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Functional characterization of cutaneous mechanoreceptor properties in aged rats

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Abstract

We investigated the effects of aging on rapidly (RA) and slowly adapting (SA) cutaneous mechanoreceptors by means of single fiber recordings and evoked sensory nerve action potentials (EAPs) of the hindpaw of the N. plantaris in adult and old Wistar rats. EAPs revealed comparable shapes and amplitudes in all animals of all age groups. In old rats, conduction velocities were slightly (15%) lengthened. The mechanoreceptor composition was different from adults, resulting in a lower number of SA units. We were not able to detect significant differences in the sizes of receptive fields and in the thresholds between old and adult animals. The absence of significant age-related changes in the cutaneous periphery of the hindpaw is discussed in respect to the previously reported alterations of cortical receptive field properties in old rats.

Keywords: Rapid/slow adapting fibers; N. plantaris; Synaptic transmission; Mechanical thresholds; Conduction velocity; Somatosensory cortex; Receptive fields; Use-dependent plasticity

We have recently reported the impact of aging onto the cortical organization by analyzing receptive fields (RFs) and representational maps of the hindpaw of primary somatosensory cortex of rats older than 24 months [2,5,14,16]. We found an overall degradation of cortical functional organizations, reflected by a several-fold enlargement of RFs and by a breakdown of the topography of representational maps. In addition, cortical response latencies were up to 40% lengthened [5,16].

In order to interpret these changes in terms of either cortical contributions reflecting age-related modifications of the sensorimotor performance, or alternatively in terms of effects taking place already at the level of the sensory periphery, we re-examined the effects of aging on the properties of cutaneous mechanoreceptors of the hindpaw in rats older than 24 months.

In the glabrous skin of the hindpaw rapidly adapting receptors of the Meissner type, vibration sensitive Pacinian corpuscles and slowly adapting receptors of both Type I and Type II classes were described [3,7–9,12,17]. However, little is known about functional properties of receptive fields of the low threshold mechanoreceptors and their possible changes during aging [6,13]. We therefore compared receptive field sizes, thresholds and receptor compositions of cutaneous low threshold mechanoreceptors in old and normal adult animals. In addition, we studied conduction velocities by means of evoked whole nerve sensory action potential recordings to assess possible age-related changes of the conduction properties of peripheral nerves.

We recorded evoked sensory nerve action potentials and single fiber potentials of the N. plantaris in a total of 19 male Wistar rats of different age groups. Six old rats of 24–27 months of age and one 30 month old animal were compared to 12 adult animals of two age groups of 6 ($n = 6$) and 3 months ($n = 6$). Animals were anaesthetized with urethane (1.5 g/kg, i.p.) and held under urethane anesthesia during the entire course of the experiments. Treatment of all animals was within the regulations of the National Institution of Health Guide and Care for Use of Laboratory Animals (Rev. 1987) (for details of the experimental procedures see [11,14]).

The N. plantaris and the sciatic nerve were covered with

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warm mineral oil. Temperature was measured immediately before a recording session. The N. plantaris was electrically stimulated (bipolar silver wire electrodes, electrode distance about 2 mm, cathode proximal to the recording electrode, rectangular pulses of 0.1 ms duration) and the evoked nerve action potentials were recorded from the intact sciatic nerve (monopolar silver hook electrode, with currents twice the threshold values). Conduction velocities were corrected for a body temperature of 36°C according to Paintal [10].

For single fiber recordings (silver wire hook electrodes), the perineural sheath was removed and fine nerve filaments were isolated. The amplified signals were fed through a window discriminator and the output TTL signals were stored in a PC computer.

