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## Activation properties of trigeminal motoneurons in participants with and without bruxism

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**D'Amico JM, Yavuz ŞU, Saraçoğlu A, Atış ES, Gorassini MA, Türker KS.** Activation properties of trigeminal motoneurons in participants with and without bruxism. *J Neurophysiol* 110: 2863–2872, 2013. First published September 25, 2013; doi:10.1152/jn.00536.2013.—In animals, sodium- and calcium-mediated persistent inward currents (PICs), which produce long-lasting periods of depolarization under conditions of low synaptic drive, can be activated in trigeminal motoneurons following the application of the monoamine serotonin. Here we examined if PICs are activated in human trigeminal motoneurons during voluntary contractions and under physiological levels of monoaminergic drive (e.g., serotonin and norepinephrine) using a paired motor unit analysis technique. We also examined if PICs activated during voluntary contractions are larger in participants who demonstrate involuntary chewing during sleep (bruxism), which is accompanied by periods of high monoaminergic drive. In control participants, during a slowly increasing and then decreasing isometric contraction, the firing rate of an earlier-recruited masseter motor unit, which served as a measure of synaptic input to a later-recruited test unit, was consistently lower during derecruitment of the test unit compared with at recruitment ( $\Delta F = 4.6 \pm 1.5$  imp/s). The  $\Delta F$ , therefore, is a measure of the reduction in synaptic input needed to counteract the depolarization from the PIC to provide an indirect estimate of PIC amplitude. The range of  $\Delta F$  values measured in the bruxer participants during similar voluntary contractions was the same as in controls, suggesting that abnormally high levels of monoaminergic drive are not continually present in the absence of involuntary motor activity. We also observed a consistent “onion skin effect” during the moderately sized contractions (<20% of maximal), whereby the firing rate of higher threshold motor units discharged at slower rates (by 4–7 imp/s) compared with motor units with relatively lower thresholds. The presence of lower firing rates in the more fatigue-prone, higher threshold trigeminal motoneurons, in addition to the activation of PICs, likely facilitates the activation of the masseter muscle during motor activities such as eating, nonnutritive chewing, clenching, and yawning.

motoneurons; pain; sleep bruxism; plateaus

JAW MUSCLES ARE INVOLVED IN both simple and complex oral-motor behaviors, such as eating, drinking, talking, and breathing, as well as nonfunctional activities such as tooth grinding and clenching. These muscles can be characterized into either jaw-closing or jaw-opening muscles. In this study we focused on the properties of motoneurons innervating the jaw-closing

masseter muscle. The masseter muscle is a multipennate structure with different compartments having various proportions of muscle fiber types and directions, with each compartment activated in different ways depending upon the motor task (Gaudy et al. 2000; Nordstrom and Miles 1991; Ogawa et al. 2006; van Eijden and Turkawski 2001). Motoneurons innervating the masseter muscle are located dorsally and laterally in the rostral and middle thirds of the trigeminal motor nucleus (Westberg and Kolta 2011). These motoneurons receive excitatory glutamatergic and inhibitory glycinergic/GABAergic inputs from premotor neurons located in areas surrounding the principal motor nucleus (Anacleit et al. 2010; Nakamura et al. 2008; Katakura and Chandler 1990; Turman and Chandler 1994). In addition, trigeminal motoneurons receive direct serotonergic inputs from the nuclei raphe obscurus, raphe pallidus, and raphe dorsalis (Kolta et al. 1993; Li et al. 1993), as well as norepinephrine inputs from the locus subcoeruleus, A5 and A7 cells, and sparse innervation from the locus coeruleus (Fenik et al. 2002; Fort et al. 1990; Schwarz and Peever 2010).

Similar to motoneurons innervating the limb muscles, trigeminal motoneurons display bistable membrane properties such as plateau potentials and burst oscillations where long-lasting periods of depolarization can occur under low levels of synaptic drive (Hsiao et al. 1998). These properties are mediated by voltage-activated, sodium and calcium persistent inward currents (PICs) that are, in turn, facilitated by serotonin and norepinephrine receptors located on the motoneuron (Schwarz et al. 2008). For example, application of serotonin can induce a negative slope region in the current-voltage relationship of trigeminal motoneurons that is subsequently abolished when the persistent L-type  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  currents are blocked with nimodipine and tetrodotoxin, respectively (Hsiao et al. 1997, 1998). Given the demonstration of strong PIC activation in animals, we examined if trigeminal motoneurons in the human also exhibit indirect evidence of PIC activation by using a paired motor unit analysis technique developed for limb muscles (Gorassini et al. 2004). Evidence for PIC activation, namely motor unit activity that persists under levels of synaptic drive lower than that needed to initially recruit the motor unit (i.e., self-sustained activity), was examined during isometric, voluntary contractions onto a bite bar (Türker et al. 2004).

