

Differential Effects of Age on Subpopulations of Hippocampal Theta Cells

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MIZUMORI, S. J. Y., C. A. BARNES AND B. L. McNAUGHTON. *Differential effects of age on subpopulations of hippocampal theta cells.* NEUROBIOL AGING 13(6) 673-679, 1992.—The possible contribution of age-related changes in the firing properties of hippocampal theta cells to spatial learning deficits was addressed in the present study. The behavioral correlates of theta cells in strata oriens, pyramidale, and granulosum were compared as young and old rats performed a radial maze spatial working memory task. Behaviorally, the old animals made significantly more errors on the maze and required more time to solve the task than did young animals. Firing rates were compared in four different locomotion states: still, running radially inward and radially outward, and forward motion. The discharge rates of theta cells in strata pyramidale and granulosum were significantly modulated by these movements in both age groups. Stratum oriens theta cells recorded from young animals, on the other hand, were not movement-sensitive, while similar cells from old animals demonstrated exaggerated responses to movement. In old animals, the mean discharge rates were higher in stratum granulosum and lower in stratum oriens than in the young rats. The discharge rates of cells in stratum pyramidale did not differ between age groups. These region specific changes in the firing characteristics of hippocampal theta cells are likely to have important consequences for information processing in this structure.

Interneurons Single units Spatial learning GABA

SPATIAL cognitive dysfunction is often observed with old age in humans (e.g., 12,52,54,57,66,67) and rodents (e.g., 9,24,30,59). Several age-related changes in neurobiological markers of hippocampal function are correlated with memory impairment in old animals. For example, correlations between choice accuracy and neurochemical changes (e.g., 16,30,31,56), electrophysiological changes (e.g., 6,8,17,19,38), and neuroanatomical changes (e.g., 27) have been made and these may be indicative of a functional relationship with spatial learning ability.

Single units recorded in hippocampus of freely-moving rodents tend to exhibit one of two discharge patterns. Hippocampal pyramidal cells are known under some behavioral conditions to fire high frequency bursts with declining spike amplitude. Hence, these cells are referred to as complex-spike (CS) cells (20,21,58). A second class of cells fires only single spikes and are typically most active in the presence of theta rhythm in the EEG. These theta cells are believed to correspond to one or more types of interneuron. Theta and CS cells differ in the duration of their extracellularly recorded action potentials. They also differ markedly in their behavioral correlates: CS cells exhibiting pronounced spatial selectivity, whereas theta cells exhibit, at best, poor spatial selectivity (36,43). The most frequently observed behavioral correlate of theta cells is an increase in firing during some kinds of loco-

motion. A minor class of cells, the so-called "theta-off" cells (13,14,15,20,46) show the opposite behavioral correlate, i.e., a reduction in firing in the presence of the theta rhythm and locomotion. These cells are rarely encountered in dorsal hippocampus and are not included in the present study.

Considerable electrophysiological evidence supports the identification of theta cells with inhibitory interneurons. Both exhibit relatively high spontaneous discharge rates, low thresholds for activation, and a relative absence of spike accommodation (21,25,32,47,48,60,61). Also, nonpyramidal (theta) cells are involved in both feedforward and recurrent inhibitory pathways mediated by GABA (1,2,13,18,35,47,64,68).

Proper regulation of the hippocampal inhibitory system is critical for normal hippocampal function. GABA antagonists may induce aberrant electrical activity in hippocampus and also spatial learning deficits (4).

Therefore, it is conceivable that age-related alterations in theta cell function contribute to the spatial memory impairment typically observed in old animals. Of interest in this regard is the recent finding by Banay-Schwartz et al. (5) that the hippocampus of old rats contain less GABA than young rats. Also, somatostatin (a neuropeptide that colocalizes with GABA in nonpyramidal cells (63) has been shown to be reduced in elderly humans (11). Cytochemical changes alone, however, are not sufficient to determine the functional state of

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inhibitory networks: Barnes (6) did not find age-related changes in paired-pulse inhibition of the population field response in the fascia dentata.