After isolation, single cutaneous units were qualitatively characterized by gentle brushing or tapping with a blunt rod to determine the corresponding RF (for details see [11]). To exclude recordings from others than cutaneous Aβ fibers (i.e. C-mechanoreceptors and β-mechanoreceptors), response latencies due to electrical stimulation of the RFs were additionally measured via thin needle electrodes inserted into the skin area of the RFs (duration, 0.1 ms; amplitude, 1–10 mA). Latencies of Aβ fibers were expected to be <2 ms. Units were classified according to their responses to sustained pressure as rapidly (RA) or slowly adapting (SA) using stimulus durations of 6–8 s delivered at a repetition rate of 10 s via an electromagnetic tactile stimulator, stimulus amplitude was 0.5–1 mm. A set of calibrated 'von Frey' hairs was used to estimate mechanical thresholds. RFs were mapped with a blunt rod (tip diameter 0.3 mm). Location and extension of the RFs were drawn on a figurine of the hindpaw.

For cortical recordings, action potentials were extracellularly recorded at depths of 600–800 microns using glass micro-electrodes (1–2 MΩ) filled with 3 M NaCl (for details of cortical electrophysiology see [14]). Cortical RFs were determined by hand-plotting in the way described for the mechanoreceptor RFs. For calculation of RF sizes, RF drawings were corrected for the individual footsize and planimetrically analyzed. Statistical analysis was performed using a battery of tests (GB-STAT 5.0). After the experiments, the animals were sacrificed by an overdose of barbiturate.

Table 1 summarizes the results obtained from evoked sensory nerve action potential recordings (EAPs). EAPs were of comparable shape and amplitude, with a smaller ongoing negative deflection followed by a large positive potential. Within each age group, the duration of the positive peak, its amplitude and the threshold of the nerve action potential varied widely, but were not significantly different. Conduction velocities in the 6 month old rats were slightly higher (minimum, 43.1; maximum, 52.0) than in the 3 month (minimum, 37.7; maximum, 48.5) and aged rats group (minimum, 35.2; maximum, 46.2). Differences between the 6 month and the 24–27 month old rats were significant (t-test, $P = 0.001$).

Using a single fiber preparation, we recorded the responses of SA and RA low threshold cutaneous mechanoreceptors to tactile stimulation of the hindpaw plantar surface. Using constant pressure, SA fibers showed ongoing activity during the entire period of stimulation. In contrast, in RA fibers sustained pressure evoked transient responses at the beginning and the end of the stimulation only (Fig. 1). No significant differences of response strength could be found within the three age groups. In old rats, the mechanoreceptor composition of the hindpaw glaborous skin was different from that found in normal adult animals. In the 24–27 month old rats, 29% of the recorded units ($n = 18$) were SA and 71% RA fibres ($n = 44$). In contrast, in the 6 month old rats, 49% were SA ($n = 32$) and 51% RA fibres ($n = 33$). About the same proportions were found in the 3 month old rats. The differences were significant for the old rat group compared to the 6 month old group (χ^2 test, $P = 0.02$). Occasionally encountered hair receptors were rejected ($n = 5$ of the total sample).

The RFs of the RA and SA units were located on the glaborous skin of the rat hindpaw. No differences were found concerning the RF locations in old rats compared to the normal adult animal group. RF locations of RA-fibers were predominantly found on the distal parts of the toes and on the central pads. The vast majority of the SA-fiber RFs were located on the pads and only few SA-fibers had RFs on the digits. In each age group, the RF sizes of the SA and RA units were separately calculated (Fig. 2). Examples of RFs are shown in Fig. 3. In general, RF sizes were in the range of 1–5 mm^2 skin area. Large receptive fields (≥6 mm^2) were found nearly exclusively among the RA-fibers ($n = 13$). It can be assumed that they

Table 1

Comparison of mean values (± SD) of peak amplitude (mV), duration (μs), electrical thresholds (μA), and conduction velocities of evoked nerve potentials in normal adult and old rats

Age	Peak (mV)	Duration (μs)	Threshold (μA)	CV_m (m/s)	CV_{36} (m/s)	Temperature (°C)
3 months	0.88 ± 0.32	499 ± 86	45.7 ± 29.3	39.2 ± 3.1	43.1 ± 4.1	34 ± 0.7
6 months	0.93 ± 0.47	455 ± 37.01	36.4 ± 26.8	41.6 ± 4.6	46.8 ± 3.6	33.32 ± 0.83
24–27 months	0.84 ± 0.46	590 ± 73.8	49.4 ± 21.7	37.1 ± 3.2	40.4 ± 4.0	34.2 ± 0.69

Conduction velocities (CV_m) are shown for a mean nerve temperature (°C) and corrected for a body temperature of 36°C (CV_{36}). In addition, the actual mean temperature is given.