Motor units innervated by trigeminal motoneurons are recruited in an orderly fashion, with small motor units having

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small spike amplitudes and twitch tensions being recruited at lower bite forces than larger motor units (Goldberg and Derfler 1977; Yemm 1977). Once securely recruited, masseter motor units can fire steadily for at least 5 min during a static contraction (Farella et al. 2011). Motor unit firing rates increase linearly with increasing force, with higher threshold units having a larger range of firing rate modulation than lower threshold units and with most units reaching a plateau in firing near 30 imp/s (Derfler and Goldberg 1978; Lund et al. 1979). In this study we examined further the relationship between firing rate and recruitment threshold, specifically if masseter motor units display an “onion skin effect,” a phenomenon where higher threshold units fire at lower rates than lower threshold units (De Luca and Hodge 2010; De Luca and Erim 1994). The onion skin effect has been observed in various motor units from the upper and lower limbs with the parent motoneuron originating in the spinal cord (De Luca and Hodge 2010; Kanosue et al. 1979; Monster and Chan 1977; Tanji and Kato 1973). We wanted to examine here if the onion skin effect was present in motor units innervated by motoneurons located in the pons.

Lastly, we indirectly examined, via  $\Delta F$  measurements, whether individuals with bruxism who experience involuntary (self-sustained) teeth grinding and clenching during sleep (Lobbezoo et al. 2006) display persistent increases in monoaminergic drive to their trigeminal motoneurons. During sleep, muscles are typically atonic; however, there are periods of rhythmic masticatory muscle activity characterized by phasic (teeth grinding) and tonic (clenching) bursts of activity (Ancelet et al. 2010; Kato et al. 2007, 2011) that coincide with the presence of microarousals (Halasz et al. 2004; Quattrochi et al. 2000). Interestingly, the discharge of neurons in the raphe nuclei, locus coeruleus, subcoeruleus, and A5/A7 cells, which release PIC-facilitating serotonin and norepinephrine to the trigeminal motoneuron pool, increases during microarousals (Leung and Mason 1999; Sakai and Crochet 2001; Takahashi et al. 2010). Individuals with bruxism experience increased numbers of microarousals during sleep (Kato et al. 2001, 2003, 2011) and likely increases in monoaminergic drive to trigeminal motoneurons. Thus we examined with paired motor unit analysis if participants with bruxism display larger estimates of PIC amplitude during voluntary contractions compared with nonbruxing controls to determine if tonically elevated levels of monoaminergic drive to trigeminal motoneurons are present in bruxers, even in the absence of microarousals and rhythmic masticatory muscle activity. Parts of the data from this article have been published in abstract form (Yavuz et al. 2010).

## METHODS

**Participants.** Protocols were approved by the Human Ethics Committee of Ege University in accordance with the Declaration of Helsinki. All participants provided informed written consent before the experiment. Nine nonbruxer (NBrux) control participants and 13 bruxer (Brux) participants were examined [NBrux =  $26 \pm 3.7$  yr (range 24 to 35), 2 males; Brux =  $22 \pm 3.1$  yr (range 19 to 29), 5 males,  $P = 0.02$ ]. Although the Brux group was significantly younger by 4 yr compared with the NBrux group, we do not expect this small age difference to play a large role in our estimates of PIC amplitude and motor unit firing properties. Brux participants were assessed by a clinician (A. Saraçoğlu). Because overnight observation was not performed, evidence for well-developed/stiff and tired/painful (especially in the morning) masseter muscles was used to diagnose sleep

bruxism, in addition to examining evidence for flat and highly polished occlusal surfaces (bruxofacets). The level of bruxism was then scaled from 0 to 5 using a visual analog scale with 0 = no bruxing, 1 = no pain and no tooth abrasion, 2 = light pain and no tooth abrasion, 3 = mild pain and some tooth abrasion, 4 = severe joint pain and tooth abrasion, and 5 = continuous bruxing. Participants having a click within the joint and deviant or limited jaw openings were excluded to rule out joint pain that was not mediated by bruxism (Dworkin and LaResche 1992). There were five Brux-2, three Brux-3, and five Brux-4 participants in this group.

