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# THE AGING HIPPOCAMPUS: A MULTI-LEVEL ANALYSIS IN THE RAT

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**Abstract—In the current experiment we conducted a multi-level analysis of age-related characteristics in the hippocampus of young adult (3 months), middle-aged (12 months), and old (24 months) Fisher 344×Brown Norway hybrid (FBNF1) rats. We examined the relationships between aging, hippocampus, and memory using a combination of behavioral, non-invasive magnetic resonance imaging and spectroscopy, and postmortem neuroanatomical measures in the same rats. Aging was associated with functional deficits on hippocampus-dependent memory tasks, accompanied by structural alterations observed both *in vivo* (magnetic resonance imaging-hippocampal volume) and postmortem (dentate gyrus neuronal density and neurogenesis). Neuronal metabolic integrity, assessed by levels of *N*-acetylaspartate with magnetic resonance spectroscopy, was however, preserved. Further, our results suggest that neurogenesis (doublecortin) seems to be related to both performance deficits on hippocampus-dependent tasks and hippocampal volume reduction. The observed pattern of age-related alterations closely resembles that previously reported in humans and suggests FBNF1 rats to be a useful model of normal human aging.** 

**Key words: FBNF1, Morris water task, transverse patterning, MRI, MRS, neurogenesis.**

Alzheimer's disease (AD), believed to be a main cause of age-related cognitive deficits, has devastating effects on the lives of patients and their caregivers. There is also a significant loss of quality of life and suffering associated with memory decline in the elderly not afflicted by one of the age-related dementias. These problems are steadily increasing in magnitude as the mean population age is on the rise. Before rational approaches to diagnosis and treatment can be developed, it will be essential to understand the healthy brain as a part of the normal aging process and as distinct from explicit disease or pathology.

Although some aspects of cognition are relatively immune to ill effects of aging, there is now consensus that certain aspects of learning and memory decline with age. The substrates of these age-related cognitive deficits remain uncertain, despite the recent proliferation in neuroscience techniques which has led to an accumulation of converging evidence on neural, physiological, and cognitive changes with aging (see Driscoll and Sutherland, 2005c for a review). Moreover, numerous reports on human, and just as many if not more on non-human animal aging are available, but despite the abundance of information there are clearly missed opportunities for the two domains to inform one another. One caveat in the aging literature rests on the fact that aspects of brain aging are often studied in isolation. Furthermore, tests used to assess learning and memory or to measure aspects of neural function or structure in humans are very different from those employed to test non-human animals making it hard to generalize or make cross-species comparisons.

Human studies have several important limitations, including the fact that a wide range of relevant genetic and environment factors is especially difficult to disentangle. A particular advantage of rodent studies is that the contributions of genetic and environmental factors can be investigated or more stringently controlled. One way to enhance the effectiveness of animal models in informing human and clinical approaches is to use animal and human tasks that permit an evaluation of similar cognitive processes across species and to evaluate in the animal model some of the noninvasive neuroimaging modalities commonly used in human clinical work. Bridging basic neuroscience and clinical discovery will continue to be extremely important for the development of new therapeutic regimens, especially given the sense of urgency in this area of research.

In order to address some of the questions that arose from human studies of aging, in our own work (Driscoll et al., 2003) and in general (Driscoll and Sutherland, 2005c), and to better describe the changes that occur with age at cellular and subcellular levels, we turn to an animal model of aging and propose a multi-level analysis in the rat. Here we focus our considerations on the hippocampus. This is not to say that the rest of the brain is spared by aging. Our focus is based on the fact that (1) some age-related cognitive changes, normal and pathological, have been linked to the hippocampal for-

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*Abbreviations:* ANOVA, analysis of variance; BrdU, bromodeoxyuridine; CA, cornu ammonis; Cho, choline; Cre, creatine; DCX, doublecortin; DG, dentate gyrus; EM, electron microscopy; FBNF1, Fisher 344×Brown Norway hybrids; IBD/NRC, Institute for Biodiagnostics/National Research Council of Canada; ICV, intracranial volume; MRI, magnetic resonance imaging; MWT, Morris water task; NAA, *N*-acetylaspartate; NIA, National Institute on Aging; PBS, phosphate-buffered saline; ROI, region of interest; TPD, transverse patterning discriminations; $V_{dis}$, disector volume of tissue; $^{1}$H MRS, proton magnetic resonance spectroscopy.

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mation (e.g. Geinisman et al., 1995), (2) there is now agreement that the hippocampus is critically involved in memory for certain types of information (Nadel and Moscovitch, 2001), and (3) the hippocampus has maintained a central role in memory research over the past 40 years in both human and non-human domains.

The main goal of the present study was to investigate the hippocampus more systematically to identify those changes that may be specifically related to deficits in hippocampus-dependent memory, which in turn would provide us with a future direction for identifying causes of age-related cognitive decline. Our secondary goal was to add to the development of a rat model of hippocampal aging that has established similarities to the changes seen in elderly humans. To that end we assessed hippocampal functional, biochemical, and structural integrity of Fisher 344×Brown Norway (FBNF1) hybrid rats, bred by the National Institute on Aging (NIA) for aging research purposes. We used memory tasks, noninvasive imaging, and stereology techniques on postmortem tissue that can be applied to both human and non-human animals.