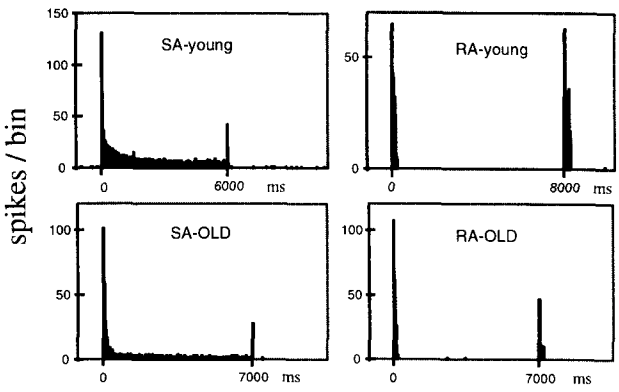

Fig. 1. Examples of PSTHs (post stimulus time histograms) of RA- and SA-responses to sustained pressure (times indicated on abscissa) in normal adult (6 months) and old (24–27 months) animals. Bin width, 10 ms. No differences in amplitude and discharge patterns were found between adult and old animals.

most probably reflect activation of Pacinian corpuscles. The old animal group differed only slightly and the obtained differences were not significant.

The mechanical thresholds of the RA and SA fibers were measured by means of calibrated 'von Frey' hairs. In all age groups, thresholds of the RA-fibers ranged from 0.7 to 42 mN, whereas the thresholds of SA-fibers ranged from 0.7 to 20 mN. Statistical analysis did not reveal significant differences between the two age groups, neither in terms of mean values nor in terms of the distributions.

To provide further evidence for the differential effects of aging on receptive field properties recorded at the level of the cutaneous mechanoreceptors and primary somatosensory cortex, we recorded in an individual animal (30 months old) both cortical and cutaneous mechanoreceptor RFs (Fig. 3). These results confirmed the considerable cortical RF enlargements in old rats [14], while no significant changes at the periphery could be detected. In this particular old animal, we found a mean RF size of 2.5 ± 1.03 mm^2 for the SA-fibers and 3.05 ± 0.80 mm^2 for the RA-fibers, which is in accordance with the group

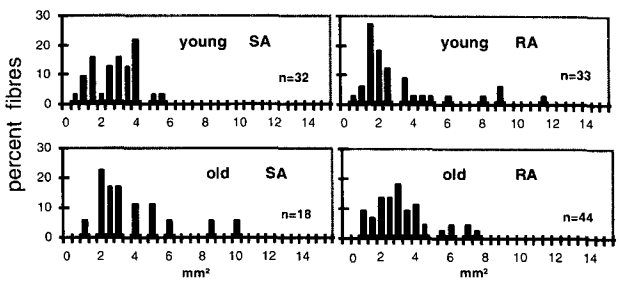

Fig. 2. Receptive field size distributions were separately calculated for SA- and RA-fibers (bin width 0.5 mm^2 skin surface) for normal adults (6 months) and old animals (24–27 months). RF sizes were in the range of 1–5 mm^2 skin area. Large receptive fields (>6 mm^2) were found usually among the RA-fibers, most probably reflecting activation of Pacinian corpuscles. Typical examples of RFs are shown in Fig. 3. RF sizes of the old animals differed only slightly from the adult group and the obtained differences were not significant.