This study tested the hypothesis that age-related changes in theta cell function contribute to the spatial performance deficit of old animals. Recently, Lavoie et al. (39) reported that, although the firing rates of strata granulosum and pyramidale theta cells are significantly modulated as animals move through their environment, the firing rates of stratum oriens theta cells are not. This regional variation in the behavioral correlates of hippocampal theta cells was taken into consideration in this analysis of theta cell activity recorded as young and old rats performed a spatial maze task.

METHOD

Subjects

Male, Fischer-344 rats (retired breeders) were obtained from Harlan Sprague-Dawley Laboratory at 9 months (mature young) and 24 months (old) of age. The animals were allowed to adapt to laboratory conditions for at least 2 weeks before testing began. During this time, food and water were provided ad lib. Lights were on in the colony room from 7 a.m. to 7 p.m., and recordings were carried out during the early part of the light cycle. For maze training, access to food was restricted such that the body weights of the rats were maintained at about 80% of their ad lib weights. The animals were singly housed throughout the experiment. Units were recorded at approximately 11 and 26 months of age. Both young and old rats were part of a larger investigation of age-related changes in medial septal influences on hippocampal neural activity. The cells described in this report were recorded before the septal manipulations.

Behavioral Apparatus

The animals were trained to perform multiple trials daily on an 8 arm radial maze (53). The maze consisted of a round central platform (19.5 cm) from which eight arms (58 cm \times 5.5 cm) radiated outward. A food cup was located at the distal end of each arm. The maze was elevated 79 cm above the floor and was open except for 0.5 cm rails attached to the sides of each arm. To control access to food reward, each arm was hinged such that the proximal portion could be lowered or raised flush with the central platform. Control of maze arms was accomplished by a remote switch. The maze was situated in a room (420 cm \times 420 cm) which contained several items that could serve as extramaze visual cues, such as a ladder, table, chair, and an experimenter. A single 60 W light mounted on one wall provided room illumination.

Behavioral Training

All rats underwent maze training before surgical implantation of electrodes. The training protocol proceeded essentially as described by Mizumori et al. (49). At the beginning of a trial, four randomly selected arms were presented simultaneously to the rat (sample phase). After the rat obtained chocolate milk reward from these arms, it was restricted to the central platform for 1 min. Following this delay period, all eight arms were presented simultaneously (memory phase). The rat's task was to select those arms that had not yet been chosen that trial. Re-entries into arms previously visited that trial were counted as errors. A 2 min period intervened between trials. Rats were trained to perform 10 trials within 1 h for 3 consecutive days. Following acquisition of this criterion, free access to food was

permitted for 1–2 days. Recording electrodes were then surgically implanted. Following recovery from surgery, animals performed 15 trials daily on the maze.

Surgical Procedures

Rats were initially anesthetized with 33 mg/kg Nembutal (50 mg/ml) followed by supplements of 0.05 ml as needed. Small burr holes were drilled in the skull to permit placement of electrodes at the following stereotaxic coordinates (55): A-P -4.0 mm posterior to bregma and ± 2.5 mm lateral to the midsagittal suture. Electrodes were initially placed 1 mm into the neocortex overlying dorsal hippocampus. The final recording depth was determined in relation to stratum pyramidale which could be identified clearly on the basis of electrophysiological recording. A reference electrode was placed in corpus callosum (A-P -6.5 mm; L 2.0 mm). A ground lead was soldered to a jewelers screw attached to the skull. Because subjects of this study also participated in tests of reversible septal inactivation effects on hippocampus, guide cannulae were implanted to permit local application of tetracaine (for further details regarding tetracaine manipulations, see ref. 48). Septal cannulae and recording sites were comparable between age groups. Dental acrylic was used to anchor the recording assembly to the skull. Amphenol pin connectors attached to each electrode were inserted into a connecting socket (50) that was permanently fastened to the rat's head. After surgery, the eyes were washed with ophthalmic solution and 0.1 ml Bicillin was administered into each hindleg.

Unit Recording Procedure

Electrode construction. Single units were recorded using the stereotrode method (44). Specifically, unit activity was independently monitored on two adjacent lacquer-coated tungsten wires (25 μ m dia; California FineWire), which were twisted together, dipped in EpoxyLite, and then baked. The electrode tips were gold plated to give final impedances to 100–200 k Ω (tested at 1 kHz). Each stereotrode was threaded through 30 ga. stainless steel tubing, then mounted on a moveable microdrive (42). Reference electrodes consisted of 114 μ m dia Teflon-coated stainless steel wire (A–M Systems).

