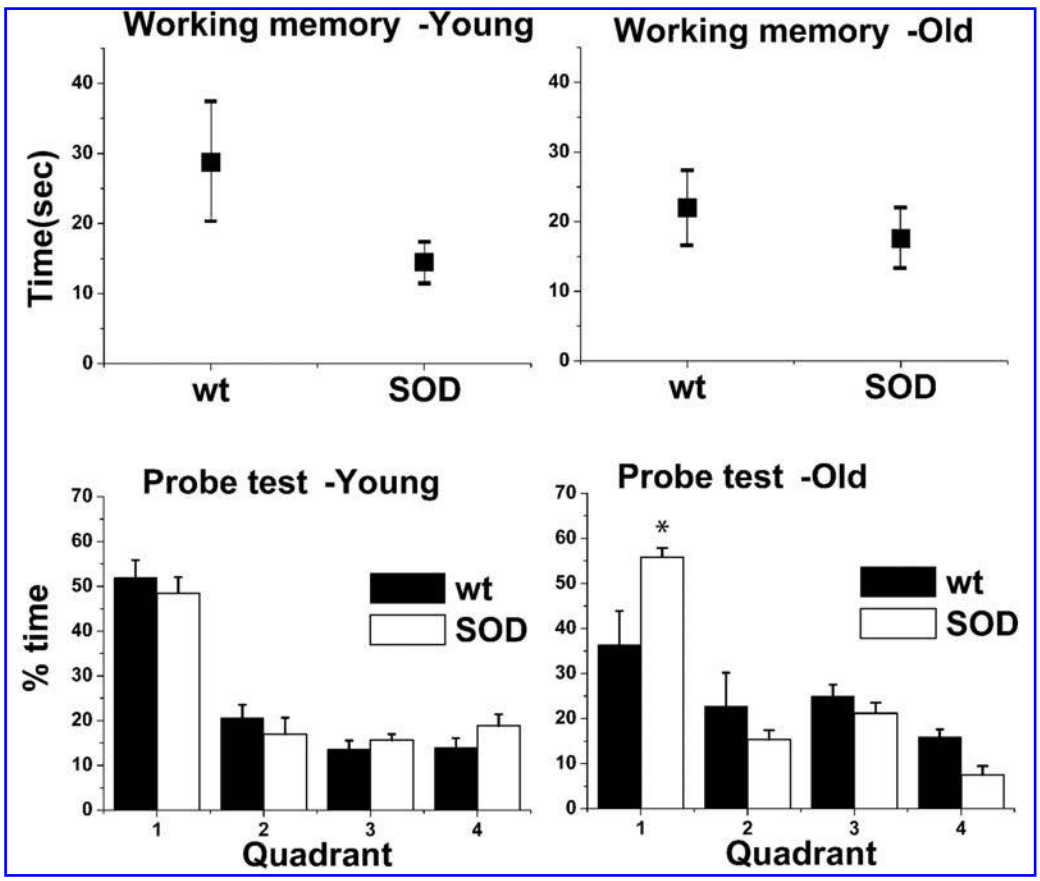

FIG. 2. Working memory and retention in water maze. The *top panels* show the latency to find the hidden platform on the second of two trials in which the platform was placed in a new location. This test measures working memory (*left,* young mice, *right*, old mice.) The *bottom panels* show the percent of time spent in each quadrant of the maze in a probe test conducted in the absence of the platform. This test measures the degree of memory retention of the former position of the platform (*left*, young mice, *right*, old mice).

genotype effect [$F(1,10) = 1.502$, $p > 0.248$], and no significant interaction [$F(3,8) = 1.425$, $p > 0.306$] (Fig. 1).

We did not compare the old and the young groups because different training paradigms were used to prevent exhaustion of the old mice.

Aged SOD mice exhibit better working memory and retention than aged wt

Following the second trial of the 4th day of training, the platform was removed and the time spent by the mice in each of the quadrants of the maze was measured. The young mice spent more time in the quadrant in which the platform was previously located, demonstrating memory retention of the platform location [$F(3,56) = 67.533$, $p < 0.0001$] (Scheffe' post hoc test for quadrant 1 compared with all other quadrants, $p < 0.00001$). The young wt and SOD mice did not differ significantly in the percent of time spent in that quadrant.