In line with our human studies (see Driscoll and Sutherland, 2005c for a review), we employed the Morris water task (MWT; Morris, 1981; virtual MWT used in human studies, Driscoll et al., 2003) and transverse patterning discriminations (TPD; Alvarado and Rudy, 1995; Driscoll et al., 2003, 2004, 2005b for a computerized version used in human studies) to investigate hippocampus-dependent memory, and magnetic resonance imaging (MRI) and single voxel proton magnetic resonance spectroscopy ($^1$H MRS) to assess hippocampal structural and biochemical integrity in the same animals *in vivo*. In addition, we measured several cellular and subcellular features in postmortem hippocampal tissue in the same rats, using light and electron microscopy (EM) and immunohistochemical techniques. The strength of our study is in the fact that we measure multiple selected changes in the same tissue both *in vivo* and postmortem from systems to subcellular levels in the animals that have also been behaviorally characterized, using methods that closely resemble those used in human studies.

Specifically, we predict a deficit in performance on hippocampus-dependent tasks in the aged rats while the performance on hippocampus-insensitive tasks should be preserved. Given that hippocampal structural changes associated with aging are often reported in both human and nonhuman animals, we expected that they would be present in the aged FBNF1 rats and predict performance on hippocampus-dependent tasks. If truly a correlate of memory decline in aging humans (Driscoll et al., 2003) and a key component in normal hippocampal functioning, *N*-acetylaspartate (NAA) is expected to exhibit a similar pattern in the aged rats. Although adult neurogenesis has not been directly related to aging and memory deficits in humans, it has been suggested to decline with aging and hence we expect not only a reduction but also a relationship to memory deficits.

## EXPERIMENTAL PROCEDURES

### Animals

Thirty FBNF1 female rats (NIA/Harlan, Indianapolis, IN, USA) were divided into three groups: Young adult, Middle-aged, and Old, 10 rats in each group. Rats were three (250–300 g), 12 (350–400 g), and 24 (375–450 g) months of age respectively at the start of the experiment. Rats were weighed upon the entrance into the colony and again approximately 3 months later (prior to bromodeoxyuridine (BrdU) injections) and remained within 10% of their body weight during this time. In addition, the rats were monitored by the animal colony care staff for any visible signs of weight loss. They were maintained at the University of Lethbridge vivarium, housed in pairs in hanging plastic cages in a room with an ambient temperature of 21 °C, 35% relative humidity, 12-h light dark cycle, food and water available *ad libitum*. All testing procedures were carried out at the University of Lethbridge, except MRI and spectroscopy, which were conducted at the Experimental Imaging Centre, University of Calgary.

All rats were free of cataracts. One old rat died during the MRI scanning procedures, had extensive brain damage due to stroke, and was removed from analyses. Two more rats died of old age at different points in the study. These two rats did not show any signs of illness during behavioral testing, and following death a necropsy was performed by an on-staff veterinarian. The rats appeared to have been healthy and there were no signs of brain tumors, hence the rats were not excluded from behavioral analysis. All rats were cared for by an animal care technician and two research assistants at all times. The staff was responsible for feeding, watering, grooming (teeth and nails), and general health monitoring of the animals during and in between testing. All procedures involving animal care and experimentation were carried out in accordance with guidelines provided by the National Institutes of Health and approved by the institutional animal care committees at the University of Lethbridge and the University of Calgary. Care was taken to minimize the number of animals used, as well as their pain and suffering.

### General procedure

The rats were delivered to the University of Calgary where the MRI/MRS procedures were carried out following a week of acclimation. Rats were then transferred to the University of Lethbridge, acclimated and handled for two weeks prior to behavioral testing. One month following behavioral testing, all rats were injected with BrdU and allowed to survive for five weeks, at which point they were killed and their brains extracted. One hemisphere from each brain was committed to immunohistochemical and the other to EM procedures in a counterbalanced manner.

### MWT

The MWT (Morris, 1981) is a well-established test of hippocampus-dependent cognition. There is a deficit in performance on the standard version of this task after hippocampal damage (Morris et al., 1982; Sutherland et al., 1982) or in old age (Gage et al., 1984; Pelleymounter et al., 1987; Rapp et al., 1987; van der Staay, 2002). All rats were trained in a modified version of the MWT in which the platform moved to a new location every second day according to a random sequence of locations. For the first two days the platform remained in the same location. On day 3 the platform moved to a new location for another two days and so on until four locations were completed (8 consecutive days). Rats were pre-trained on the task for 2 days prior to data acquisition on a platform location that did not appear during actual testing. After completion of hidden platform training, we administered one day of visible platform training. Rats received eight trials on each day and were released twice from each cardinal compass point around the perimeter of the pool in a pseudorandom sequence. A maxi-