**Motor unit recordings.** Each participant sat in a dental chair adjusted for height so that the horizontal plane of his/her upper dental arch was aligned with the upper bite plate of a custom-built mastication apparatus (Türker et al. 2004). Bite plates were coated with a semirigid dental impression material (3M Express; 3M ESPE, St. Paul, MN) that was moulded to each participant's teeth to ensure that contact force and jaw position were similar across participants. The bite bar was coupled to a handmade force transducer [Kyowa (KFG-5-120-C1-11) strain gauge] to monitor the force profiles of the bite. Participants were given a visual display of their exerted bite force on a computer screen. A triangular line was drawn on a transparency and overlain on the computer screen. The participants were instructed to produce a force profile that followed the drawn line with the offset, vertical, and horizontal scales of the computer display adjusted to match the initial level, strength, and speed of the contraction, respectively. The strength and acceleration of the contraction were adjusted to ensure that at least two motor units (a control and test unit, see *Estimation of PIC amplitude*) were recruited during the ascending phase of the contraction. The strength of the contraction was expressed as a percentage of their maximum voluntary contraction (%MVC), which was obtained by averaging the bite force from three maximum contractions. On average, the peak of the contractions was 15–20% MVC, lasted for 15–20 s, with a rate of contraction/relaxation of 1–2% MVC/s.

Intramuscular electrodes were used to record single motor unit action potentials in the masseter muscle. TeXon insulated (except for their tips) silver bipolar wire electrodes (100- $\mu$ m diameter with insulation; 70- $\mu$ m core diameter) were inserted into the deep masseter muscle using a sterile 25-G needle. The needle was then withdrawn, leaving the fish-hooked wires in the belly of the muscle (Prasartwuth et al. 2008). Surface electromyography (EMG) was recorded from the masseter muscle, amplified by 1,000 times and bandpass filtered between 20 and 500 Hz. Intramuscular EMG signals were amplified by 300 times and high-pass filtered at 100 Hz. EMG and force signals were amplified using a CED1902 Quad-system and digitized using a CED Power1401–8 channel converter and Spike 2 (Version 6.07) software using a sampling rate of 20 kHz for the intramuscular EMG, 2 kHz for the surface EMG, and 2 kHz for the force signal. A lip-clip (see Türker et al. 2004 for details) was used as a ground.

**Data analysis.** Data were analyzed offline using spike discrimination software (Spike 2; Cambridge Electronic Design, Cambridge, UK). Single motor unit action potentials were selected by first setting a horizontal threshold that was at least 3 standard deviations above background noise. The selected motor units were then visually sorted based on waveform shape. When possible, the same two units were tracked for every participant (NBrux participants: 39/45 motor unit pairs; Brux participants: 56/65 motor unit pairs).

**Estimation of PIC amplitude.** The amplitude of PIC ( $\Delta F$ ) activation was estimated using the paired motor unit analysis technique (Gorassini et al. 2002, 2004) as follows. The times of occurrences for the single motor unit action potentials obtained in Spike 2 were exported via a text file to Matlab for further analysis in a custom-written Matlab program (The MathWorks, Natick, MA). The instantaneous firing rates of the units were then calculated as the reciprocal of each interspike interval. The firing rate profile of a lower threshold control motor unit was used as a measure of the synaptic input to the motoneuron pool and specifically, to a relatively higher threshold

motor unit, termed the test unit. To calculate the firing rate of the control unit at recruitment and derecruitment of the test unit, a fifth-order polynomial was used to smooth the firing rate profile. The smoothed firing rate of the control unit at recruitment and derecruitment of the test unit was determined automatically, and the  $\Delta F$  measurement was calculated as the difference in smoothed firing rate of the control unit when the test unit was derecruited compared with when it was recruited, i.e.,  $\Delta F = F_{\text{derecruitment}} - F_{\text{recruitment}}$ . The  $\Delta F$ , therefore, corresponds to the reduction in synaptic input needed to counteract the depolarization from the PIC and provides an indirect estimate of PIC amplitude.