The old mice also spent more time in the quadrant that had formerly contained the platform [$F(3,32) = 25.189$, $p < 0.0001$]. Old wt mice spent only 36.4 ± 7.5% of the time in the correct quadrant whereas old SOD mice spent 55.9 ± 2.02%. The interaction between quadrant and genotype was significant [$F(3,32) = 4.936$, $p < 0.006$] and the difference in time spent in the correct quadrant was close to significance [$t(4) = 2.498$, $p < 0.059$], demonstrating better memory of the learned task by the old SOD mice (Fig. 2). Interestingly, a similar phenomena was recently found in aged mice overexpressing extracellular SOD (19).

After completion of the probe test, the mice were tested in two trials for a new location of the platform. The latency to find the platform on the second of these trials was considered a measure of the working memory. In both age groups, there were no significant differences between the SOD and the wt mice. However, there was a clear tendency for the SOD mice to be faster than the wt in finding the platform on the second trial (Fig. 2).

The rate of neurogenesis is dramatically reduced in aged individuals

In many recent studies (16, 22, 45), neurogeneis in the adult brain has been measured by detecting the incorporation of a thymidine analog 5′-bromo-2′-deoxyuridine (BrdU) to dividing cells with consequent counter detection of neuronal markers. It has been shown recently (6, 37) that Doublecortin (DCX), a microtubule-associated phosphoprotein required for neuronal migration and differentiation specifically labels newly generated neurons in the DG (for a recent thorough study using BrdU and DCX on aging rodents, see Ref. 31). This labeling, unlike BrdU labeling is neuron specific, it is time specific insofar as it labels neurons in the first three weeks of differentiation, it labels neuronal processes that enables visualization of dendritic structures, and it does not require additional labeling of the neurons with a neuronal marker.

We stained hippocampal slices taken from young and old wt and SOD mice with anti DCX antibodies. Figure 3 shows the extensive specific labeling of new neurons found in both wt and SOD young adult dorsal hippocampus (Figs. 3A and 3B). By comparison, old mice of both genotypes expressed an extremely low number of DCX-labeled neurons (Figs. 3C and 3D). Four sections from dorsal hippocampus (per mouse) of 4 young mice of either genotype and 6 old mice from either genotype were stained for DCX and the labeled cells bearing neuronal morphology were counted in each section in both hemispheres. Cells counted were those for which a distinct cell body was visible in stratum granulosum with ex-

