Cerebral Cortex May 2010;20:1208-1216
doi:10.1093/cercor/bhp185
Advance Access publication September 10, 2009

Effects of Aging on Paired-Pulse Behavior of Rat Somatosensory Cortical Neurons

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Aging affects all levels of neural processing including changes of intracortical inhibition and cortical excitability. The paired-pulse stimulation protocol, the application of 2 stimuli in close succession, is used to investigate cortical excitability. The paired-pulse behavior is characterized by the fact that the second response is significantly suppressed at short interstimulus intervals (ISIs) but approaches the first response with increasing ISIs. However, there are controversial reports about the influence of age on paired-pulse behavior. We therefore used pairs of tactile stimuli (ISIs from tens to hundreds of milliseconds) to record extracellular responses of somatosensory cortical neurons of young and aged rats. Paired-pulse behavior was quantified as the ratio of the amplitude of the second response divided by the first. For all ISIs, we found significantly higher ratios in the old animals indicating reduced paired-pulse suppression (PPS). Evaluation of the single response components revealed a significant reduction of the response to the first stimulus for old animals but no age-dependent decrement to the second. Changes in PPS are usually mediated by modulating the second response characteristics. Thus, our data demonstrate reduced PPS due to an overall reduction of the first response as a form of modified PPS developing at old age.

Keywords: aging, cortical excitability, intracortical inhibition, rat, somatosensory cortex

Introduction

Aging exerts major reorganization and remodeling at all levels of brain structure and function. Although there is a growing body of information about age-related changes at the cellular and molecular levels, little is known about how aging affects the way in which neurons process and integrate sensory information.

Numerous lines of evidence converge on the observation that during aging intracortical inhibition is particularly affected. For example, a recent ultrastructural study revealed a significant age-related decline in the numerical density of presumptive inhibitory synapses of sensorimotor cortex (Poe et al. 2001) demonstrating a deficit in the intrinsic inhibitory circuitry of the aging neocortex. This observation is in agreement with recent reports about an improvement of visual cortical function in senescent monkeys after application of γ-aminobutyric acid (GABA) and its agonist muscimol on cells in primary visual cortex (V1) (Leventhal et al. 2003). Comparing stimulus selectivity of cells in V1 in young and old macaque monkeys revealed evidence for a significant degradation of orientation and direction selectivity in old animals (Schmolesky et al. 2000) consistent with an age-related degradation of intracortical inhibition. Reduced inhibition was also reported in the secondary visual cortex of senescent monkeys (Yu et al. 2006).

Studies of primary somatosensory cortex (SI) of aged rats have reported substantial, age-related enlargements of hindpaw receptive fields (RFs) (Spengler et al. 1995; Godde et al. 2002; David-Jurgens et al. 2008). Because γ-aminobutyric acid–mediated (GABAergic) mechanisms are crucially involved in the control of the size and shape of cortical RFs (Hicks and Dykes 1983; Chowdhury and Rasmusson 2002), it is reasonable to conclude that the observed RF enlargement in the old animals is due to alterations in GABAergic transmission. Age-related declines in a subpopulation of GABAergic neurons containing the calcium-binding protein parvalbumin in SI is direct evidence for altered GABAergic transmission (Jürgens and Dinse 1998). Using somatosensory evoked potential mapping in combination with electric source localization, it was shown that the distance between the dipoles of the index and the little fingers increased in elderly subjects, parallel to a decline of tactile acuity (Kalisch et al. 2008). In adults, map expansion is typically associated with a gain in perceptual performance (Elbert et al. 1995; Pleger et al. 2001). To explain this atypical relation between map expansion and perceptual performance, it was suggested that the age-related reduction of intracortical inhibition mechanisms results in the disintegration of cortical maps. Based on computational approaches, it has been demonstrated that less distinctive cortical representations emerge as a typical outcome of age-related alterations, where distinctiveness of cortical maps was assumed to correlate with behavioral measures (Li and Sikstrom 2002).

During the last several years, stimulation with pairs of stimuli in close succession (paired-pulse stimulation) has become a common tool to investigate short-term plasticity. This is a useful technique to investigate changes in, and the balance between, cortical excitation and intracortical inhibition. Paired-pulse suppression (PPS) describes the phenomenon that at short interstimulus intervals (ISIs) neuronal responses to the second stimulus are significantly reduced. PPS is quantified in terms of the ratio of the amplitude of the second response divided by the first. That means that large ratios are associated with reduced PPS, and small amplitude ratios are associated with stronger PPS. However, whether and how aging affects paired-pulse behavior remains largely elusive. The available data from human motor cortex of elderly subjects are controversial. According to one study, the motor-evoked potential (MEP) magnitude of intracortical PPS elicited by transcranial magnetic stimulation (TMS) was significantly smaller in elderly as compared with young adults (Peinemann et al. 2001). In contrast, a study employing a different stimulation device found no evidence for age-related changes of intracortical PPS. The MEP amplitude ratios were similar in young and aged subjects, although the raw amplitudes were significantly

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smaller in the aged group (Oliviero et al. 2006). Another TMS study based on a large population of young and elderly subjects reached a similar conclusion, reporting a large variability of paired-pulse behavior on the one hand and no influence of age on paired-pulse behavior on the other hand (Wassermann 2002). So far, no data for the somatosensory system have been reported.