For each participant, five contraction trials were selected to calculate the mean  $\Delta F$ . Only contraction trials with symmetrical force profiles were included to ensure equal rates of increases and decreases in synaptic input to the motoneurons. Contractions with abrupt increases or decreases in the force profile, which can effect recruitment and derecruitment of motor units (Nordstrom and Miles 1991), were omitted. Only trials where the control unit fired for at least 2 s before the test unit was recruited were included to ensure that the PIC was fully activated in the control motoneuron given that the calcium component of the PIC can take at least 500 ms to activate (Li et al. 2004; Moritz et al. 2007). This ensured that any changes in firing rate of the control motor unit only reflected changes in synaptic input onto its motoneuron and not from abrupt depolarizations produced during PIC activation. In total, 45 contraction trials ( $5 \times 9$  participants) were used to calculate the mean  $\Delta F$  for the NBruX group and 65 trials ( $5 \times 13$  participants) were used to calculate the mean  $\Delta F$  for the BruX group.

**Common synaptic drive to control and test units.** To ensure that the firing rate of the control motor unit approximated the synaptic input to the test motor unit, we needed to ensure that both units were receiving a common synaptic drive (De Luca and Erim 1994) by determining if both units were being modulated in a similar manner. To do this, the smoothed firing rate of the control unit (fit with a 5th order polynomial) was plotted against the smoothed firing rate of the test unit and the coefficient of determination ( $r^2$ ) of the rate-rate plot was measured. Only trials where  $r^2 \geq 0.7$  were used, ensuring that at least 70% or more of the rate modulation of the test unit could be accounted for by the rate modulation of the control unit. Fifteen of the 110 unit pairs analyzed had  $r^2$  values  $< 0.7$ , which may have resulted from recording units from different functional compartments in the masseter muscle (see Introduction).

**Onion skin effect.** To measure the onion skin effect, we compared the relationship between the mean firing rate and recruitment thresholds for the control and test motor units in a pair from the nine NBruX participants. As mentioned earlier, the higher threshold test motor units were recruited at least 2 s or more after the control units during the ascending phase of the contraction. The recruitment threshold for all motor units was measured as the force at which the motor unit began to fire, expressed as %MVC. Mean firing rates were calculated in a time period when both the control and test motor units were active during the contraction. This ensured that firing rates were measured during equivalent levels of synaptic drive. In addition, firing rates were only measured after the units were securely recruited. For example, slow start-up firing rates of the test motor unit were excluded. In 10 of the 45 NBruX contractions analyzed, a second test

motor unit (*test-2*) that was recruited after the first test unit (*test-1*) was also analyzed and compared with the original control and *test-1* motor units. One participant was excluded as an outlier because the average force, expressed as %MVC, was two times higher than the rest of the participants, most likely due to an underestimation of the true MVC in this participant. In total, the mean firing rate and recruitment threshold of 40 control, 40 *test-1*, and 10 *test-2* motor units were measured.

To determine if motor units within a pair that had large differences in recruitment thresholds also had large differences in mean rates (and vice versa for motor units with small differences in recruitment thresholds), the difference in mean firing rate between the two units in a pair (e.g., *test-1* minus control, *test-2* minus control, and *test-2* minus *test-1*) was plotted against the difference in recruitment threshold between the two units in a pair and a correlation coefficient ( $r$ ) was calculated (60 motor unit pairs in total). Differences in firing rates and recruitment thresholds were measured between motor unit pairs for each participant, rather than comparing values across all motor units in a group, to reduce intersubject variability that can influence the onion skin effect (De Luca and Hostage 2010).

**Statistics.** All statistics were performed using SigmaPlot 11 software (Systat Software). Data presented in text and in Fig. 1, C and D, are means  $\pm$  SD, and data in Figs. 2, C and D, and 4C, are presented as means  $\pm$  SE. Normality for the distribution of  $\Delta F$ , recruitment threshold and mean firing rate values was tested with the Shapiro-Wilk test. One-way ANOVA was used on normally distributed data (e.g., mean firing rates of control, *test-1* and *test-2* units), whereas a one-way ANOVA on ranks was used for nonnormally distributed data (e.g., recruitment thresholds between control and test units). Post hoc Student's  $t$ -tests (Bonferroni corrected) and Dunn's test were used to determine if there were differences in the mean  $\Delta F$  values and motor unit firing properties (e.g., mean rates, difference in recruitment times of control and test units, etc., see Table 1) between the NBruX and BruX groups. Linear regression analysis was used to determine if the differences in recruitment thresholds between control and test units varied linearly with the difference in their mean firing rates and if there was a relationship between  $\Delta F$  values and the peak %MVC force produced during a contraction for both the NBruX and BruX participants. Significance was set to  $P \leq 0.05$ .