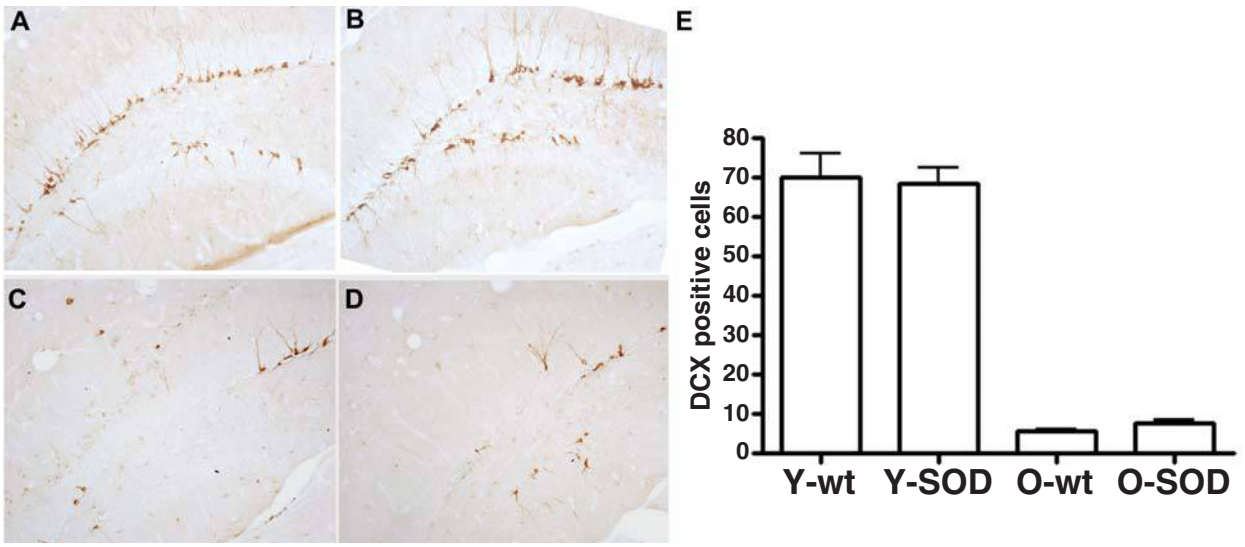

FIG. 3. Neurogenesis in DG granular layer. Coronal sections (16 μm) from young and old wt and SOD transgenic mice were immunostained using antibodies raised against doublecortin (DCX). The stained neurons are new neurons that are within the first 3 weeks of differentiation. (**A**) young wt, (**B**) young SOD, (**C**) old wt, (**D**) old SOD. *Bar* = 500 μm. The quantified results from four sections of 4 young and 6 old mice of wt and SOD genotypes are shown in (**E**). The graph shows the average number of DCX labeled cells in one unilateral section of the hippocampus.

tending neurites. Cells of similar size and morphology were evident across genotypes and ages. The average number of DCX positive cells in a hemisection was 70 ± 6, 68.5 ± 4, 5.5 ± 0.5, and 7.5 ± 1 cells for young wt, young SOD, old wt and old SOD, respectively (Fig. 3E). Two-way generalized linear model (GLM) revealed significant main effect of age [$F(1,16) = 63.277$, $p < 0.0001$] on neurogenesis. A considerable decrease of over 90% in the rate of neurogenesis is evident when comparing old sections of either genotype with the young ones. Both the effects of genotype [$F(1,16) < 1$] and the interaction between genotype and age [$F(1,16) < 1$] were not significant.

The total number of cells in the DG is not affected by the rate of neurogenesis

A 90% reduction in the rate of DG neurogenesis may affect the overall number of DG cells. We therefore attempted to determine whether a decrease in the overall cell number of proportional magnitude accompanied the decrease in neurogenesis. To estimate the number of neuronal cells in the DG, we stained adjacent sections to those stained for DCX with an antibody specific for the neuronal marker NeuN. We found no significant differences in the width of the granular layer of the DG (Fig. 4), there were no apparent differences in the density or size of DG granular layer cells across age groups and genotypes. This demonstrates that the dramatic difference seen in the rate of neurogenesis between old and young mice does not result in an overall reduction in the number of DG granular neurons.

Neurogenesis in aged mice does not correlate with spatial memory performance

We have demonstrated a marked reduction in neurogenesis in 2-year-old mice compared to young adults with a slightly higher level of neurogenesis measured for SOD mice as compared to wt. We have also demonstrated that old SOD mice were better than wt in their performance of spatial memory tasks. These findings may suggest that the improved behavior of the SOD mice is due to the slightly higher level of neurogenesis. We therefore attempted to correlate the average number of new cells in the hippocampal sections with the time spent in the correct quadrant on the probe trial—a quantitative measure of the spatial learning (Fig. 5). Interestingly, we found no significant correlation between the number of new neurons and the performance in the spatial learning task (Pearson correlation = 0.100955, $p > 0.781$). Although the average rate of neurogenesis was greater for the old SOD mice, the variability in this group was also larger (standard deviation wt = 2.3, SOD = 6.3). Consequently, both groups include mice with similar learning ability and very different neurogenesis rates, and this was more evident in the SOD group. This lack of correlation indicates that the better spatial performance exhibited by the SOD mice cannot be ascribed to a higher level of neurogenesis.