Single unit monitoring. A headstage was attached to the connecting socket on the rat's head for all postsurgical maze training sessions. The headstage was comprised of 5 FETs and an infrared light-emitting diode for behavioral monitoring (48). The incoming signals of the stereotrode pair of wires (X and Y) were processed independently. They were amplified 5–10 K, then filtered (at half amplitude) at 600–800 Hz (high pass) and 6 kHz (low pass). The analog signals were subsequently passed through a window discriminator such that a 1 msec sampling period began when a signal from either channel surpassed a predetermined threshold. The maximum and minimum voltages of each signal, as well as the latencies of these values from the onset of the sampling period, was calculated in hardware (FMZ Electronics Co.), then passed on to a PDP-11 computer for storage and offline data analysis.

Unit identification and analysis. Single units identified as theta cells typically exhibited relatively short duration single spikes (200–300 μ sec; measured as the time between points of maximum and minimum voltages of a signal). These cells often exhibited relatively high spontaneous discharge rates with an absence of spike accommodation. Those units recorded within 100 μ m of complex-spike cells were classified as stratum pyramidale theta cells. Theta cells found more than 100–200 μ m dorsal to complex-spike cells were classified as

TABLE 1
MEAN (\pm SE) SPIKE WAVEFORM AND FIRING RATE CHARACTERISTICS OF THETA CELLS
IN YOUNG AND OLD RATS

Age/Cell Type	<i>n</i>	Amplitude (μ V)	Duration (μ seconds)	Mean Rate (HZ)	Maximum Rate (HZ)
Young					
s. granulosum	24	83.9 (4.2)	326.9 (28.2)	7.6 (1.6)	73.3 (6.1)
s. oriens	15	88.9 (6.3)	263.1 (22.7)	9.5 (2.3)	67.0 (11.9)
s. pyramidale	11	72.8 (8.8)	228.8 (12.3)	13.7 (3.8)	82.1 (12.6)
Old					
s. granulosum	20	148.6* (28.3)	282.2 (21.6)	16.3* (3.8)	100.0 (22.3)
s. oriens	10	145.3* (30.2)	263.4 (16.8)	5.7* (2.2)	33.5 (14.3)
s. pyramidale	11	109.5 (30.7)	267.7 (29.2)	16.8 (4.4)	97.3 (19.7)

*Significantly different from young, $p < 0.05$

stratum oriens theta cells while those units recorded 100–500 μ m ventral to complex-spike cells were considered stratum radiatum cells. Units encountered near the granular layer were classified as stratum granulosum theta cells.

Many theta cells increase discharge when an animal engages in voluntary movement (e.g., 58,65). Movement-sensitive discharge was evaluated by calculating average firing rates as the animal remained still at the end of the maze arms, turned around 180° to face the central platform from the arm end, moved forward inward on maze arms, crossed the central platform, and moved forward outward on maze arms. The mean rate in each behavioral condition was compared between age groups. Also, the ratio of mean rate during a particular movement condition to the mean rate during the still condition was determined. This ratio normalizes the absolute firing rates of the cells, and provides an index of the extent to which all cells within a group showed similar proportional changes in rate during movement. Given that not all theta cells exhibit clear movement-sensitivity, this was not a requirement for classification as a theta cell.

RESULTS

At the time of unit recording, the animals were performing at peak levels. The young rats ($n = 6$) made 0.45 errors (± 0.08) per trial while the old rats ($n = 5$) made 1.83 errors (± 0.73) per trial. This difference was statistically significant, $t(9) = 2.07$, $p < 0.05$. Young rats also performed the task more quickly than old rats, $t(9) = 2.48$, $p < 0.02$. On average, the young animals took 79.0 (± 5.4) s to complete a trial, while the old rats required 113.8 (± 14.9) s.

