ABSTRACT

Objective. There is growing interest in the impact of aging on the plasticity of pain responses. Up-regulation characterizes pain responses in clinical situations, and consequently aging effects on the development and resolution of increased sensitivity have important implications for the experience of pain in those older age groups who are more likely to suffer from chronic conditions. This study examined temporal summation of pain at different stimulus frequencies to gain further insights into the effect of age on pain plasticity.

Design. In a group of younger and a group of older subjects, trains of five brief electrical stimuli were applied to the skin over the sural nerve at frequencies ranging between 0.2 and 2.0 Hz. Nociceptive reflexes were recorded throughout the application of stimuli. Single pulses and the fifth pulse of each series were rated for pain intensity with a visual analog scale.

Results. The younger subjects demonstrated temporal summation at frequencies of stimulation that were consistent with previous reports, namely 0.33 to 2.0 Hz. The older group had a greater mean rating of the fifth pulse relative to a single pulse at all frequencies of stimulation. The behavior of the nociceptive reflex to repeated stimuli was equivalent for the two age groups, only summating at a frequency of 2.0 Hz.

Conclusions. The temporal summation of low-frequency stimuli in the older subjects suggests that aging impacts on the capacity of the nociceptive system to down-regulate subsequent to sensitization.

Key Words. Pain; Nociceptive Reflex; Temporal Summation; Aging

Introduction

Psychophysical assessments of the effects of aging on pain perception have focused on threshold measures. The examination of pain thresholds in groups of disparate age has provided important insights into the acuity for pain that includes differential age-related changes according to stimulus modality, duration, location, and class of primary afferent [1]. The implications of age-related effects on threshold measures are necessarily restricted to the normative physiological conditions that characterize tests of the acuity for
pain. Sensitivity to noxious stimuli in a clinical context can be influenced dramatically by the sequela of injury and disease. The presence or absence of aging effects on the plasticity of pain responses subsequent to tissue inflammation or nerve injury cannot be inferred from pain threshold measures under physiological conditions. It is important to extend aging research to examine effects on pain plasticity in order to enhance understanding of the clinical experience of pain in the subgroup of the population with the greatest prevalence of chronic symptoms [2].

Further insights into the association of aging and pain plasticity may be afforded through the assessment of temporal summation of pain. Responses to brief noxious stimuli delivered within a circumscribed frequency range are notable for escalating subjective intensity within five or six stimuli [3]. The psychophysical attributes of temporal summation are presumed to be a consequence of windup, an electrophysiological demonstration of up-regulation of dorsal horn responses to repetitive stimuli of C-fibers [4]. Frequency-dependent temporal summation may fall short of a definitive model of central sensitization [5,6], but does provide a well-defined epiphenomenon that permits an investigation of aging effects on the temporal profile of enhanced responses to noxious stimuli. Of particular interest is the lower boundary of frequency for temporal summation in older people. In younger subjects, temporal summation occurs within the frequency range of 0.33–2.0 Hz [3]. At interstimulus intervals of greater than 3 seconds, the sensitizing effects of earlier C-fiber inputs have abated, and potentiation of the effects of subsequent stimuli on dorsal horn cells does not occur. If dorsal horn sensitization after C-fiber input is prolonged in older people, then summation should occur at lower frequencies than the 0.33-Hz boundary previously reported for younger people. This study tests the effect of aging on the frequency range of temporal summation.

Methods

Subjects

An advertisement calling for volunteers was posted in public places in the vicinity of the National Ageing Research Institute (NARI), and circulated to people on the NARI volunteer register. Subjects did not receive compensation, other than out-of-pocket expenses, for participation in the study. The sample for the study included two groups of 15 subjects ranging in age from 18 to 40 years and 65 years and over. Screening of prospective subjects prior to recruitment excluded people with pain, and people with medical conditions and/or those using medications likely to influence pain perception. Older subjects were also administered the Mini Mental Status Examination to exclude the possibility of cognitive impairment [7]. An equal proportion of men and women were recruited for each of the two age groups.

