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PHYSIOLOGICAL COMPENSATION FOR LOSS OF AFFERENT SYNAPSES IN RAT HIPPOCAMPAL GRANULE CELLS DURING SENESCENCE

BY CAROL A. BARNES AND BRUCE L. MCNAUGHTON

From the Institute of Neurophysiology, University of Oslo, Oslo 1, Norway and the Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1*

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SUMMARY

1. The effects of senescence on the input-output characteristics of the perforant path projection to granule cells of the fascia dentata were studied in rats using extracellular techniques *in vivo*, and both extra- and intracellular recording *in vitro*.

2. Senescent animals exhibited a significant reduction in the perforant path excitatory synaptic field potential at all stimulus intensities tested. This was associated with a reduction in the size of the afferent fibre response, although there was no apparent change in the threshold for fibre activation. These data support the anatomical literature which indicates a loss of afferent synapses with advanced age.

3. For a given magnitude of afferent fibre response, however, the old animals exhibited a larger synaptic field potential, suggesting that the remaining synapses were in fact more powerful. Furthermore, the magnitude of the extracellular population spike, an index of the number of discharging granule cells, was greater in the old animals when plotted as a function of extracellular e.p.s.p. amplitude.

4. Intracellular recording from a total of 190 granule cells in the transverse hippocampal slice preparation revealed a 17 % reduction in the voltage threshold for synaptically elicited granule cell discharge, and a 13 % reduction in the latency of the action potential in old compared to young rats. Resting potentials, action potential amplitudes, whole neurone time constants, the relations between applied current and input resistance, and the discharge threshold following depolarizing current pulses, were not different between age groups.

5. These data indicate that granule cells could partly compensate for a loss of synapses during senescence by an increase in their electrical responsiveness to synaptic activation and possibly by an increase in synaptic efficacy.

INTRODUCTION

While a variety of behavioural, neuroanatomical and neurophysiological alterations are known to be associated with the process of senescence, a full understanding of the functional implications of these changes has been hampered by the difficulty

* Present address of both authors.

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of correlating physiology and anatomy in most higher centres of the nervous system. The fascia dentata of the hippocampal formation represents a relatively simple cortical structure. Its regular laminated geometry, the accessability of several distinct synaptic projection systems, and the relative uniformity of cell type make the fascia dentata a highly suitable model system for physiological and anatomical study and comparison. The present work was undertaken to investigate the effect of aging on the input-output characteristics of the perforant path – granule cell components of this structure, in attempt to relate these functional characteristics to known anatomical alterations that occur during senescence (e.g. Geinismann & Bondareff, 1976).

One of the most significant anatomical changes in the senescent fascia dentata is the loss of afferent synapses in the molecular layer. Whereas the number of granule cells and the thickness of the molecular layer appear to remain quite constant throughout the lifespan in the rat (Bondareff & Geinisman, 1976; Geinisman, Bondareff, & Dodge, 1977), Geinisman & Bondareff (1976) have found a 27% reduction in the number of synaptic contacts within the termination zone of the perforant pathway (comparing 3 month with 25 month old rats). This loss of synapses could result in a substantial change in granule cell output and integrative capabilities. The decrease in number of synapses with age may be associated with dendritic atrophy in rats (Geinisman, Bondareff & Dodge, 1978), although there is some controversy as to the extent of this atrophy in the rat (Barnes & McNaughton, 1979), and as to whether dendritic atrophy or growth dominates in human granule cell dentrites (Buell & Coleman, 1979; Scheibel, Lindsay, Tomiyasu & Scheibel, 1976). It is not clear from the anatomical literature whether the reduction of synapses is due to a reduction in the number of afferent fibres *per se*, or in the number of en passant synapses made by each fibre. It appears, however, that the space vacated by the loss of neuronal elements from the molecular layer becomes occupied by astrocytes (Landfield, Rose, Sandles & Wholstadter, 1977).

In apparent agreement with these anatomical changes, Barnes (1979) found a reduction in the magnitude of the granule cell population e.p.s.p. for a given perforant path stimulus strength in awake, unrestrained old rats. Paradoxically, however, there was no change in the stimulus current required to elicit granule cell discharge. Thus the senescent granule cells exhibited a larger population spike for a given magnitude of extracellular e.p.s.p. However, since the recording in Barnes' study was extracellular, several alternative explanations for these observations remained open.