A total of 44 stratum granulosum (young $n = 24$; old $n = 20$) and 49 CA1 theta cells (young $n = 26$; old $n = 23$) were recorded from 6 young and 5 old rats. Thus, an average of 8.3 and 8.6 cells were recorded per young and old rat, respectively. Cells in the CA1 group were divided into strata oriens and pyramidale categories. Twenty-five units were classified as oriens cells (young $n = 15$; old $n = 10$), 22 were pyramidale cells (young $n = 11$; old $n = 11$), and 2 units were classified as radiatum cells. Because both stratum radiatum cells were found in old rats, they were excluded from subsequent age comparisons. The mean spike amplitudes, durations, and firing rates of theta cells recorded from young and old rats are presented in Table 1. In addition, the maximum firing rate for any 100 msec period was calculated and can also be found in Table 1.

Mean spike amplitude was significantly larger overall for both stratum granulosum and stratum pyramidale theta cells of

old animals, $F_{\text{granulosum}}(1, 43) = 6.53$, $p < 0.02$ and $F_{\text{pyramidale}}(1, 48) = 4.89$, $p < 0.05$. The age difference in spike amplitude of CA1 cells is likely due to a change in stratum oriens cells since a significant effect of age on spike amplitude was found for these cells, $F(1, 24) = 4.67$, $p < 0.05$, and not stratum pyramidale cells. The mean discharge rate of stratum granulosum units was significantly greater in old animals, $F(1, 43) = 7.60$, $p < 0.05$. Age effects were also observed for the mean firing rates in stratum oriens, $F(1, 43) = 5.29$, $p < 0.05$ but not in stratum pyramidale.

There were significant region-selective age effects on the behavioral correlates of CA1 and stratum granulosum theta cell discharge. Figure 1 shows the mean firing rates as functions of different behaviors on the maze. A significant effect of age and movement were found for stratum granulosum theta cells, $F_{\text{age}}(1, 42) = 6.18$, $p < 0.02$ and $F_{\text{movement}}(4, 42) = 14.92$, $p < 0.001$ (two-factor ANOVA, repeated measures); however, there was no significant interaction effect. CA1 theta cells as a group (i.e., oriens and pyramidale combined) showed a significant effect of behavior for both young and old rats, $F_{\text{young}}(4, 25) = 5.94$, $p < 0.001$ and $F_{\text{old}}(4, 22) = 3.28$, $p < 0.02$; however, there was no age effect nor behavior by age interaction effect. When the CA1 group was divided into stratum oriens and pyramidale subpopulations (Fig. 2), it was found that stratum pyramidale cells of both young and old animals were sensitive to movement, $F_{\text{young}}(1, 10) = 6.45$, $p < 0.001$ and $F_{\text{old}}(1, 10) = 6.81$, $p < 0.001$ while oriens cells were not.

While the mean rate data provide an index of the population response to movement, a change in firing by a high rate cell will have a greater impact on the mean value than a similar percentage change by a low rate cell. Therefore, the rate data were normalized by dividing the movement rates (i.e., rates during turns, inward movement, traversals of the central platform, and outward movement) by the rate during the still condition (i.e., when the rat remained still at the arm ends). For statistical analysis, these ratio scores were transformed to logarithms. A two-factor ANOVA performed on the resultant log ratios (Fig. 3) revealed a significant overall effect of movement on stratum granulosum cells, $F(3, 42) = 38.21$, $p < 0.001$. No effect of age on movement sensitivity was observed. That is, stratum granulosum cells recorded from both young and old animals were movement sensitive, $F_{\text{young}}(3, 23) = 6.70$, $p < 0.001$ and $F_{\text{old}}(3, 19) = 10.65$, $p < 0.001$.

In contrast to stratum granulosum cells, the log ratios of CA1 theta cell rates exhibited a significant age effect, $F(1, 47) = 4.92$, $p < 0.05$. However, there were neither movement effects nor age by movement interaction effects. Given that the