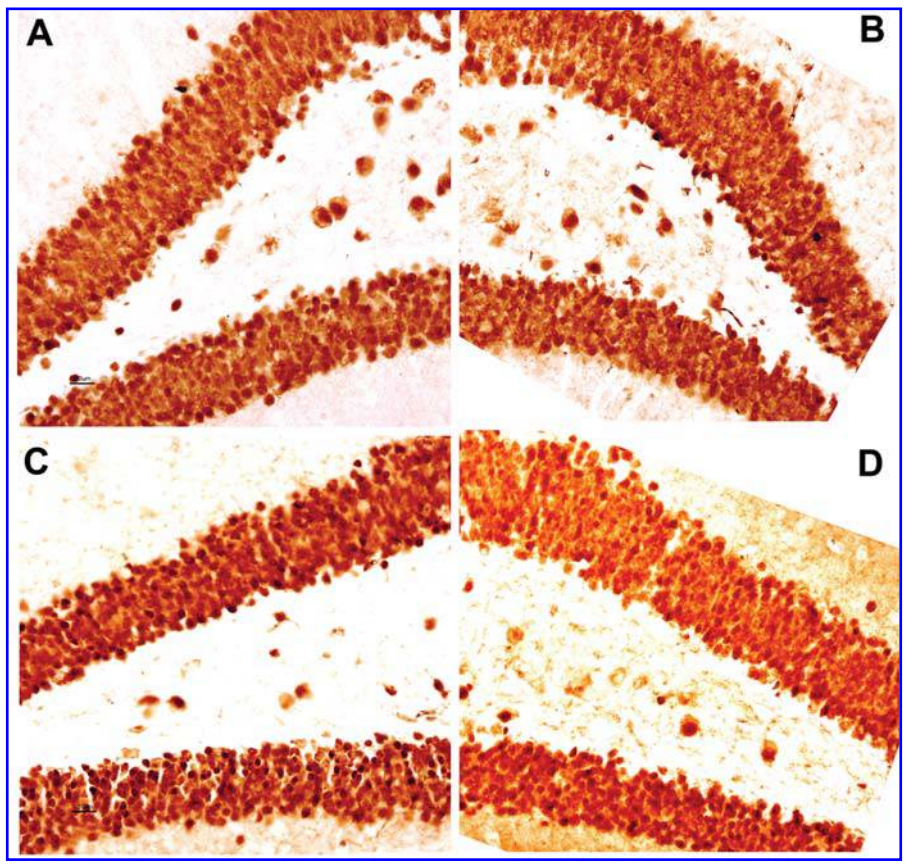

FIG. 4. Neuronal density in the DG. Coronal sections (16 μm) from young and old wt and SOD transgenic mice were immunostained using antibodies to the neuronal marker NeuN. No gross morphological alterations are seen as a consequence of age or genotype. (**A**) young wt, (**B**) young SOD, (**C**) old wt, (**D**) old SOD. *Bar*, 100 μm.

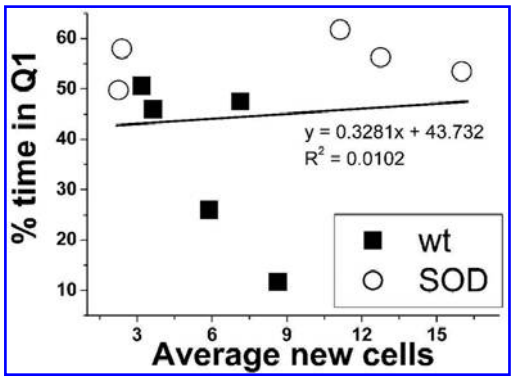

FIG. 5. Correlation between memory and neurogenesis in old mice. On the graph is plotted the rate of neurogenesis for each mouse as measured by the average number of cells counted in DCX stained sections as a function of performance in the water maze as measured by the percent of time spent in the "correct" quadrant of the water maze in the absence of the platform.

DISCUSSION

In the present study, we used young SOD overexpressing mice that have shown impairment in hippocampal LTP (17, 27) as well as aged wt mice that are also impaired in LTP. We have shown recently (21) that slices from old SOD mice expressed LTP that was *higher* than that shown for old wt slices and was similar to that shown for slices from young wt mice. We hypothesized that the ability of SOD slices to express LTP derived from the combined effect of age-dependent mitochondrial breakdown with the transgenically driven overproduction of H_2O_2. This combined effect allowed for transient H_2O_2 fluxes necessary for hippocampal synaptic plasticity. This possible mechanism may also underlie the ability of old SOD mice to learn the water maze task in the current study.

We show here that SOD and wt mice have a similar rate of neurogenesis in both old and young subjects. As shown previously by others (5), we find a dramatic 90% reduction in the rate of neurogenesis in 2-year-old wt and SOD mice. This reduction in the rate of neurogenesis is not accompanied by a reduction in the overall amount of granular neurons in the DG as seen in NeuN stained sections. The old SOD mice exhibited superior spatial learning performance in the water maze compared to wt controls. The performance in the water maze did not correlate with the rate of neurogenesis measured in the brains of these subjects, indicating that neurogenesis is probably not a crucial factor in hippocampus dependent tasks in the old animal.