We therefore examined paired-pulse behavior at the level of single neurons of somatosensory cortex by studying cortical responsiveness to a second cutaneous stimulus applied at different ISIs in young and aged rats. These experiments revealed substantial similarities in the overall ISI dependence across the age range studied. In contrast to previous reports (Geissler et al. 2007; Hoffken et al. 2007), the reduction in PPS observed at all ISIs tested was due to changes in response magnitude of the first response. Although in aged animals RFs were significantly enlarged, we found no correlation between RF size and PPS, indicating that spatial and temporal aspects of tactile processing are differentially affected by age.

Materials and Methods

Subjects

This study presents the data from 9 young (3–9 months, 200–500 g) and 10 old (20–33 months, 300–500 g) rats (Wistar and Long-Evans). Prior experiments revealed no differences in RF properties and neuronal response characteristics after tactile stimulation between both strains. The 50% probability of survival in an aging colony is about 31 months for Wistar rats (Snyder et al. 1990). The animals were kept under standard housing conditions. Treatment of all animals was within the regulations of the European Communities Council Directive of November 24, 1986 (86/609/EEC), and all experiments were approved by the German Animal Care and Use Committee.

Anesthesia and Animal Preparation

Animals were initially anesthetized with urethane (Sigma, St Louis, MO; 20% in ringer solution, 1.5 g/kg intraperitoneally) together with Ketamine (ketamine-hydrochloride 10%, Pfizer [Karlsruhe, Germany], 0.1 g/kg intramuscularly), and supplements were provided as needed based on eyelid and limb reflexes. Although the use of urethane is experimentally useful (Maggi and Meli 1986), there is still some controversy about its influence on the GABAergic system. For example, modest changes in both the inhibitory and the excitatory system were reported (Hara and Harris 2002), whereas other studies found no alterations of $GABA_A$- or $GABA_B$-mediated inhibitory or glutamate-mediated excitatory synaptic transmission by urethane in cortical neurons of the rat (Sceniak and Maciver 2006). On the other hand, the use of ketamine will introduce interferences with *N*-methyl-D-aspartic acid receptors (Yamakura et al. 2000). Pentobarbital substantially prolongs evoked inhibitory conductances (Wehr and Zador 2005). Preliminary research in our laboratory suggests that the effects of anesthesia on PPS are small. Somatosensory evoked potentials recorded in elderly human subjects after paired-pulse median nerve stimulation reflect changes in paired-pulse ratios comparable to aged rats (Lenz et al. 2009).

Body temperature was maintained at 37.5 °C by a thermostatically controlled heating pad. The cranial bone was dissected, and the cisterna magna was punctuated, thereby lowering intracranial pressure, minimizing pulsations, and avoiding edema. A craniotomy was performed over the right somatosensory cortex (about 0.5 mm rostral to 1 mm caudal from bregma), and the dura mater was removed. To prevent the cortical surface from drying, the area of the craniotomy was filled with warm silicone oil (Silicone DC 200 fluid 50 cst, Serva, Heidelberg, Germany). The left hindlimb was fixated on the hairy skin to ensure a stable position for tactile stimulation of the glaborous side of the paw.

Stimulation and Recording

Single cells or multiunit activity consisting of a small number of action potentials clearly above background were recorded extracellularly at depths of about 700 μm (layer IV) of the hindpaw representation of primary somatosensory cortex. Recordings were made using glass microelectrodes filled with 3 M NaCl. Electrodes had a resistance of 1–1.5 MΩ. Signals were amplified, high pass filtered, and monitored on an oscilloscope and audio monitor. Microelectrodes were positioned with a DC motor–driven x-, y-micromanipulator (Märzhäuser, Wetzlar, Germany) and run vertically through the cortex using a microstepper with a minimal step length of 1 μ (Physiological Institute, University of Mainz). Data presented in this study are based on a total of 179 recording sites (75 from young, 104 from old animals).

After discriminating action potentials according to amplitude, they were digitalized as transistor–transistor logic pulses and stored in a laboratory PC with a temporal resolution of 1 ms (data acquisition software "korr," Happy Mutants Bio Corporation, Ltd, Mainz, Germany). Poststimulus time histograms (PSTHs) could be displayed online. Spike acquisition and tactile stimulation were controlled by a Master 8 (A.M.P.I., Jerusalem, Israel) stimulus generator. Tactile stimuli could be applied by means of an electromagnetic stimulator. The diameter of the probe was 2 mm. Probe indentation was approximately 500 μm.

Paired-pulse stimuli were applied to RF centers located on a digit of the hindpaw. Eight-microsecond stimuli with ISIs of 35, 50, 70, 100, 150, and 200 ms were repeated 32 times for each recording. For analysis, we calculated the maximal response strength (amplitude) in terms of spikes per bin to each of both stimuli in the PSTHs using a bin width of 1 ms. The ratio (A_2/A_1) between the amplitude of the second (A_2) and first (A_1) response was taken as measure of the PPS of SI neurons. Accordingly, A_1 and A_2 as well as paired-pulse ratios reflect averages following accumulation of responses from 32 trials.

Measurement of RF Sizes

To define the size of cutaneous RFs on the glabrous skin of the hindpaw of the same neurons that were tested for paired-pulse behavior, we used the handplotting technique (Merzenich et al. 1984). In this technique, RFs are defined as those areas on the skin at which just visible skin indentation using a small probe with a nodular tip of 1-mm diameter evoked a reliable neuronal discharge. Other studies have shown that just visible indentation is in the range of 250–500 μm, which is in the middle of the dynamic range of cutaneous mechanoreceptors (Gardner and Palmer 1989). The location and the extent of RFs were then transferred in a schematic drawing of the paw. RF size was analyzed by calculating the skin area in square millimeter by planimetry.