The present study replicates and extends the extracellular observations both in intact animals and in the hippocampal slice preparation, and provides at least a partial explanation for these phenomena. In addition we provide some normative data on the electrical excitability and synaptic transmission in granule cells which extends the preliminary intracellular observations of a number of workers (Andersen, Holmqvist & Voorhoeve, 1966; Assaf & Kelly, 1979; Dudek, Deadwyler, Cotman & Lynch, 1976; Lømo, 1970).

METHODS

Subjects

The *in vivo* and *in vitro* studies reported here were carried out in two different populations of rats. The *in vivo* studies employed male Long Evans rats of the Charles River strain obtained from Quebec Breeding Farms Ltd, Canada (thirty-one rats aged 12 months; thirty rats aged 30 months). For the *in vitro* studies, barrier reared Wistar albino rats bred by TNO-Zeist, The Netherlands, were used (sixteen aged 10 months; sixteen aged 31 months). Since the expected life of these rats is approximately 3 years, the old rats were near the end of their lifespan while the young rats had lived one third their expected lives.

Extracellular studies

In vivo. The methods used for chronic implantation of recording and stimulating electrodes are described in detail in Barnes (1979). Surgery was carried out using sodium pentobarbitone (Nembutal) general anaesthesia. Both electrodes consisted of 114 μm o.d. Teflon-coated stainless-steel wire. The recording electrode was positioned in the attached (i.e. upper) blade of the dorsal fascia dentata immediately below the granule cell body layer. This was accomplished by monitoring multiple unit injury discharge during penetration (McNaughton & Barnes, 1977). The stimulating electrode was located in the angular bundle so as to maximize the perforant path response as described by McNaughton & Barnes (1977). The locations of the electrodes were verified histologically at the end of the experiment.

Electrophysiological measures were taken in the awake freely moving state 1 month after surgery. The procedure for testing was as follows. The stimuli consisted of balanced diphasic square-wave constant-current pulses. With a pulse width set at 10 μsec (half cycle) the current was adjusted to a level just above the threshold for a detectable extracellular e.p.s.p. (young $\bar{X} = 295\ \mu A \pm 25$; old $\bar{X} = 297\ \mu A \pm 18$). Stimulus–response curves were then obtained by increasing the pulse duration in steps of 10 μsec from 20 to 250 μsec. The interval between each increase in stimulus intensity was 15 sec. The entire series was repeated 10 times, and the responses at corresponding stimulus levels were averaged by digital computer.

The field e.p.s.p. was measured at a fixed latency of 2 msec after stimulus onset. This provides a valid measurement of the relative e.p.s.p. amplitude between age groups since it has been shown previously (Barnes & McNaughton, 1979) that the field e.p.s.p. rise times are unaffected by senescence. At the latency used, the field e.p.s.p. measurement was not contaminated by the presence of a population spike. The magnitude of the population spike, a measure of the number of discharging granule cells (Andersen, Bliss & Skrede, 1971), was taken as the area under the tangent line joining the onset and offset. Previous studies have shown this measurement to correlate highly with height measurements, but to be somewhat less variable (Barnes, 1979). These analyses were performed automatically by digital computer.

In vitro. The methods used for the *in vitro* studies were similar to those used by Skrede & Westgaard (1971). Rats were killed with an overdose of ether, the brain was removed, and the hippocampi were rapidly dissected free. Transverse slices approximately 440 μm thick were cut on a Sorvall tissue chopper and were incubated in a standard chamber at 33 ± 1°C. The perfusion fluid contained the following (concentrations in mM): NaCl 124; $CaCl_2$ 2; KCl 2; KH_2PO_4 1·25; $MgSO_4.7H_2O$ 2; $NaHCO_3$ 26; glucose 10. The slices were allowed to equilibrate for 1·5 hours before electrophysiological measurements were begun. The data were obtained in a 'semi-blind' fashion in these experiments inasmuch as the experimenter who selected which slice to record from, and who made the actual measurements, did not know the group to which the animal under study belonged. The data were decoded only after all experiments were completed and all measurements recorded.

Two recording pipettes filled with 4 M-potassium acetate were used (5–10 MΩ) for extracellular recording. One was positioned in the middle of the molecular layer, and the other immediately below it in the layer of granule cell bodies. Synaptic responses were elicited via an etched tungsten stimulating electrode located in the molecular layer approximately 500 μm from the recording electrodes, and at the same depth as the dendritic recording pipette. The records obtained from the molecular layer permitted measurement of the presynaptic fibre potential (Andersen *et al.* 1966; Lømo, 1971) and the negative-going extracellular e.p.s.p. at the site of

synaptic activation. The electrode in the granule layer recorded the positive-going extracellular e.p.s.p. at the major current source, as well as the population spike. The signals from both electrodes were simultaneously sampled by digital computer at a rate of 10 points/msec. The procedure for stimulus–response determination was similar to that employed for the intact preparations with the exception that in approximately half the cases stimulus pulse height was manipulated rather than pulse width. Since the results of these two methods were comparable, the data were pooled. The amplitude of the population e.p.s.p. was taken at a latency of 0·8 msec following its onset. The magnitude of the presynaptic fibre response was taken as the difference in height between prestimulus base line voltage and peak, since the onset was often obscured by the stimulus artifact.