Procedure

Measurement of Nociceptive Reflex (RIII)

During testing, the subject lay on their left side. Both legs were placed on top of each other for support and the knees placed at an angle between 100 and 130 degrees. Towels were placed between the knees to provide firm and stable cushioning, and pillows provided support for the upper body. Skin posterior to the right lateral malleolus was abraded and degreased to decrease impedance. Stimulating electrodes (2 × Ag-AgCl) were placed 2.5 cm apart in proximity to the retromalleolar aspect of the sural nerve. Stimuli consisted of five 1-ms pulses with an internal frequency of 330 Hz (13-ms duration). Following preparation to decrease skin impedance, recording electrodes (2 × Ag-AgCl) were placed over the musculotendinous junction of the ipsilateral biceps femoris. Electromyographic activity was recorded for 200-ms periods after stimuli. Signals were filtered (50–200 Hz) and amplified (2 × 10^{6}). The RIII reflex was quantified as the root mean square (RMS) of signal intensity during a 70- to 130-ms poststimulus window [8].

Psychophysical Ratings and Delivery of Repetitive Stimuli

Sensations associated with the delivery of electrical stimuli were rated on a 10-cm visual analog scale (VAS). The VAS was anchored at either end with the words “not felt” or “worst possible pain.” A vertical line at the midpoint of the VAS was labeled with the words “just noticeable pain” [9]. All pen marks on the printed VAS forms were made by subjects while in the side-lying posture. A brief training session was conducted before RIII threshold testing began. The purpose of the training was threefold: (1) it familiarized subjects with electrical stimulation, helping reduce anxiety; (2) it provided subjects the opportunity to develop internal consistency in rating and distinguishing between stimuli—the distinction between definite sensation, just noticeable pain, and definite pain was repeated until the subjects were sure of these
terms and used them confidently; and (3) it allowed the experimenter to establish the approximate stimulus intensities to commence the staircases to determine RIII and pain thresholds.

Pain and RIII thresholds were determined with a double-random staircase. Repetitive stimuli during tests of temporal summation were set at 110% of individual RIII thresholds. Each test of temporal summation incorporated five stimuli, the last of which was rated. A single, rated pulse was also delivered prior to each set of five stimuli. Sets of five stimuli were presented at frequencies of 0.2, 0.25, 0.33, 1, and 2 Hz on five occasions each in pseudorandom order. An interval of at least 40 seconds was maintained between successive stimulus sets.

Analysis
Mean differences in thresholds (pain and RIII) and the effects of age were tested with repeated-measures analysis of variance (ANOVA). The effects of age and stimulus frequency on pain ratings and RIII reflexes were assessed with repeated-measures ANOVA. Post hoc tests (paired t-tests) with Bonferroni corrections for multiple comparisons were used to establish within-group differences in the dependent variables (pain ratings and RIII RMS) for the final pulse of each of the stimulus frequencies (i.e., 2, 1, 0.33, 0.25, and 0.2 Hz). Pearson r correlation coefficients were calculated for the relationship between pain and RIII thresholds, as well as the relationship between RIII magnitude and VAS ratings of a single pulse, in both age groups.

Results
The mean age of the younger group was 25.0 (±4.5, range 18–32) years, and the mean age of the older group was 70.3 (±4.0, range 65–79) years. The proportions of men in both groups were 53%. The racial distribution of the younger and older groups was consistent with the broader Australian community, in that almost all subjects were of either Anglo-Saxon or European heritage. The years of education in the younger group were generally higher than that of the older group.