Statistical Analyses

In the present study, values are given as mean ± standard error of the mean, unless otherwise noted. For statistical analyses, values of young and old animals were compared with unpaired, 2-tailed student *t*-test unless otherwise mentioned. To test the influence of the factor age on the maximal neuronal response strengths of both response components (A_1 and A_2), we performed a 2-way repeated-measure analysis of variance (ANOVA) with age as between-subject factor and A_1 and A_2 as within-subject factors. To test for differences in variability between age groups, we used the *F*-test. For correlation analyses, we calculated Pearson's linear correlation coefficients.

Results

Comparison of PPS in Young and Old Animals

Typical examples of PSTHs from a young and an old animal are shown in Figure 1 for various ISIs. For illustration of the ISI dependence of the PPS, we calculated "paired-pulse curves" (Fig. 2) by plotting the mean amplitude ratios (second response amplitude divided by first response amplitude) obtained from the PSTHs as a function of different ISIs. In general, the PPS of neurons recorded in the hindpaw representation of

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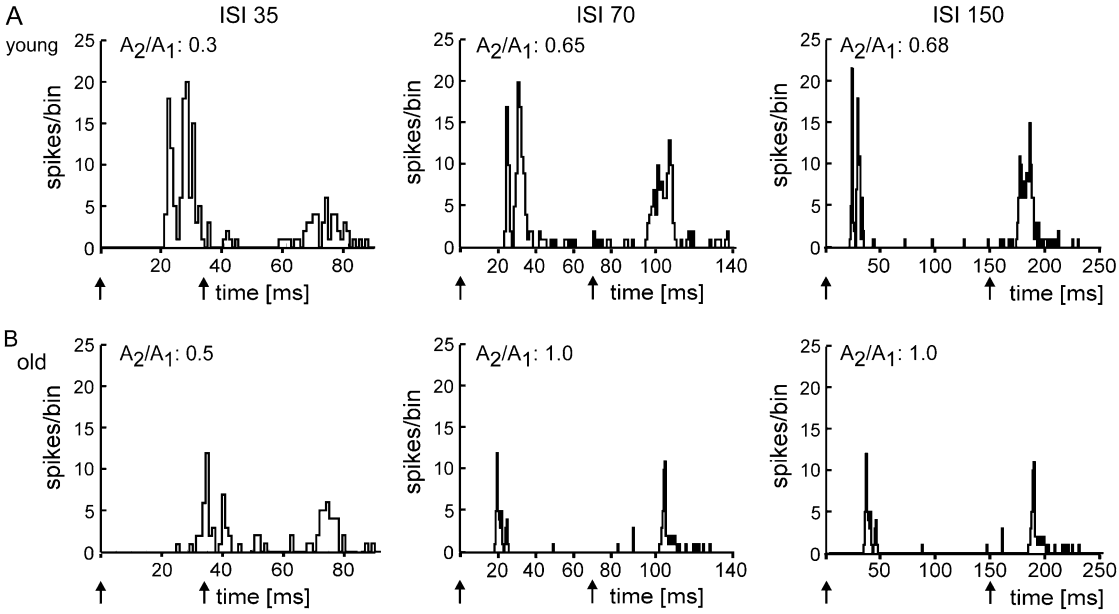

Figure 1. Examples of PSTHs (*A* and *B*). PSTHs of the neuronal responses after tactile paired-pulse stimulation with different ISIs. Stimulus duration was 8 ms. The number of spikes/bin (bin width = 1 ms) is plotted against time. Arrows indicate onset of tactile stimulation. Additionally, the ratio calculated for the amplitudes of the second and first response peaks is shown for each single PSTH. (*A*) PSTHs of neuron responses recorded in a young animal. (*B*) PSTHs of neuron responses recorded in an old animal.

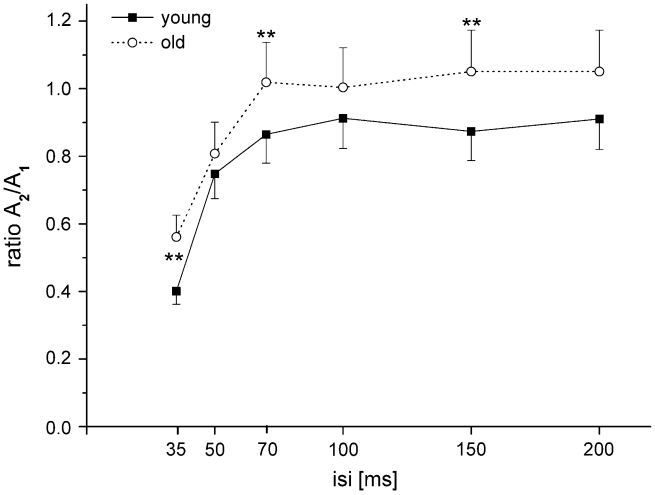

Figure 2. Mean paired-pulse curves of young and aged rats. Mean amplitude ratios of young (solid line, black squares) and old (dotted line, open circles) animals are plotted against the ISI. Vertical bars show standard error of the mean. Asterisks mark significantly different ratios resulting from comparison of age groups at individual ISIs (*$P < 0.05$, **$P < 0.001$).

somatosensory cortex of the old animals resembled that found for young animals: As a rule, the degree of PPS was dependent on the ISI. Substantial PPS was usually present at short ISIs between 35 and 70 ms. At ISIs between 70 and 100 ms, on the other hand, the responses to the paired stimuli became approximately equal in size. On average, we observed very little evidence for significant paired-pulse facilitation, where the second response was larger than the first one (amplitude ratio > 1).