Intracellular studies

In vitro. Intracellular recordings from granule cells were obtained from the same slices as the extracellular measures. Recording micropipettes were filled with 4 M-potassium acetate and had a resistance at 1 kHz of 80–120 MΩ. Afferent fibres were activated via the same stimulating electrode as used for extracellular studies. Resting membrane potentials were referred to the DC level recorded when the electrode was at the surface of the slice. Immediately after penetration of the cell, resting potentials were invariably low. Under the influence of 0·4–0·6 nA of hyperpolarizing current passed through the recording electrode, these typically improved steadily towards a stable maximum which was attained between 3 and 7 min following initial penetration. The measures reported here were taken after the resting potential had stabilized and hyperpolarizing current had been removed. Cells were rejected from the study if the height of the synaptically elicited action potential was less than 50 mV from onset to peak in the absence of applied current.

A battery of tests was administered to each acceptable cell in the following order. (1) Measurement of action potential amplitude from onset to peak, and the base-width. (2) Measurement of the resting potential. (3) Measurement of the peak amplitude of the e.p.s.p. at the threshold for synaptically elicited granule cell discharge. (4) Measurement of the rheobasic current (defined as the maximum amount of current that could be applied without granule cell discharge on four out of five consecutive current pulses). (5) Current–voltage relations were determined using 100 msec square-wave pulses delivered in a randomized series between the rheobasic current and approximately −1·0 nA. At each current level the average of five voltage transients was recorded. Bridge balance was monitored continuously and adjusted as necessary. Apparent time constants were determined as the time required for the voltage transient following a −0·2 nA current pulse to reach 2/e of its steady value measured at 90 msec following pulse onset. (6) Measurement of the effect of negative and positive steady current levels from −0·8 nA to approximately rheobase on the magnitude of the e.p.s.p. These data were used to compute the apparent reversal potential for the e.p.s.p.

The entire battery required between 40 and 55 min for all tests to be completed. A total of ninety-six young and ninety-four old granule cells meeting our criterion were tested. In some cases the penetration was lost before completion of the series, so that the number of cells contributing to each average measurement varied. These numbers will be specified in the results.

RESULTS

Extracellular

The amplitude of the field e.p.s.p. as a function of stimulus intensity is shown in Fig. 1 for the *in vivo* and the *in vitro* preparations (Fig. 1 *A*, *D* respectively). In both experimental conditions the young animals exhibited a larger extracellular synaptic response than the old animals for a given stimulus intensity. The waveforms of the extracellular responses are shown in parts *B*, *D*, *E* and *F* of Fig. 1. The asterisks indicate the position of the negative-going population spike superimposed on the field e.p.s.p. and the dashed lines indicate the time at which the field e.p.s.p. amplitude was measured.