The mean thresholds for pain did not differ significantly from the thresholds for the RIII in the amalgamated groups ($F_{1,27} = 1.6, P = 0.21$). A trend for higher mean thresholds in the older group did not reach significance ($F_{1,27} = 3.4, P < 0.08$), nor was there any interaction between age and type of threshold ($F_{1,27} = 0.2, P = 0.64$) (Table 1). Mean ratings of single pulses did not differ between the two age groups ($t_{29} = 0.29, P = 0.78$). Frequency was a significant factor in determining pain ratings of the final stimulus of sets of five stimuli ($F_{5,24} = 5.6, P > 0.001$). Age was not a significant factor for pain ratings ($F_{1,28} = 0.07, P = 0.8$), but there was a significant interaction between age and frequency ($F_{5,24} = 3.9, P > 0.01$). Higher frequencies were associated with increased pain ratings of the final stimulus. The final stimulus was rated as significantly more intense for all frequencies compared with a single pulse in the older group (0.2 Hz $t_{14} = 4.3, P < 0.001$; 0.25 Hz $t_{14} = 5.6, P < 0.0001$; 0.33 Hz $t_{14} = 6.1, P < 0.0001$; 1 Hz $t_{14} = 6.3, P < 0.0001$; 2 Hz $t_{14} = 9.1, P < 0.0001$), whereas the younger subjects demonstrated temporal summation only for frequencies of 0.33 Hz or greater (0.2 Hz $t_{14} = 0.9, n.s.$; 0.25 Hz $t_{14} = 0.7, n.s.$; 0.33 Hz $t_{14} = 5.1, P < 0.0001$; 1 Hz $t_{14} = 8.7, P < 0.0001$; 2 Hz $t_{14} = 10.3, P < 0.0001$) (Table 1 and Figure 1). Frequency was also a significant factor for the RIII ($F_{5,24} = 5.6, P > 0.001$). The mean RIIIs associated with the final pulse of sets with a frequency of 2 Hz were greater than the average single pulse for each of the age groups (Figure 2). However, neither age ($F_{1,28} = 1.1, P = 0.31$) nor the interaction of age and frequency ($F_{5,24} = 0.65, P = 0.67$) were significant factors for the magnitude of the RIII.

The pain and RIII thresholds did not correlate in either group (younger group $r = 0.27, P = 0.3$; older group $r = 0.25, P = 0.4$). The relationship between the RMS of a single pulse and VAS rat-

Table 1 Mean values (SE) for thresholds, ratings, and reflexes for the two age groups

<table>
<thead>
<tr>
<th></th>
<th>Younger Group</th>
<th>Older Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thresholds (mA)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>14.0 (1.09)</td>
<td>16.2 (1.52)</td>
</tr>
<tr>
<td>RIII</td>
<td>14.9 (0.95)</td>
<td>18.3 (1.70)</td>
</tr>
<tr>
<td><strong>Pain ratings (0–10)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single pulse</td>
<td>58.1 (3.26)</td>
<td>53.8 (3.05)</td>
</tr>
<tr>
<td>0.2 Hz</td>
<td>58.8 (3.47)</td>
<td>60.3 (3.34)</td>
</tr>
<tr>
<td>0.25 Hz</td>
<td>58.9 (3.73)</td>
<td>63.3 (3.73)</td>
</tr>
<tr>
<td>0.33 Hz</td>
<td>61.9 (3.31)</td>
<td>63.7 (3.51)</td>
</tr>
<tr>
<td>1.0 Hz</td>
<td>66.2 (3.27)</td>
<td>68.4 (3.50)</td>
</tr>
<tr>
<td>2.0 Hz</td>
<td>69.3 (2.93)</td>
<td>70.9 (3.60)</td>
</tr>
<tr>
<td><strong>RIII magnitude (RMS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single pulse</td>
<td>3.1 (0.77)</td>
<td>4.4 (0.76)</td>
</tr>
<tr>
<td>0.2 Hz</td>
<td>2.9 (0.80)</td>
<td>3.4 (0.57)</td>
</tr>
<tr>
<td>0.25 Hz</td>
<td>2.9 (0.80)</td>
<td>3.5 (0.59)</td>
</tr>
<tr>
<td>0.33 Hz</td>
<td>3.1 (0.75)</td>
<td>3.7 (0.53)</td>
</tr>
<tr>
<td>1.0 Hz</td>
<td>4.7 (0.73)</td>
<td>6.8 (1.43)</td>
</tr>
<tr>
<td>2.0 Hz</td>
<td>9.6 (1.94)</td>
<td>10.8 (2.23)</td>
</tr>
</tbody>
</table>