At all ISIs tested, the mean amplitude ratios of the neurons recorded in old animals were higher than those of the young animals, indicating less PPS in old age (Fig. 2). Repeated-measures ANOVA on ratio values with ISIs as the repeated factor and age group as the between-subject factor revealed significant main effects for the factors age ($F_{13,982}$, $P < 0.0001$) and ISI ($F_{53,738}$, $P < 0.0001$) with no significant age × ISI interaction ($F_{0,979}$, $P = 0.435$). Post hoc comparisons between age groups at each of the individual ISIs revealed significant differences at ISIs of 35, 70, and 150 ms (Bonferroni-adjusted $P = 0.0083$). Typically, PPS in both young and old animals showed a substantial intra- and interindividual variability. However, data from the aged individuals displayed a larger interindividual variability than young individuals. In fact, high interindividual variability is regarded as a characteristic property of elderly populations (Rapp and Amaral 1992; Gallagher and Rapp 1997). An increase in variability occurred most notably at short ISIs of 35 and 50 ms. Group differences in variability were significant for the ISIs of 35 and 50 ms (*F*-test; 35 ms: $P < 0.001$; 50 ms: $P < 0.05$).

To test the influence of age on PPS, we calculated Pearson's linear correlation coefficients between the amplitude ratio (A_2/A_1) and age parameters. Scatterplots depicting the relation between age and amplitude ratio are shown for the ISIs of 35 and 200 ms in Figure 3. For both ISIs, we found a significant positive correlation with age (A_2/A_1 35 ms: $r = 0.210$, $P < 0.001$; A_2/A_1 200 ms: $r = 0.178$, $P < 0.05$). One-way ANOVA of the factors age (months) and paired-pulse ratios revealed significant age effects for both ISIs (ISI 35 ms: $F = 3{,}392$; $P < 0.001$; ISI 200 ms: $F = 2{,}636$; $P < 0.05$).

Impact of Age on Stimulus Number

In principle, an increase of the amplitude ratio between the first and second response (i.e., a reduction of PPS) can be achieved by 2 different types of alterations of the response behavior: either by an increase of the second response or by a reduction of the first response. Analyzing our data revealed that the amplitude of the response to the first stimulus plays a crucial role in controlling PPS in general and in mediating

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changes of PPS during aging in particular. In both young and old animals, small first peak amplitudes were associated with minimal PPS, whereas large first peak amplitudes were linked to stronger PPS (Fig. 4). Perhaps, due to strongly suppressed responses to the second stimulus and/or a large degree of variability, the relation between the magnitude of the first response and the degree of PPS was not significant at an ISI of 35 ms; however, our data are significant at an ISI of 200 ms.

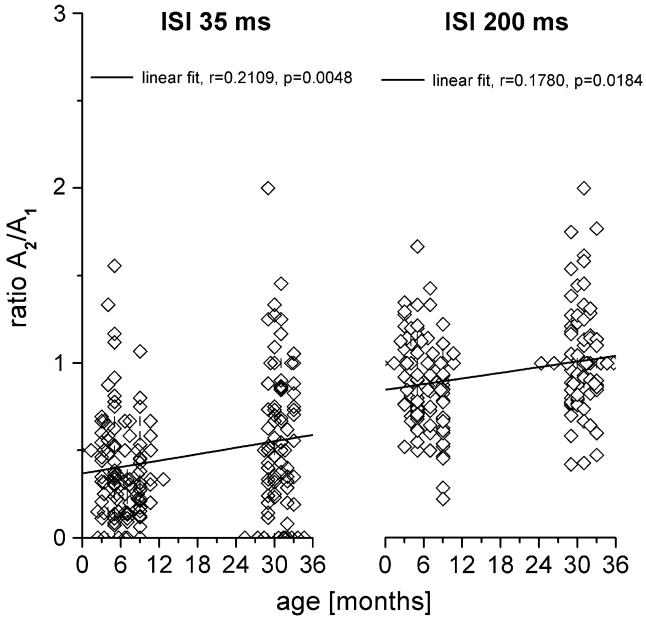

Figure 3. Amplitude ratios as a function of age. Amplitude ratios of all recording sites of young and old animals are plotted against age (overlapping data are separated by a small upright line). The black line represents the best linear fit. Amplitude ratios obtained at ISI 35 ms (left) and at ISI 200 ms (right).

To test the impact of age on the first and second individual response amplitudes, we performed a 2-way repeated-measure ANOVA with age as between-subject factor and A_1 and A_2 as within-subject factors. There is a significant interaction of the factors age and amplitude for ISIs 35 and 200 ms, indicating that the amplitudes of A_1 and A_2 diverge as age increases (35 ms: $F_{1,176} = 13.719$, $P < 0.001$; 200 ms: $F_{1,173} = 10.449$, $P < 0.001$). We also compared the mean amplitudes of the first and second response peaks of the young and the old rats (Fig. 5). We found significantly smaller amplitudes of the first response in old animals (*t*-test, $P < 0.05$) but no significant age group differences in the size of the amplitude of the second response peak (*t*-test, $P = 0.083$). Interaction between age and first and second response amplitude is illustrated in Figure 6.

Comparison of RF Size in Young and Old Animals

In the present study, in addition to assessing PPS, we measured RF size at each recording site. The averaged RF size recorded in young animals was 70.76 and 143.24 mm^2 in the group of aged animals (Fig. 7*A*, *t*-test, $P < 0.0001$). Age and RF size were highly correlated ($r = 0.65$, $P < 0.0001$). Similar to the increased interindividual paired-pulse ratio variability of aged individuals, we observed a significant increase in variability for the RF size factor in the aged animal group (*F*-test: $P < 0.0001$).