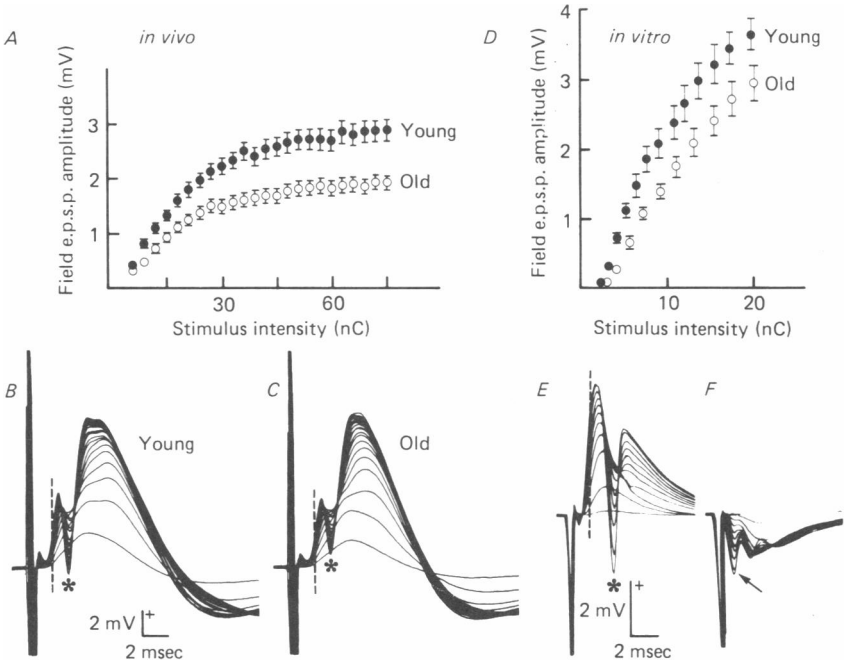

Fig. 1. The relation between field e.p.s.p. amplitude and perforant path stimulus intensity in young (●) and old (○) rats is shown for the *in vivo* (*A*) and *in vitro* (*D*) preparations. For comparison, stimulus intensity is expressed as the product of current and duration since these two parameters were varied differently in the two preparations (see Methods). For the *in vivo* experiment, response wave forms were averaged across animals within the young (*B*) and old (*C*) groups. Superimposed traces at the various stimulus levels recorded simultaneously from the granule layer (*E*) and the molecular layer (*F*) are shown from a single slice preparation. The dashed lines in *B*, *C* and *E* indicate the time of measurement of the field e.p.s.p. (2 msec after stimulus onset). The sharp negative deflexions in *B*, *C*, and *E* are population spikes (asterisks) whereas the early negative deflexion in *F* represents the presynaptic fibre response (arrow).

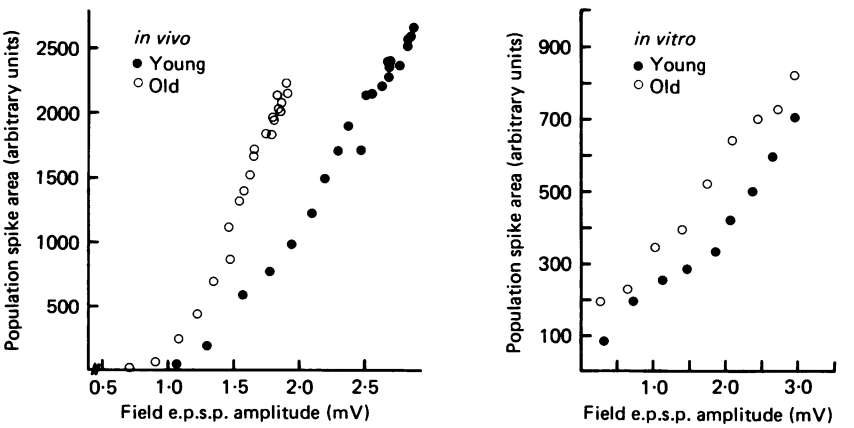

Fig. 2. Relationship between the mean amplitude of the population spike (the area under the tangent line joining onset and offset) and the mean amplitude of the extracellular e.p.s.p. recorded from the granule cell body layer in young (●) and old (○) rats. The old animals show more granule cell discharge for a given field e.p.s.p. amplitude in both the awake and hippocampal slice preparations.

Fig. 2 shows the relationship between the magnitude of the population spike and the amplitude of the extracellular e.p.s.p. recorded in the granule layer. In both experimental conditions the old animals exhibited a greater population spike for a given field e.p.s.p. amplitude.