RMS, root mean square; VAS, visual analog scale.
ings also failed to reach significance in both groups (younger group \( r = -0.08, P = 0.8 \); older group \( r = -0.15, P = 0.6 \)).

Discussion

This study has revealed an age-related difference in pain responses to repeated noxious stimuli. Temporal summation of pain responses to electrical stimuli occurred at much lower frequencies in the older group compared with younger subjects. The frequency-related differences between the groups were confined to perceptual processes; temporal summation of the RIII was only in evidence at 2 Hz for both young and old subjects.

Time is an important factor in the delineation of pain experiences among young and older people. From a psychophysical perspective, age-related differences in pain responses under physiological conditions are most pronounced for very brief stimuli. As stimuli become more durable, then differences between young and old people in threshold measures fail to materialize [10]. When noxious stimuli are of a sufficient magnitude or duration to precipitate sensitization, then the experience of older people diverges again from younger people. The persistence of secondary hyperalgesia after topical application of capsaicin is much longer in older people compared with young subjects, despite similar indices of primary hyperalgesia and equivalent levels of peak sensitivity in the secondary region [11]. The important distinction between the old and young does not appear to be the absolute level of pain experienced subsequent to stimulation, but the time course of pain responses.

The demonstration of an aging effect on temporal summation has added to a growing body of evidence implicating disturbances of nervous system plasticity in the pain experience of older people. The most parsimonious explanation for the temporal summation of lower-frequency stimuli in the older group is age-related impairment of descending inhibitory mechanisms. The propensity of dorsal horn neurons to undergo transient membrane potential changes subsequent to peripheral inputs is subject to descending modulation. In animal models of temporal summation, proximal transection of the spinal cord facilitates C-fiber mediated reflexes [12]. There is strong evidence of a progressive age-related loss of serotonergic and noradrenergic neurons in the dorsal horn [13,14], lending weight to the argument that inhibitory mechanisms could be contributing to

![Figure 1](https://example.com/image1)

**Figure 1** The pain ratings of the final pulse in a set of five pulses are expressed as a percentage of the rating of a single pulse for two age groups. The older group demonstrated significantly greater ratings of the fifth pulse relative to a single pulse for each of the five frequencies of stimulation. The younger group behaved in a manner that was consistent with previous reports, only demonstrating temporal summation for frequencies of 0.33 Hz or greater. *Greater mean response compared with single pulse at Bonferroni corrected level for 10 comparisons (\( P < 0.005 \)). RMS, root mean square.

![Figure 2](https://example.com/image2)

**Figure 2** The magnitude of nociceptive reflexes elicited by a single pulse and by the final pulse of a set of five stimuli at a range of frequencies in two age groups. The magnitude of the reflex is expressed as the root mean square of signal intensities in a time window of 70 to 130 ms post stimulus. It is apparent that there was no difference between the age groups in the respective profile of responses to the range of interstimulus intervals. A significant mean increase in response relative to a single pulse was evident for the final pulse of the stimuli delivered at 2.0 Hz for each of the age groups.
the findings of this study. Indeed, direct evaluation of endogenous inhibition of pain in groups of disparate age has demonstrated impaired function in older people [15]. It may be that membrane potential changes in dorsal horn neurons subsequent to C-fiber stimulation are more likely to persist in the absence of countervailing descending inputs or local inhibitory modulation. Enduring effects of peripheral stimulation mean that subsequent stimuli will potentiate at greater intervals, leading to an age-related summation of lower-frequency C-fiber inputs. While there is some appeal in an argument that postulates reported aging effects for pain summation to documented age differences in descending inhibition, this connection is largely speculative and the method employed in this study does not exclude alternative explanations. It is possible that the interaction between age and stimulus frequency may be dependent on putative age-related changes in other aspects of dorsal horn or supraspinal function.