No Correlation between PPS and RF Size in Young and Old Animals

The enlargement of RFs in old animals demonstrated in our present and previous studies might be a consequence of reduced, presumably, GABAergic inhibition. A similar argument can be made for the reduction of PPS found in the old animals. We calculated correlation coefficients to analyze a possible relation between RF size and PPS from both young and old animals (Fig. 7*B*). There was no correlation between PPS and RF size ($r = 0.0029$, $P = 0.968$).

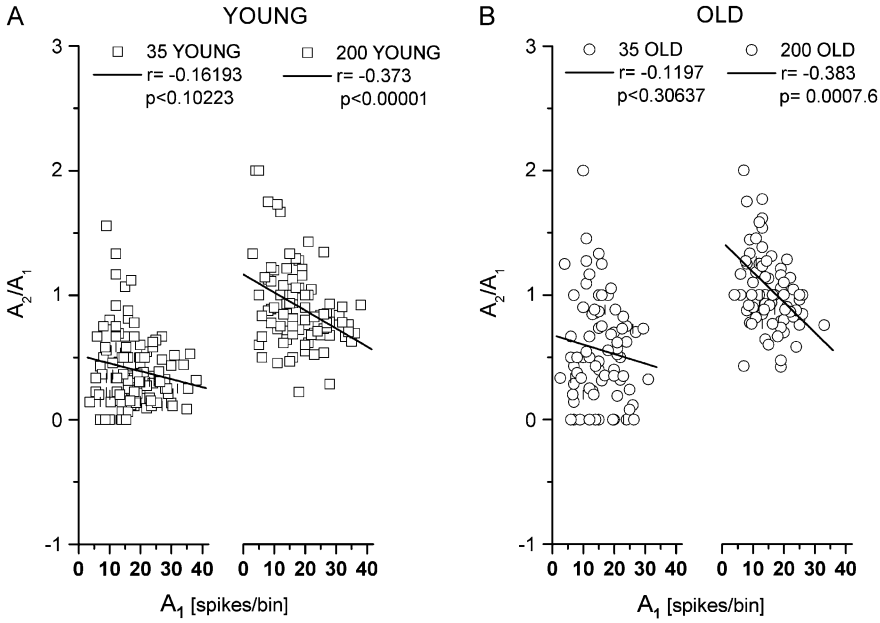

Figure 4. Amplitude ratios as a function of the response amplitude to the first stimulus (*A* and *B*). Amplitude ratios of all recording sites of young (squares) and old (circles) animals are plotted against the size of the first response amplitude (overlapping data are separated by a small upright line). The black lines represent the best linear fit. (*A*) Data of the young animals. Amplitude ratios obtained at ISI 35 ms (left) and at ISI 200 ms (right). (*B*) As in (*A*) for the old animals.

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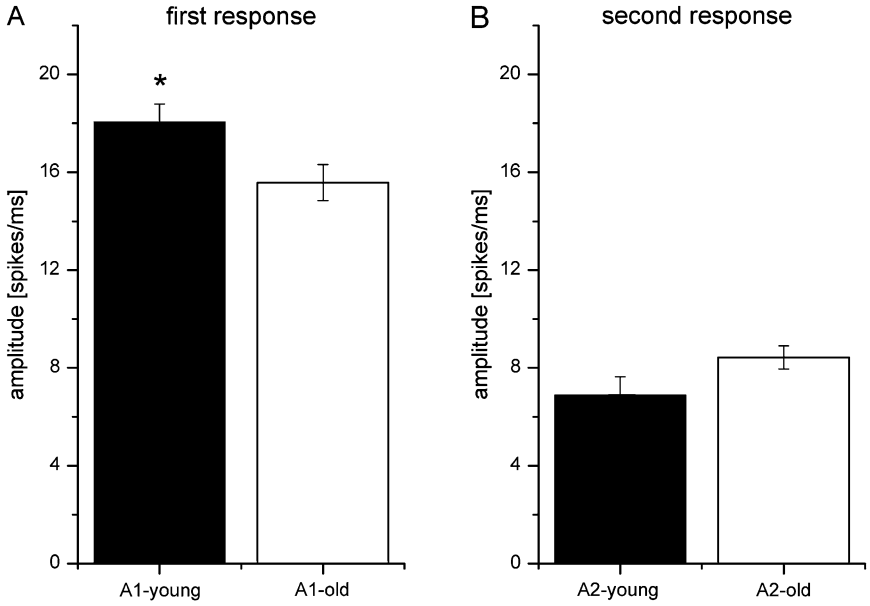

Figure 5. Mean response amplitudes in young and old animals (*A* and *B*). Mean amplitudes of young (black) and old (white) animals. Error bars show standard error of the mean. (*A*) Mean values for the first response amplitude and (*B*) for the second response amplitude. Significant group differences are indicated by asterisks (*$P < 0.05$).