The functional significance of adult neurogenesis is the subject of numerous current studies (12). The recent finding that aging is associated with a drastic reduction in the rate of neurogenesis is especially intriguing in light of the high rate of neurogenesis found in young adults (8), predicting a function for the new neurons. Indeed, at least two studies were conducted looking into functional consequences of the decline in the rate of neurogenesis, with opposite results (13, 32). Some studies have shown that neurogenesis rate does not correlate with success in learning the water maze (30, 42). Still others reported that such a correlation does exist (3, 13, 23, 29, 34, 38, 43, 46). Our finding supports the view that adult neurogenesis is an interesting phenomena that is probably not necessary for execution of spatial memory functions in the hippocampus. We do, however, suggest a critical role for the management of ROS as a factor in age-dependent cognitive decline.

ABBREVIATIONS

BrDU, bromo deoxyuridine; DCX, doublecortin; DG, hippocampal dentate gyrus; LTP, long-term potentiation; ROS, reactive oxygen species; SOD, superoxide dismutase.

REFERENCES

1. Akman C, Zhao Q, Liu X, and Holmes GL. Effect of food deprivation during early development on cognition and neurogenesis in the rat. *Epilepsy Behav* 5: 446–454, 2004.
2. Altman J and Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124: 319–335, 1965.
3. Ambrogini P, Cuppini R, Cuppini C, Ciaroni S, Cecchini T, Ferri P, Sartini S, and Del Grande P. Spatial learning affects immature granule cell survival in adult rat dentate gyrus. *Neurosci Lett* 286: 21–24, 2000.
4. Barnes CA. Aging and the physiology of spatial memory. *Neurobiol Aging* 9: 563–568, 1988.
5. Bondolfi L, Ermini F, Long JM, Ingram DK, and Jucker M. Impact of age and caloric restriction on neurogenesis in the dentate gyrus of C57BL/6 mice. *Neurobiol Aging* 25: 333–340, 2004.
6. Brown JP, Couillard–Despres S, Cooper–Kuhn CM, Winkler J, Aigner L, and Kuhn HG. Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol* 467: 1–10, 2003.
7. Bruel–Jungerman E, Laroche S, and Rampon C. New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur J Neurosci* 21: 513–521, 2005.
8. Cameron HA and McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol* 435: 406–417, 2001.
9. Cameron HA and McKay RD. Restoring production of hippocampal neurons in old age. *Nat Neurosci* 2: 894–897, 1999.
10. Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, and During MJ. VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 36: 827–835, 2004.
11. Cui X, Zuo P, Zhang Q, Li X, Hu Y, Long J, Packer L, and Liu J. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-alpha-lipoic acid. *J Neurosci Res* 84: 647–654, 2006.
12. Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, and Malenka RC. Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* 42: 535–552, 2004.
13. Drapeau E, Mayo W, Aurousseau C, Le Moal M, Piazza PV, and Abrous DN. Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. *Proc Natl Acad Sci USA* 100: 14385–14390, 2003.
14. Driscoll I, Howard SR, Stone JC, Monfils MH, Tomanek B, Brooks WM, and Sutherland RJ. The aging hippocampus: a multi-level analysis in the rat. *Neuroscience* 139: 1173–1185, 2006.
15. Epstein CJ, Avraham KB, Lovett M, Smith S, Elroy–Stein O, Rotman G, Bry C, and Groner Y. Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome. *Proc Natl Acad Sci USA* 84: 8044–8048, 1987.
16. Eriksson PS, Perfilieva E, Bjork–Eriksson T, Alborn AM, Nordborg C, Peterson DA, and Gage FH. Neurogenesis in the adult human hippocampus. *Nat Med* 4: 1313–1317, 1998.