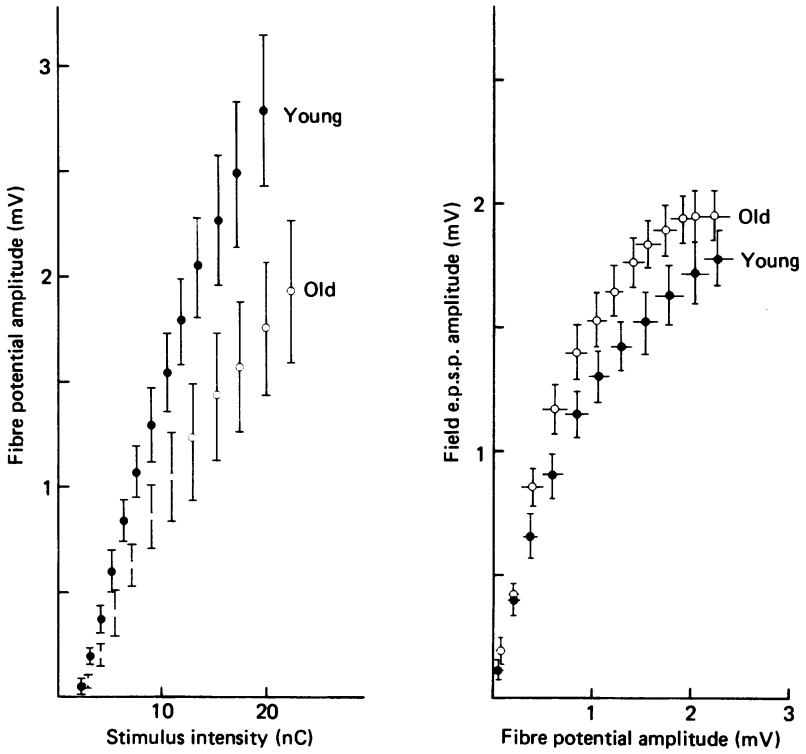

Fig. 3. *A*, relationship between the amplitude of the fibre response (the compound action potential of the perforant path fibres) and stimulus strength recorded in the molecular layer of the fascia dentata in sixteen young (●) and sixteen old (○) rats *in vitro*. While the threshold stimulus for the fibre response is equivalent between age groups, the young animals show a larger response at stimulus intensities above threshold. *B*, relationship between the fibre response amplitude and the field e.p.s.p. amplitude recorded as in *A*. For a given fibre response amplitude the old animals show a larger synaptic response.

Fig. 3*A* shows the relationship *in vitro* between the magnitude of the fibre response and stimulus strength (the arrow in Fig. 1*F* indicates the position of the fibre response). Although both curves extrapolate to the same threshold stimulus (intercept young and old = 2 nC) at higher stimulus levels, the young animals consistently exhibited a larger fibre response. Since the threshold for activation of the perforant path fibres is the same for both age groups, the difference at higher levels of stimulation is not likely to be due to a difference in the excitability of individual fibres, but rather to different numbers of fibres being excited. The ratio of the field e.p.s.p. recorded in the molecular layer to the corresponding fibre potential amplitude is plotted as a function of stimulus strength in Fig. 3*B*. These data show that for a

given fibre response the old animals actually had a larger synaptic response. In twenty slices (twelve young and eight old) an input–output curve was obtained comparing the amplitude of the intracellular e.p.s.p. with the magnitude of the fibre response recorded in the molecular layer. While the variance in these data was much greater than that for extracellular data (due partly to the smaller n), the mean

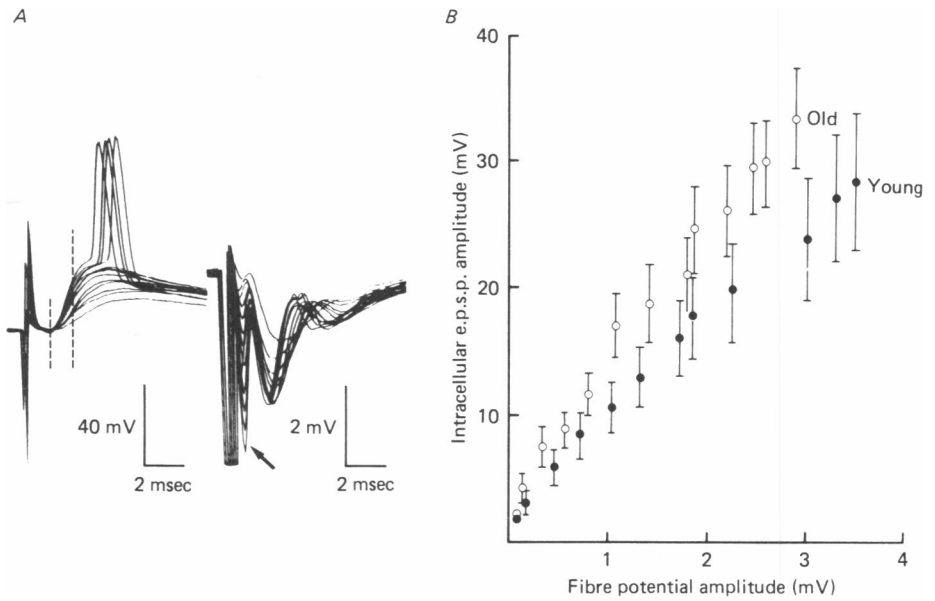

Fig. 4. *A*, effect of increasing the stimulus intensity on the intracellularly recorded granule cell response, and on the extracellular response recorded simultaneously in the molecular layer. Note from this record that the latency of the action potential in the intracellular record corresponds well with the latency of the population spike in the extracellular record. Note also that the fibre potential (arrow) is not contaminated by directly activated action potentials in the granule cells. This was true of all acceptable cells from which intracellular recordings were obtained. The dashed lines indicate the points from which the e.p.s.p. amplitude measures were taken. *B*, relationship between intracellular e.p.s.p. amplitude and the extracellularly recorded fibre potential amplitude recorded from young (●) and old (○) rat slices.