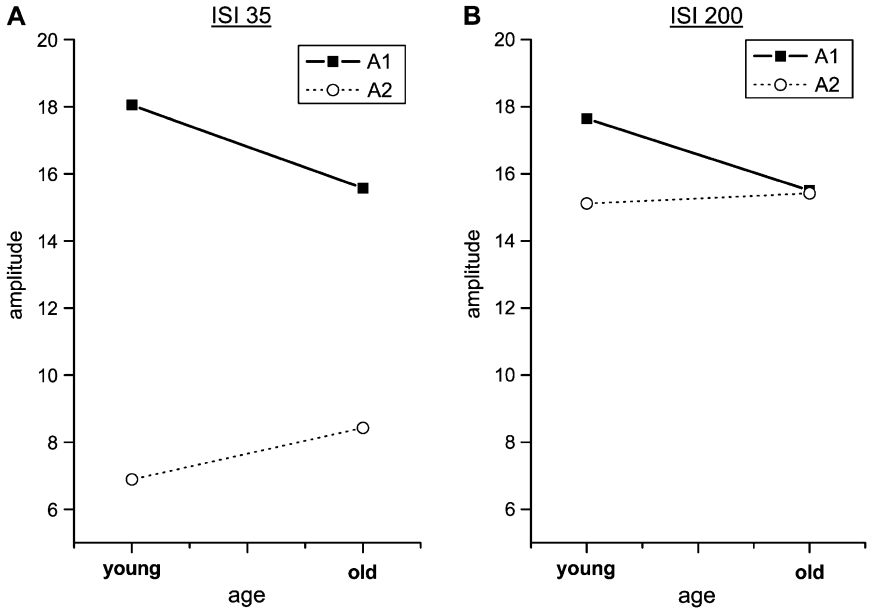

Figure 6. Interaction between age and response magnitude (*A* and *B*). Interaction of the factors age and amplitude of the first (A_1, solid black line) and second (A_2, dotted line) response peak. (*A*) Data refer to ISI 35 ms. (*B*) Data refer to ISI 200 ms.

Discussion

We used a paired-pulse protocol consisting of paired tactile stimulation with different ISIs to study age-related alterations in PPS of SI neurons recorded in young and old rats. We analyzed neuronal responses in terms of maximal response strength (amplitude). PPS was quantified as the ratio of the amplitude of the second response divided by the first. For all ISIs, we found significantly higher ratios in the old animals as compared with young rats, indicating reduced PPS. However, the dependence of the neural responsiveness on different ISIs was similar in young and old animals. Our results provide new evidence of an age-specific modulation of PPS: In old animals, the response to the first stimulus is reduced, whereas the response to the second stimulus is not affected by age.

Terminology

Many related terms are used to describe aspects of neural excitability and inhibition. We use the term "paired-pulse behavior" to describe the overall response dependency on different ISIs. We use the term "PPS" to refer to a reduction in the neuronal response to the second of a pair of 2 successive stimuli, a phenomenon often referred to as "forward

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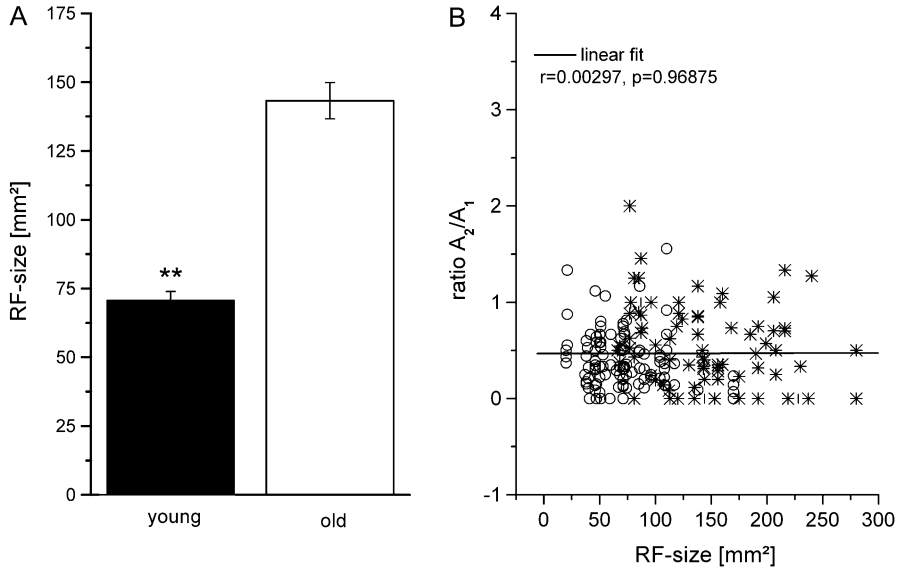

Figure 7. *A*) Mean RF size in young (black) and old (white) rats. Error bars show standard error of the mean. Significant group differences are indicated by asterisks (**$P < 0.001$). (*B*) Amplitude ratios as a function of RF size. Amplitude ratios obtained at ISI 35 ms of young (open circles) and old (stars) animals are plotted against RF size. The black line represents the best linear fit.

suppression." We will use "inhibition" to refer to one possible candidate for this suppression, namely, GABAergic intracortical inhibition arising from postsynaptic activation of $GABA_A$ receptor– or $GABA_B$ receptor–gated channels. By "synaptic depression," we refer to a reduction in synaptic drive. Mechanisms mediating synaptic depression include postsynaptic receptor desensitization, presynaptic depletion of releasable vesicles, or other presynaptic mechanisms depressing vesicle release (Bellingham and Walmsley 1999). The fact that the second response of 2 stimuli given in short succession is strongly suppressed has been denoted as "short-term depression," a special form of short-term plasticity, to describe changes of neural behavior resulting from prior activity (Zucker and Regehr 2002).