Previous comparisons of temporal summation in different age groups have reported comparable, decreased, and elevated pain ratings of repeated stimuli in older subjects [16,17]. The disparate results of earlier studies are difficult to reconcile, but may be a function of differences in stimulus intensity and localization. Unlike the response-dependent approach used in the current experiment, previous reports employed thermal stimuli of fixed intensity. For stimuli of 47°C and 50°C applied to the arm, older subjects experienced a greater degree of temporal summation [16]. This aging difference for summation is not apparent for thermal stimuli applied to the arm in excess of 50°C [16,17]. It may be that a threshold effect is in operation among older people, whereby the factors that facilitate summation at lower frequencies may enhance the likelihood of summation for stimuli of lower absolute intensities. However, the absence of temporal summation to 51°C stimuli applied to the leg in older people is not compatible with this tentative hypothesis [17].

The magnitudes of the RIII and pain thresholds in the two groups were compatible with previous reports of responses to electrical stimuli [18,19], although there was a trend toward higher pain and RIII thresholds in the older group. Differences in RIII thresholds were the primary factor contributing to the trend. Post hoc power analyses suggest that a doubling of the sample size would be sufficient to produce a significant between-group effect for RIII thresholds, whereas more than 80 subjects per group would be required in order to demonstrate an aging effect on pain thresholds, given the reported mean differences and variances. The absence of an age-related difference in electrical pain threshold is consistent with the majority of previous studies using this stimulus modality [20]. Advanced age has yet to be systematically examined with respect to the RIII reflex, but the result of this study points toward a small elevation in threshold with increasing age.

The absence of an age-related change in summation of nociceptive reflexes reflects the primacy of C-fiber input in the initiation and maintenance of central sensitization [21–24]. The respective conduction velocities of Aδ and C-fiber inputs to the dorsal horn create a disassociation between reflexive and higher-order outputs. The presumed up-regulation of second-order neurons in the spinal cord may sensitize these cells to further C-fiber stimulation, but be of insufficient magnitude to enhance responses to Aδ inputs that occur within a discrete time window relative to the later arrival of C-fiber mediated stimuli. It is possible that the summation of nociceptive reflexes apparent in both groups at the highest frequency is mediated by a mechanism that is independent of aging effects. Temporal summation of Aδ-mediated nociceptive reflexes evoked at 2 Hz has been reported under physiological conditions in humans [8,25], but temporal summation at stimulus intensities below activation levels for C-fibers is usually only associated with up-regulated states such as hyperalgesia subsequent to tissue inflammation in animal models [26,27]. Alternatively, countervailing effects of diminished myelinated fiber input [28] and impaired recovery after perturbation in the older group could result in no net effect for repeated Aδ input to the dorsal horn. Irrespective of the underlying mechanisms, it would appear that age-related changes operate differentially with respect to the central responses mediated by myelinated and unmyelinated primary afferents.

The results of this study highlight an important distinction between younger and older people in their capacity to experience alterations of pain responses subsequent to persistent peripheral inputs. Studies of the epidemiology of pain suggest that older people are more prone to chronic symptoms [2]. The greater vulnerability to chronic pain in older people is presumably a consequence of increased risk for disease in this age group. However, aging effects on the nociceptive system may further confound the experience of
older people by prolonging recovery subsequent to up-regulation of pain responses. The agenda for future aging and pain research should shift focus to the mechanisms that contribute to sensitization of the nociceptive system, with the ultimate objective of reducing the likelihood of suffering in those older people with the greatest risk of chronic pain.

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References