amplitude of the intracellular e.p.s.p. for a given fibre response was nonetheless greater in all cases in the old slices (see Fig. 4). Furthermore the magnitudes of the differences between age groups were comparable between the extracellular and intracellular experiments (that is, the percentage difference between the mean values for this relation between age groups ranged from 4 to 16 % in the extracellular case, and 3–20 % in the intracellular case).

Intracellular

The age group comparisons for the intracellularly recorded data (see Methods) are summarized in Table 1. Except for those measures which were significantly different between age groups ($P < 0{\cdot}05$), the pooled means will be reported here. Individual group means can be seen in Table 1.

Activation of fibres in the molecular layer with low intensity stimuli resulted in a monophasic e.p.s.p. with an average peak latency (measured from apparent e.p.s.p. onset) of 4·9 msec, and width at half amplitude of 10·0 msec. These measures were taken from the e.p.s.p.s immediately below cell discharge threshold. With increasing stimulus intensities, the granule cells discharged a single action potential. Typically,

TABLE 1. Summary of intracellular response characteristics of granule cells from young (Y) and old (O) rats. Asterisks denote statistically significant differences between age groups and n the number of cells contributing to the mean value

		Mean	S.E. of mean	n	% Change
Resting potential (mV)	Y	−68·2	±0·9	96	
	O	−66·9	±1·0	94	−2
Spike height (mV)	Y	62·7	±0·7	96	
	O	62·4	±0·8	94	−5
Spike width (msec)	Y	1·27	±0·02	88	
	O	1·27	±0·02	87	0
Spike latency (msec)	Y	3·92	±0·17	94	
	O	3·40	±0·11	92	−13*
Spike undershoot (mV)	Y	14·5	±0·6	94	
	O	13·8	±0·7	92	−5
E.p.s.p. peak latency (msec)	Y	4·99	±0·23	94	
	O	4·68	±0·20	92	−6
E.p.s.p. half width (msec)	Y	10·6	±0·7	94	
	O	9·5	±0·9	92	−10
Orthodromic threshold (mV)	Y	24·1	±0·9	94	
	O	20·0	±1·0	92	−17*
Direct threshold (mV)	Y	20·0	±1·2	86	
	O	18·3	±1·3	75	−9
Input resistance (MΩ)	Y	55·3	±2·9	84	
	O	53·8	±3·4	77	−3
Time constant (msec)	Y	11·2	±0·5	82	
	O	11·3	±0·6	70	1
Reversal potential (mV)	Y	−18·9	±1·4	66	
	O	−18·2	±2·0	62	−4

these action potentials had their onset near the peak of the e.p.s.p, where the rate of change of membrane voltage was close to zero. Although the resting potentials were not different between age groups (−68 mV) the sizes of the e.p.s.p.s at discharge threshold in the old group were significantly less than in the young rats (20·0 and 24·1 mV respectively). Also, the latency of the action potential at threshold was reduced in the old group (old 3·40 msec; young 3·92 msec). The height (63 mV) and base widths (1·27 msec) of the action potentials were not different between groups. Examples of above and below threshold responses from the two age groups are shown in Fig. 5.

Further increases in the stimulus intensity resulted in an increase in the amplitude of the e.p.s.p. and a decrease in the latency of the action potential (see intracellular example in Fig. 4*A*). Even at the highest stimulus intensities multiple discharge was rare, occurring in only seven of 190 stable cells. Interestingly, six of these cells were from the old group. Multiple discharges of 2 or 3 spikes, however, were more frequently

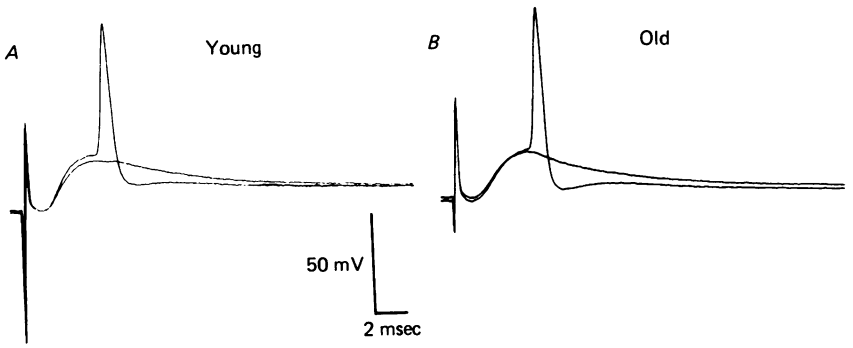

Fig. 5. Representative intracellular responses from granule cells in the fascia dentata elicited by perforant path stimulation. In each example a just subthreshold response is shown superimposed on a just suprathreshold response. Note that the action potential is initiated at the peak of the e.p.s.p., and is followed by a pronounced hyperpolarizing afterpotential ('spike undershoot').