Potential Mechanisms Underlying Paired-Pulse Behavior

Our data provide evidence that aging affects PPS. However, the question remains, whether GABAergic or which other mechanisms contribute to the observed age-related alterations. Despite substantial experimental and theoretical work, even in young animals, the mechanisms mediating paired-pulse behavior are not fully understood. There is agreement that presynaptic mechanisms play a crucial role in PPS (Hashimoto and Kano 1998). As to the contribution of GABAergic inhibition, Wehr and Zador (2005) reported that in rat auditory cortex GABA receptor–mediated inhibition does not play a major role in forward suppression for ISIs beyond 100 ms. For longer ISIs, synaptic depression is assumed to be responsible for the observed PPS (Wehr and Zador 2005). In somatosensory cortex, synaptic depression is responsible for a form of rapid sensory adaptation that is similar and perhaps analogous to forward suppression in the auditory system (Chung et al. 2002). In the visual cortex, suppression is also more consistent with thalamocortical synaptic depression than with inhibition (Carandini et al. 2002; Freeman et al. 2002). Because of differences in the time course of recovery between cortical and thalamic cells, however, it seems unlikely that inheritance of thalamic response properties fully accounts for long-lasting forward suppression (Wehr and Zador 2005). Other lines of evidence suggest alterations in the composition of synaptic connections to explain changes in PPS. For example, in auditory cortex slices, low-probability connections were associated with either paired-pulse facilitation or PPS and a nondecremental response to 20-Hz spike trains, whereas high-probability connections were characterized by marked suppression (Atzori et al. 2001). In addition, there is evidence for the involvement of $GABA_B$ receptors in regulation of PPS, as presynaptic blockade of $GABA_B$ receptors causes a decrease in synaptic release probability consistent with the presynaptic inhibition of glutamate release (Porter and Nieves 2004). Furthermore, it was reported that the amount of paired-pulse facilitation is inversely related to the initial release probability of the synapse (Dobrunz and Stevens 1997). Under the assumption that the response amplitudes used in our study can be equated with synaptic release probability measurements, a decrease in the amplitude of the first response can account for the decrease in PPS.

Besides the contribution of GABAergic mechanisms, there is also evidence for the involvement of glutamatergic transmission in the paired-pulse phenomenon. For example, presynaptic group II and III metabotropic glutamate receptors can have a strong inhibitory effect on transmitter release (Takahashi et al. 1996; von Gersdorff et al. 1997) implying a crucial role in modulating paired-pulse behavior. Beyond direct effects, it is possible that more subtle feed-forward circuits of excitatory and inhibitory interneurons influence paired-pulse behavior. Because our experiments used paired-pulse protocols at a systemic level to investigate aging effects on cortical excitability, our data do not provide information about the possible underlying mechanisms.

Other Conditions Affecting PPS

Besides the influence of age, other conditions such as learning, environmental enrichment, or brain injury affect PPS. Percaccio

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et al. (2005) studied temporal information processing in the auditory cortex of rats that were housed in an enriched environment. The authors reported that enrichment increased the response to the first tone thereby enhancing the degree of PPS. With respect to our data, this study is important because it shows that PPS can be modulated by altering the response strength of the first stimulus. In a subsequent paper, this group provided evidence that the increase of response strength to the first stimulus as a result of housing animals under enriched conditions is most presumably mediated by changes in glutamatergic but not in GABAergic transmission (Nichols et al. 2007).

In the recent years, PPS has been increasingly studied in humans as well. Application of tactile coactivation, a passive stimulation protocol, has been shown to improve tactile discrimination performance of the fingers in parallel to an expansion of the cortical maps in SI finger representation (Pleger et al. 2001, 2003; Dinse et al. 2003). Hoffken et al. (2007) used electrical median nerve stimulation and tactile coactivation to demonstrate reduced PPS of somatosensory evoked potentials. Specifically, they demonstrated that the individual gain in perceptual performance was positively correlated with the degree of suppression. In contrast to the results shown here, the response magnitude of the first stimulus remained unaffected. Instead, the coactivation-induced suppression was due to an increased response to the second stimulus.

Damage of cortical tissue is known to lead to hyperexcitability that dramatically alters PPS. Autoradiography studies in which cortical lesions were induced by photothrombosis or freezing revealed a downregulation of $GABA_A$ receptors and PPS (Qu et al. 1998; Que et al. 1999; Schmidt et al. 2006). In another study, reduced PPS was demonstrated in 7-week-old rats after hypoxic ischemic brain injury indicative of hyperexcitability (Geissler et al. 2007). In contrast to the current results, the magnitude of the first peak remained unaffected, but the response to the second stimulus was substantially enhanced. This implies that the alterations of PPS measured after cortical lesions or tactile coactivation are controlled by other mechanisms than those involved in changes induced by environmental enrichment or aging.

Taken together, PPS can be altered in at least 3 qualitatively different ways: first by changing the response to the first stimulus or, alternatively, by changing the response magnitude of the second stimulus. A third possibility to influence PPS arises from changes in the effectiveness of the inhibitory influence of the first response on the second response.

Reduced First Response Amplitudes—Subcortical or Cortical Effects?