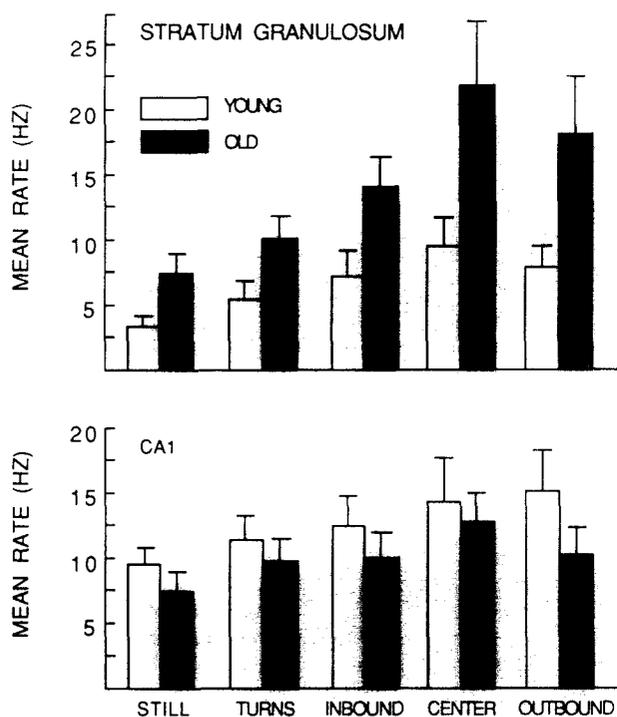


FIG. 1. Mean (\pm SE) discharge rates of stratum granulosum and CA1 (strata oriens, pyramidale, and radiatum combined) theta cells recorded from young and old animals as they performed a maze task. Mean rates were calculated as a function of specific behaviors exhibited by the rat on the maze. Specifically, firing rates were determined when the rat remained STILL at the arm ends, and during 180° TURNS at the arm ends, forward movement INBOUND on maze arms, traversals of the CENTER platform, and forward movement OUTBOUND on maze arms. The firing rates of stratum granulosum and CA1 theta cells varied significantly as a function of behavior for both young and old animals. However, the overall mean firing rate of stratum granulosum theta cells of old animals was higher than similar cells recorded from young animals.

strata oriens and pyramidale cells were differentially sensitive to movement (Fig. 2), it is possible that these CA1 subpopulations of cells differentially contributed to the total CA1 response during movement. Thus, the log ratios were analyzed individually for strata oriens and pyramidale theta cells. Stratum oriens theta cells of old rats exhibited an exaggerated response to movement compared to similar cells recorded from young animals, $F(1, 23) = 6.91, p < 0.02$ (Fig. 4), which showed no significant effect of behavior. Interestingly, the maximum movement effect in old rats was observed during turning behavior at arm ends. This stands in contrast to the pattern of movement effects for most other categories of theta cells. Stratum pyramidale cells from both young and old rats showed significant modulation by movement, $F_{\text{young}}(3, 10) = 3.05, p < 0.01$ and $F_{\text{old}}(3, 19) = 11.29, p < 0.01$. No significant age effects were observed for stratum pyramidale cells.

DISCUSSION

The present study addressed the possibility that age-related changes in the activity of hippocampal interneurons (theta cells) contributes to the spatial memory impairments of old animals. Theta cells recorded from strata oriens and pyramidale of CA1, and stratum granulosum of young and old rats were