17. Gahtan E, Auerbach JM, Groner Y, and Segal M. Reversible impairment of long-term potentiation in transgenic Cu/Zn-SOD mice. *Eur J Neurosci* 10: 538–544, 1998.
18. Gross CG. Neurogenesis in the adult brain: death of a dogma. *Nat Rev Neurosci* 1: 67–73, 2000.
19. Hu D, Serrano F, Oury TD, and Klann E. Aging-dependent alterations in synaptic plasticity and memory in mice that overexpress extracellular superoxide dismutase. *J Neurosci* 26: 3933–3941, 2006.
20. Jaako–Movits K and Zharkovsky A. Impaired fear memory and decreased hippocampal neurogenesis following olfactory bulbectomy in rats. *Eur J Neurosci* 22: 2871–2878, 2005.
21. Kamsler A and Segal M. Paradoxical actions of hydrogen peroxide on long-term potentiation in transgenic superoxide dismutase-1 mice. *J Neurosci* 23: 10359–10367, 2003.
22. Kawai T, Takagi N, Miyake–Takagi K, Okuyama N, Mochizuki N, and Takeo S. Characterization of BrdU-positive neurons induced by transient global ischemia in adult hippocampus. *J Cereb Blood Flow Metab* 24: 548–555, 2004.
23. Kempermann G and Gage FH. Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. *Eur J Neurosci* 16: 129–136, 2002.
24. Kim H, Lee MH, Chang HK, Lee TH, Lee HH, Shin MC, Shin MS, Won R, Shin HS, and Kim CJ. Influence of prenatal noise and music on the spatial memory and neurogenesis in the hippocampus of developing rats. *Brain Dev* 28: 109–114, 2006.
25. Kleindienst A, McGinn MJ, Harvey HB, Colello RJ, Hamm RJ, and Bullock MR. Enhanced hippocampal neurogenesis by intraventricular S100B infusion is associated with improved cognitive recovery after traumatic brain injury. *J Neurotrauma* 22: 645–655, 2005.
26. Lee HH, Kim H, Lee JW, Kim YS, Yang HY, Chang HK, Lee TH, Shin MC, Lee MH, Shin MS, Park S, Baek S, and Kim CJ. Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. *Brain Dev* 28: 147–154, 2006.
27. Levkovitz Y, Avignone E, Groner Y, and Segal M. Upregulation of GABA neurotransmission suppresses hippocampal excitability and prevents long-term potentiation in transgenic superoxide dismutase-overexpressing mice. *J Neurosci* 19: 10977–10984, 1999.
28. Lu D, Mahmood A, Qu C, Goussev A, Schallert T, and Chopp M. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. *J Neurotrauma* 22: 1011–1017, 2005.
29. Lu L, Bao G, Chen H, Xia P, Fan X, Zhang J, Pei G, and Ma L. Modification of hippocampal neurogenesis and neuroplasticity by social environments. *Exp Neurol* 183: 600–609, 2003.
30. Madsen TM, Kristjansen PE, Bolwig TG, and Wortwein G. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. *Neuroscience* 119: 635–642, 2003.
31. McDonald HY and Wojtowicz JM. Dynamics of neurogenesis in the dentate gyrus of adult rats. *Neurosci Lett* 385: 70–75, 2005.
32. Merrill DA, Karim R, Darraq M, Chiba AA, and Tuszynski MH. Hippocampal cell genesis does not correlate with spatial learning ability in aged rats. *J Comp Neurol* 459: 201–207, 2003.
33. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11: 47–60, 1984.
34. Nilsson M, Perfilieva E, Johansson U, Orwar O, and Eriksson PS. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J Neurobiol* 39: 569–578, 1999.
35. Pourie G, Blaise S, Trabalon M, Nedelec E, Gueant JL, and Daval JL. Mild, non-lesioning transient hypoxia in the newborn rat induces delayed brain neurogenesis associated with improved memory scores. *Neuroscience* 140: 1369–1379, 2006.
36. Raber J, Rola R, LeFevour A, Morhardt D, Curley J, Mizumatsu S, VandenBerg SR, and Fike JR. Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat Res* 162: 39–47, 2004.
37. Rao MS and Shetty AK. Efficacy of doublecortin as a marker to analyse the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. *Eur J Neurosci* 19: 234–246, 2004.
38. Rola R, Raber J, Rizk A, Otsuka S, VandenBerg SR, Morhardt DR, and Fike JR. Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. *Exp Neurol* 188: 316–330, 2004.
39. Scerri C, Stewart CA, Breen KC, and Balfour DJ. The effects of chronic nicotine on spatial learning and bromodeoxyuridine incorporation into the dentate gyrus of the rat. *Psychopharmacology (Berl)* 184: 540–546, 2006.
40. Serrano F and Klann E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Res Rev* 3: 431–443, 2004.
41. Shimazu K, Zhao M, Sakata K, Akbarian S, Bates B, Jaenisch R, and Lu B. NT-3 facilitates hippocampal plasticity and learning and memory by regulating neurogenesis. *Learn Mem* 13: 307–315, 2006.
42. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, and Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410: 372–376, 2001.
43. Snyder JS, Hong NS, McDonald RJ, and Wojtowicz JM. A role for adult neurogenesis in spatial long-term memory. *Neuroscience* 130: 843–852, 2005.
44. Ueda S, Sakakibara S, and Yoshimoto K. Effect of long-lasting serotonin depletion on environmental enrichment-induced neurogenesis in adult rat hippocampus and spatial learning. *Neuroscience* 135: 395–402, 2005.
45. Van Kampen JM, Hagg T, and Robertson HA. Induction of neurogenesis in the adult rat subventricular zone and neostriatum following dopamine D receptor stimulation. *Eur J Neurosci* 19: 2377–2387, 2004.
46. van Praag H, Christie BR, Sejnowski TJ, and Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA* 96: 13427–13431, 1999.
47. Wang R, Dineley KT, Sweatt JD, and Zheng H. Presenilin 1 familial Alzheimer's disease mutation leads to defective associative learning and impaired adult neurogenesis. *Neuroscience* 126: 305–312, 2004.
48. Wati H, Kudo K, Qiao C, Kuroki T, and Kanba S. A decreased survival of proliferated cells in the hippocampus is associated with a decline in spatial memory in aged rats. *Neurosci Lett* 399: 171–174, 2006.
49. Winocur G, Wojtowicz JM, Sekeres M, Snyder JS, and Wang S. Inhibition of neurogenesis interferes with hippocampus-dependent memory function. *Hippocampus* 16: 296–304, 2006.
50. Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, and Schwartz M. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci* 9: 268–275, 2006.