Although postnatal developmental changes of thalamocortical transmission are well investigated, little is known about possible changes developing at old age. Although paired-pulse behavior most presumably reflects intracortical processing, the observation of reduced first amplitudes at old age most likely reflects alterations of thalamocortical transmission and/or changes developing along the entire sensory pathway. According to an unpublished study in our laboratory performed in the ventroposteriolateral (VPL) nucleus of the thalamus of aged rats, response magnitudes of VPL neurons were similarly reduced as reported here for SI. However, due to the substantial corticothalamic projections, it is difficult to conclude whether this is a true subcortical alteration or a reflection of cortical changes. To show that age-related changes of cortical processing are not a simple reflection of changes occurring already at the periphery, the effects of aging on cutaneous mechanoreceptors had been investigated in adult and old rats. Evoked action potentials revealed comparable shapes and amplitudes in all animals of all age groups, and there were no differences in RF size and in threshold between old and adult animals (Reinke and Dinse 1996). Similarly, for the visual system, little age-related changes had been reported for retinal and thalamic levels (Spear et al. 1994; Kim et al. 1997). However, conduction is significantly slowed down in aged animals resulting in a lengthening of response latencies by up to 35% (David-Jurgens et al. 2008). It is conceivable to assume that as a consequence the scatter of the incoming pulses is increased that in turn impairs synaptic integration. As a result, in the old rats, the temporal precision of spiking is reduced that causes more diffuse responses characterized by smaller amplitudes.

Relation between RF Size and PPS

In this study on age-related changes of PPS, we additionally confirmed previous findings about a substantial enlargement of RFs in the hindpaw representation in aged rats (Spengler et al. 1995; Godde et al. 2002; David-Jurgens et al. 2008). In young animals, GABAergic mechanisms are known to control the size and shape of RFs (Hicks and Dykes 1983). Accordingly, as possible alterations of GABAergic processes might play a role in age-related reduction of PPS, we asked whether age-related alterations of PPS and of RFs covary. If this is the case, one would expect to find a strong correlation between age-related enlargement of RFs and a reduction of PPS. Surprisingly, the correlation analysis revealed a complete lack of relationship, indicating that both parameters are regulated independently. One possible explanation for the lack of correlation is that the different GABA receptors involved in mediating spatial and temporal inhibition are differentially affected by aging. Spatial inhibition, which modulates RF size, is mediated to a large extent postsynaptically via $GABA_A$ and $GABA_B$ receptors (Lee et al. 1994; Schwark et al. 1999; Chowdhury and Rasmusson 2002, 2003). On the other hand, PPS is to a substantial degree presynaptically controlled (Hashimoto and Kano 1998; Bellingham and Walmsley 1999). As a result, no obvious relationship between inhibition acting on RF size and on temporal aspects should result. Alternatively, it is reasonable that during aging particular $GABA_A$ receptor subunits change, thereby yielding receptors with different subunit composition (Rissman et al. 2007; Schmidt et al. 2008). In cases where these changes affect surround inhibition and PPS differently, no correlation would be found. Another explanation for the lack of correlation is that the inhibition constraining RF size is likely mediated by superficial intracortical connections, whereas the inhibition contributing to paired-pulse effects is more likely thalamocortical. Furthermore, it is conceivable that GABAergic transmission involved in controlling PPS is not fundamentally altered in the aged animals.

Possible Impact on Sensory Function in Aged Individuals

From a functional perspective, paired-pulse behavior serves as a marker of the overall status of intracortical excitability (Kujirai et al. 1993; Ziemann et al. 1996; Ilic et al. 2002; Fedi et al. 2008; Schmidt et al. 2008). Conceivably, the observed increase of excitability with age might affect multiple levels of processing and has been discussed in the context of a loss of

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neuron specificity and the resulting deterioration of sensory processing (Leventhal et al. 2003; Yang et al. 2008). Similarly, the expansion of somatosensory maps and associated reduction of tactile performance have been linked to reduced levels of suppression (Kalisch et al. 2008). Using a computational mean field approach, we have recently shown that map expansion can be paralleled by either a perceptual impairment or an improvement dependent on changes of lateral interaction processes based on Mexican hat interaction. The "aging" model reveals broader distributed representations together with reduced tactile acuity due to a broader excitatory and a weaker inhibitory component (Wilimzig et al. 2006). On the other hand, paired-pulse behavior has been linked to forward masking and temporal processing (Atzori et al. 2001; Percaccio et al. 2005; Wehr and Zador 2005). There is compelling evidence that temporal processing in elderly individuals is impaired (Mendelson and Ricketts 2001; Mendelson and Wells 2002; Conlon and Herkes 2008). Single-cell data obtained for tactile train stimulation in old rats revealed a substantial inability to faithfully follow rates of 20–30 Hz. Interestingly, paired-pulse behavior turned out to be a rather poor predictor of train behavior (David-Jurgens and Dinse 2008). Accordingly, more studies are needed to explore the relation between paired-pulse behavior and how rapidly presented sequences of stimuli are perceived.

Conclusion

The paired-pulse ratios from the old animals were higher than those from the young rats at all ISIs, indicating less suppression. The shape of the paired-pulse curve, however, remained unaltered. Accordingly, the ISI dependency characterized by substantial suppression at short ISIs and less suppression at longer ISIs is similar in young and old animals. Thus, our results revealed no evidence for age-related changes of paired-pulse behavior itself. On the other hand, we found an age-related reduction of the first response amplitude. In the case of unchanged PPS, the second response amplitude should be reduced as well as compared with young animals, which was, however, not the case. Instead, the ratio between the first and second response was higher for old animals relative to young animals. This indicates that an age-related reduction of activity as apparent in a decreased first response amplitude most likely causes less suppression of the second response amplitude as evident in an increased paired-pulse ratio in old animals. Changes of PPS might be one substrate responsible for impaired temporal processing in aged individuals.

Funding

Deutsche Forschungsgesellschaft (Da 1116/1-1 DFG, Di DFG 334/19-1).

Notes

Conflict of Interest: None declared.

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