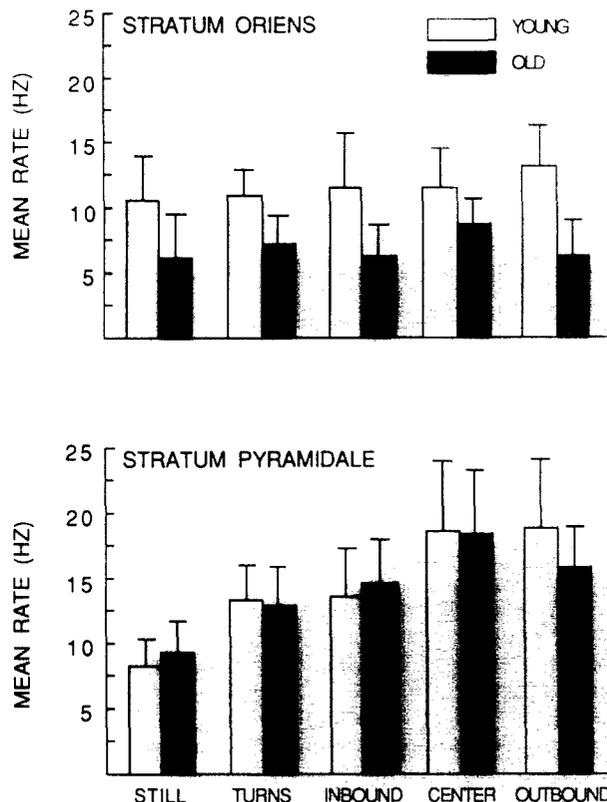


FIG. 2. The mean rates of CA1 strata oriens and pyramidale theta cells recorded as young and old animals engage in different behaviors on the maze. Stratum oriens cells were not significantly modulated by behavior for either young and old age groups. The overall mean rate, however, was lower for stratum oriens cells of old animals. Stratum pyramidale theta cells of both young and old rats demonstrated significant movement-sensitivity.

assessed for their sensitivity to particular behaviors exhibited by the rat during traversal of the 8-arm maze. The mean firing rate of stratum granulosum theta cells was significantly greater for old animals even though there was no age difference in the movement sensitivity of these cells. On the other hand, while the mean rate of stratum oriens theta cells was significantly lower in the old rats, movement sensitivity was significantly greater. Finally, there were no differences in the behavioral correlates of stratum pyramidale theta cell activity; both age groups showed significant modulation by movement.

Previous studies have demonstrated a positive relationship between velocity and firing rate of most movement-sensitive theta cells (43,46). Therefore, one explanation for the observed group differences in stratum oriens theta cells might be that old animals traversed the maze more rapidly than did the young animals. Instead, the old animals of this study required significantly more time to solve the maze than did young animals, and their overall velocity was about 20% slower. Thus, velocity per se does not adequately explain the results.

Although at present there is no explanation for the observed age effects, it is possible that changes in one or more afferent systems might be involved. For example, stratum granulosum interneurons receive primarily GABAergic input from the medial septum (22,23,40), an inhibitory influence from the supra-mammillary nucleus (29,47), and both noradrenergic and serotonergic inputs from locus coeruleus and dorsal raphe,

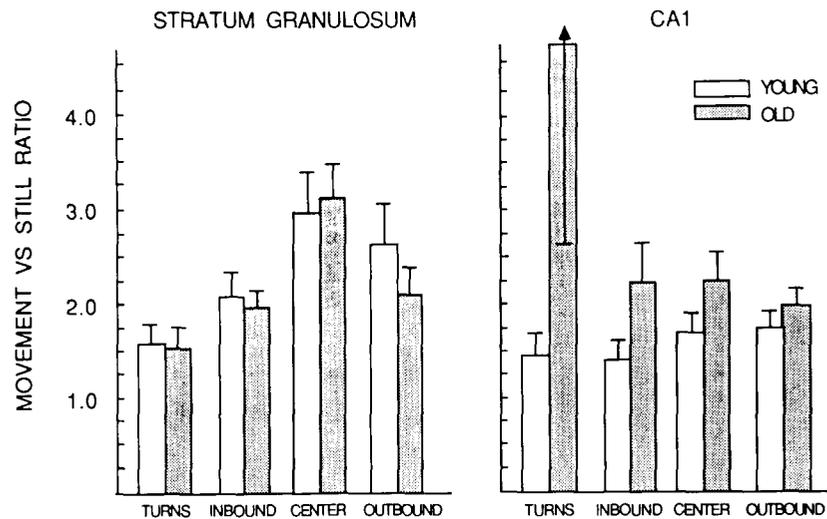


FIG. 3. The mean rates of stratum granulosum and CA1 theta cells were normalized by dividing the rates during a behavior by rates during the still condition. A significant change was observed in the movement versus still ratio for stratum granulosum theta cells of both young and old animals. CA1 theta cells (including both strata oriens and pyramidale cells) of old rats were more responsive to movements than similar cells recorded from young animals.

respectively (3,41). Alteration of any of these inputs might account for the relatively accelerated rates in old animals. Indeed, Gilad et al. (28) have reported a reduction in the number of medial septal neurons in old rats. Paradoxically, there is a reduction in the number of synapses carrying excitatory input to the dentate gyrus from the entorhinal cortex and medial septum in old rats (26,28) from which one might predict an overall decrease in firing rates of both granule cells and interneurons.