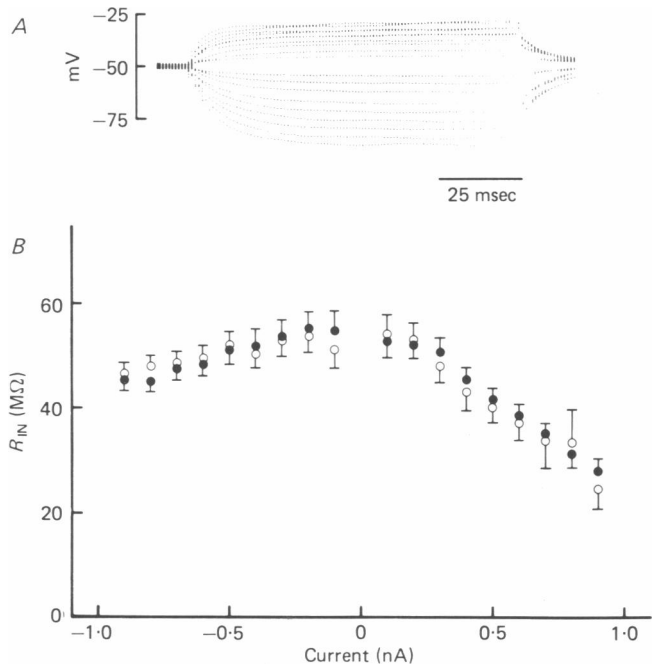

Fig. 6. *A*, representative examples of the voltage transients recorded from granule cell somata during 100 msec square wave current pulses applied through the recording pipette. Pulse amplitudes ranged from −0·9 nA up to cell firing threshold, and were delivered in a randomized order. The balance of the bridge circuit was continuously monitored and adjusted as necessary. *B*, relationship between the mean input resistance and applied current in young (●) and old (○) rats. While this relationship was quite linear in some cells, no cells showed significant increase in input resistance with depolarizing current pulses. There were no statistically significant differences between age groups.

observed immediately after penetration, before the resting potential stabilized at its maximal value. Fast prepotentials or 'D spikes' similar to those reported by Spencer & Kandel (1961) for CA1 pyramidal cells were observed in 22 % of the granule cells in this study. As with pyramidal cells, these could frequently be unmasked when the full action potential was blocked by hyperpolarizing the soma. There was no difference in the frequency of appearance of these D spikes between age groups.

With stimulation slightly above threshold, action potentials were followed in all cases by brief hyperpolarizing afterpotentials. These were 10–15 mV in amplitude and lasted approximately 4 msec. This spike undershoot was equally pronounced whether the spike was elicited synaptically or with applied depolarizing current pulses. The amplitude of the hyperpolarizing afterpotential was taken as the difference in voltage at the onset of the action potential and the peak of the undershoot. The two groups did not differ significantly (see Table 1) on this measure.

The relation between input resistance and applied current strength (Fig. 6) was very similar between the young and old rats. The membrane resistance was maximal near resting potential and decreased both with depolarizing and hyperpolarizing current pulses. There were no cases of the subthreshold anomalous rectification reported by Hotson, Prince & Schwartzkroin (1979) in CA1 pyramidal neurons. Measurement of the rate of change of membrane voltage following a −0·2 nA current step yielded a mean whole neuron time constant of 11·3 msec. This was reduced at higher current intensities, primarily due to the fall in input resistance.

Because of the membrane rectification the membrane potential could not be shifted sufficiently in the depolarizing direction to achieve complete reversal of the e.p.s.p. Nevertheless, it was possible, with applied steady current, to manipulate the membrane potential within a mean range of −106 and −49 mV. Within this range, a highly linear relationship between membrane potential and e.p.s.p. magnitude was observed (the mean r^2 values for the young and old groups were 0·924 and 0·920 respectively). The extrapolated regression lines showed no differences in the mean apparent reversal potentials (see Table 1).