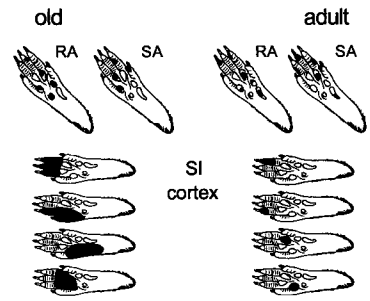

Fig. 3. Differential effects of aging on RFs of cutaneous (SA- and RA-fibers) mechanoreceptors and cortical neurons from the hindpaw representation of primary somatosensory cortex. Top, Mechanoreceptor and cortical recordings from the same individual animal (30 months). Bottom, For comparison, cortical RFs from a 5 month old animal. Examples of SA and RA RFs are from a 6 month old animal used in this study. While cortical RFs in old animals are several-fold enlarged, mechanoreceptor RF sizes are not affected, indicating that cortical age-related alterations are not a simple reflection of peripheral changes.

average described above. Similarly, we found a receptor composition different from normal adult animals with 76% of the RA and 24% of the SA type. However, the mean cortical RF size in this old animal was 63 mm^2, which is several-fold the average size found in normal adult animals (Fig. 3).

This study was designed to examine age-dependent modifications of the cutaneous peripheral nerves in aged rats and to relate these findings to earlier reports about cortical and thalamic changes during aging. In these studies, recordings in the hindpaw representation of primary somatosensory cortex in aged rats had revealed highly enlarged receptive fields and lengthened response latencies compared to normal adult animals [5,14,16]. To evaluate how far these central changes might be a simple reflection of aging processes taking place in the periphery, we focused on the one hand on receptive field sizes, thresholds and composition of single cutaneous mechanoreceptor properties and on the other hand on nerve conduction velocities. The N. plantaris was chosen, because this nerve innervates almost exclusively the plantar surface of the rat's hindlimb [4]. As the comparison with central changes of aging was our main interest, we did not attempt to characterize the single mechanoreceptors in more details [7–9,12,17].

Our observed conduction velocities of N. plantaris fibers of the normal adult animals were in the same range as those reported by [8] for single Aβ fibers in the same nerve. It has been reported that the diameters of N. plantaris fibers increase progressively up to 9 months of age with little changes thereafter, until a reduction occurs between 21 and 24 months [13,15]. Since velocity depends on fiber calibra, these findings are in good agreement with our observation that 6 month old rats showed highest conduction velocities, whereas the 24–27 month and the 3 month old animals had slightly slower conduction velocities. A similar pattern of age-dependent changes was recently reported for sciatic and caudal nerves in mice,

with only modest deterioration at high age [15]. However, our observed age-related decrease in conduction velocities is only in the range of 14%. Such a reduction is by far not sufficient to explain the prolonged latencies in the range of 40% observed in the somatosensory cortex and thalamus of aged rats [5,16]. While age-related effects on the degree of myelinization and synaptic transmission have been discussed, other factors must be involved to generate the lengthening of cortical and subcortical response latencies.

In old rats the mechanoreceptor composition of the hindpaw glaborous skin was different from that found in normal adult rats. However, it remains to be clarified whether shifts in the receptor distribution is due to a loss of peripheral slowly adapting mechanoreceptors or to changes in their response characteristics. Since Sharma et al. [13] found no significant age-related alteration of the total fiber number in the N. plantaris, it might be speculated that the shift in the composition is more likely due to changes in the response characteristics of the slowly adapting receptors. Age-related changes of neurovascular functions might be involved in such changes [6]. Besides, changes of receptor compositions are at present difficult to interpret in terms of their implications on cortical RF size.

Combined, we found little significant changes in the functional properties of peripheral cutaneous mechanoreceptor of old rats. This lack of age-related modifications is particularly unexpected in view of the dramatic alterations reported for cortical (Fig. 3) and thalamic RF properties [5,14,16], which therefore appear unlikely to be causally related to peripheral changes. However, these results provide further support for the hypothesis [1,4,16], according to which part of age-dependent modifications of cortical organization reflect central plastic adaptive capacities following changes of sensory inputs, which in turn are assumed to be mediated by age-related modifications of motor behavior.

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