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3. Wei-Hua Lee , Ashok Kumar , Asha Rani , Jose Herrera , Jinze Xu , Shinichi Someya , Thomas C. Foster . 2012. Influence of Viral Vector–Mediated Delivery of Superoxide Dismutase and Catalase to the Hippocampus on Spatial Learning and Memory During Aging. *Antioxidants & Redox Signaling* **16**:4, 339-350. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Dae Young Yoo, Bich Na Shin, In Hye Kim, Woosuk Kim, Dae Won Kim, Ki-Yeon Yoo, Jung Hoon Choi, Choong Hyun Lee, Yeo Sung Yoon, Soo Young Choi, Moo-Ho Won, In Koo Hwang. 2011. Effects of Cu,Zn-Superoxide Dismutase on Cell Proliferation and Neuroblast Differentiation in the Mouse Dentate Gyrus. *Neurochemical Research* . [CrossRef]
5. Cynthia A. Massaad , Eric Klann . 2011. Reactive Oxygen Species in the Regulation of Synaptic Plasticity and Memory. *Antioxidants & Redox Signaling* **14**:10, 2013-2054. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Jacob Raber, Laura Villasana, Jenna Rosenberg, Yani Zou, Ting Ting Huang, John R. Fike. 2011. Irradiation enhances hippocampus-dependent cognition in mice deficient in extracellular superoxide dismutase. *Hippocampus* **21**:1, 72-80. [CrossRef]
7. Kelly Fishman, Jennifer Baure, Yani Zou, Ting-Ting Huang, Marta Andres-Mach, Radoslaw Rola, Tatiana Suarez, Munjal Acharya, Charles L. Limoli, Kathleen R. Lamborn, John R. Fike. 2009. Radiation-induced reductions in neurogenesis are ameliorated in mice deficient in CuZnSOD or MnSOD. *Free Radical Biology and Medicine* **47**:10, 1459-1467. [CrossRef]
8. Amy M. Hein, M. Kerry O'Banion. 2009. Neuroinflammation and Memory: The Role of Prostaglandins. *Molecular Neurobiology* **40**:1, 15-32. [CrossRef]
9. Dr. Ariel Kamsler , Menahem Segal . 2007. Control of Neuronal Plasticity by Reactive Oxygen Species. *Antioxidants & Redox Signaling* **9**:2, 165-167. [Citation] [Full Text PDF] [Full Text PDF with Links]