The term 'apparent reversal potential' is used since the values are derived by extrapolation, and more importantly since the change in membrane potential recorded at the soma was undoubtedly greater than the actual change at the site of synaptic activation in the dendrites. Since steady current was used in the determination of the apparent reversal potential there is a possibility that our measurements were affected by changes in the internal ion concentrations due to iontophoresis of acetate or potassium from the recording pipette. If such an artifact is present, its contribution is probably small since the various levels of hyperpolarizing and depolarizing current were presented in a randomized order, and a given level was never prolonged more than the several seconds required to collect one response. Also, in control experiments, the amplitude of the e.p.s.p. was observed to remain constant when steady hyperpolarizing currents of up to 0·5 nA were applied for periods of up to 30 min. In addition, the balance of the DC bridge was checked periodically between current levels to ensure accurate reading of the voltage level.

DISCUSSION

Old animals exhibited significantly smaller field e.p.s.p.s and afferent fibre responses at a given suprathreshold stimulus intensity. These differences were not due to a change in the activation threshold of the afferent fibres. Although it is possible that

the reduction in the fibre responses in old animals may be due to differences in the sizes of the individual action potentials, it is rather unlikely that such a change could occur in the absence of some change in the excitation threshold. Thus, the most likely explanation appears to be a reduction in the number of afferent fibres in the molecular layer with senescence. This conclusion is certainly consistent with the decrease in the number of synapses in this region observed in the electronmicroscopical study of Geinisman & Bondareff (1976). It should be pointed out here that the differences in field responses observed between age groups are unlikely to be artifacts arising from changes in tissue impedance with age since such a change would have led to differences in apparent response thresholds as well. These were not observed. Furthermore, the extracellular and intracellular data were in agreement.

Although the field e.p.s.p. at a given stimulus level was reduced in old animals, the ratios of the field and intracellular e.p.s.p.s to fibre potential were nevertheless increased. This observation suggests that the afferent fibres in old animals are more effective on the average. The possible interpretations of this phenomenon are discussed below.

A clear change occurs in the excitability characteristics of the granule cells with senescence. In both experimental conditions, the old animals exhibited a larger population spike for a given population e.p.s.p. amplitude. The intracellular data confirm the suggestion that the difference is due to a recuction in the granule cell discharge threshold following synaptic activation. One possible mechanism for this effect is that the area of electrical excitability could expand to include more of the proximal dendritic tree during senescence. Such an expansion would explain both the discrepancy between orthodromic and direct threshold differences (Table 1) and the observed reduction in the spike latency in the old animals. If the spike trigger zone were located closer to the site of synaptic activation, both the spike onset latency and the apparent discharge threshold, as seen from the soma, would be reduced. This could occur in the absence of change in the threshold to direct activation at the soma using intracellular current pulses. This explanation is compatible with the results of Jefferys (1979). He showed, using extracellular current source density analysis in hippocampal slices, that the initial portion of the granule cell dendritic tree is in fact electrically excitable, although generally somewhat less so than the soma.

In the present experiment, the relation between applied current and input resistance, and whole neuron time constant were virtually identical between age groups. Nor were there any differences in the waveform characteristics of the e.p.s.p. This is in agreement with a previous report (Barnes & McNaughton, 1979) which showed that the passive cable properties of the granule cell dendrites do not alter significantly during senescence.

It seems probable that the loss of a significant fraction of the excitatory input to the granule cells would disrupt their capacity to process or to store information unless this was compensated for in some way. The lowering of the granule cell discharge threshold reported here could be viewed as one such compensatory mechanism if it were assumed that preservation of normal function required the maintenance of a constant discharge probability in the granule cells. The advantage of this form of compensation would be that both the relative and the absolute weightings of the remaining synapses in the control of granule cell output would tend to be preserved.

If this reasoning is correct, however, it is clear that the 17% reduction in discharge threshold is not sufficient to compensate completely for the approximately 33% loss of afferent fibres. Some subsidiary mechanism would still be required.

Our results show that, on the average, the afferent fibres in the old animals generate approximately 13% larger synaptic responses than in the young. Although it is possible that the increase in average strength could be due to a selective loss of weaker elements, we consider the explanation to be unlikely since it implies that all of the synapses made by a given fibre are either strong or weak. Thus, the increase in the ratio of synaptic response to fibre response probably represents a true strengthening of the remaining elements. As such, it could provide the additional compensation necessary to maintain a constant discharge probability in the partially deafferented granule cells of the senescent brain.

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