Alternatively, changes in intrinsic connectivity might also underlie the present results. For example, GABA_A receptors of stratum granulosum interneurons apparently mediate excit-

atory coupling between interneurons (45), which could change with age. In addition, dye-coupling experiments have revealed an increase in gap junctions between hippocampal principal cells in old rats (10). Nonpyramidal cells also apparently form gap junctions (62). Increased coupling among theta cells in old rats is thus another possible explanation for the increased firing rates of stratum granulosum theta cells.

Reversible inactivation of the septum eliminates the movement modulation of theta cells without affecting the (non-movement) baseline rates (48). Given that no effect of age on the movement-sensitivity of strata granulosum and pyrami-

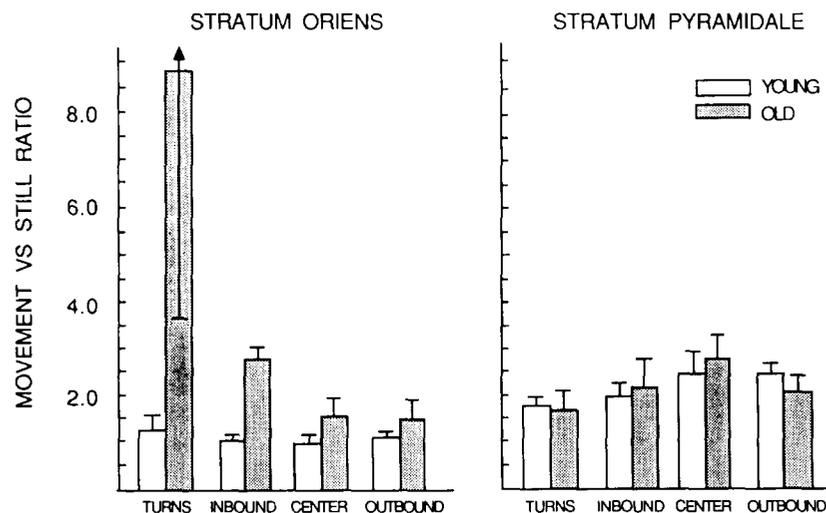


FIG. 4. Movement versus still ratios are presented for strata oriens and pyramidale subpopulations of CA1 theta cells. When compared to young animals, enhanced responsiveness to movement by oriens theta cells of old rats was observed. In contrast, no age differences in movement sensitivity were observed for stratum pyramidale theta cells, although both age groups showed significant movement-induced increase in firing rates.

dale theta units was observed in the present study, it appeared that the movement contribution of the septohippocampal pathway is largely intact in old, memory-impaired animals. This interpretation is consistent with that of Barnes et al. (7) who showed that there was no age difference in the extent to which prestimulation of the medial septal area facilitated the perforant path-induced population spike in the dentate gyrus.

The age-related changes in CA1 interneurons were specific to the stratum oriens. This regionally-selective age effect may be related to the different types of theta cell found in oriens and pyramidale. Stratum oriens interneurons are cytochemically distinct from those in stratum pyramidale: oriens cells are reactive to somatostatin while pyramidale cells are not (33). Pyramidale interneurons, on the other hand, concentrate substances (such as the calcium-binding protein parvalbumin) which are not found in stratum oriens (34,51). Although it is not known whether parvalbumin or somatostatin undergo age-related changes in rat, somatostatin has been found to be reduced in elderly humans (11). As in stratum granulosum, the lack of an age-difference in the movement-sensitivity of stratum pyramidale theta cells suggests that their medial septal

input is functionally intact in old rats. Finally, stratum oriens cells, which are generally not movement-sensitive in young rats (39), exhibited an exaggerated response to movement in the old rats of the present study. There is insufficient information on the connectivity of these cells to permit reasonable speculation as to the cause of this age effect.

Although the pattern of regional variation in interneuron firing characteristics observed in the present study undoubtedly has a substantial impact on the information processing characteristics of the hippocampal formation, it remains to be determined whether these effects are themselves detrimental or might reflect compensatory changes that moderate the effects of deterioration in other pathways. A paradox, which will require further examination, is the fact that that age effects do not appear in inhibitory responses in dentate gyrus or CA1 following electrical activation of afferent pathways (6,7,37).

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