## RESULTS

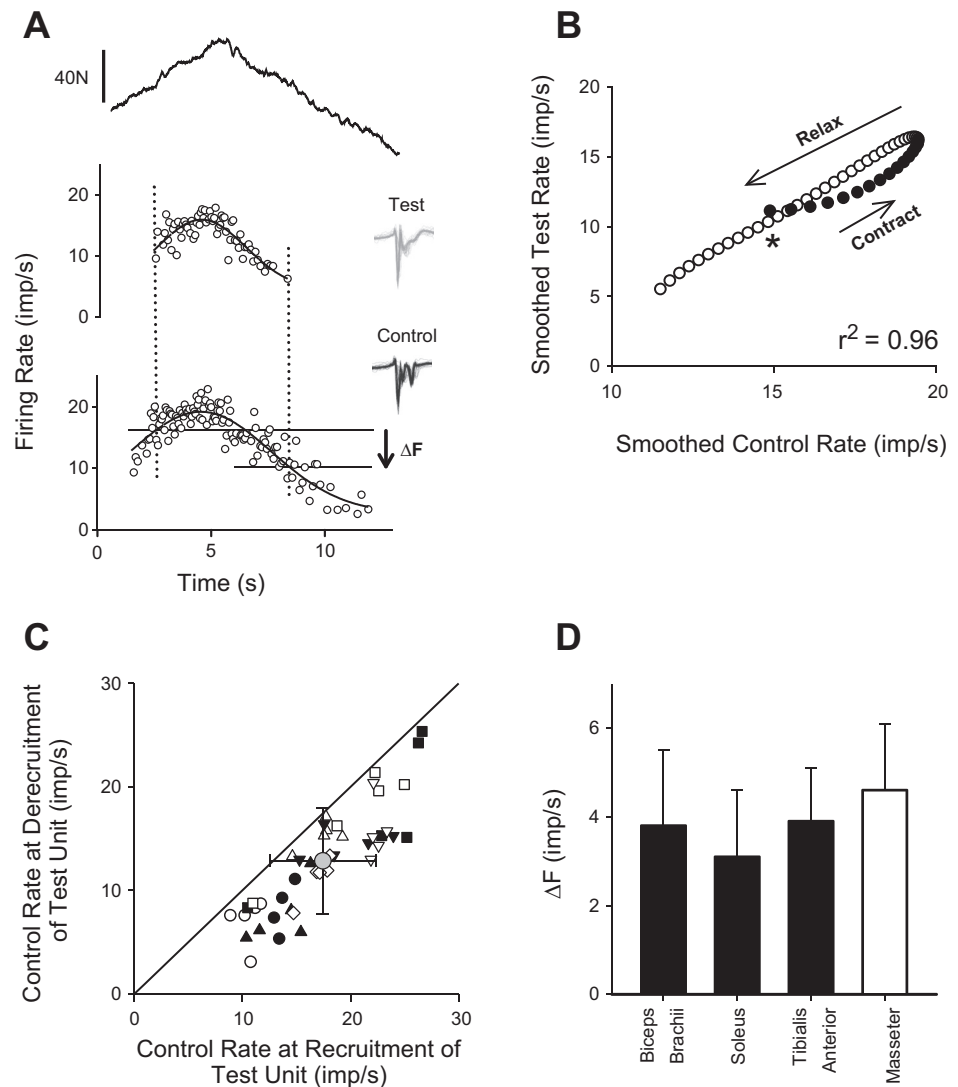
**$\Delta F$ : NBruX control participants.** In the nine NBruX control participants, estimates of PIC amplitude activated in masseter motoneurons were obtained using the paired motor unit analysis technique. Briefly, the firing rate of a lower threshold control unit was used as a measure of the synaptic input to a higher threshold test unit during a triangular voluntary contraction (Fig. 1A). As demonstrated for this NBruX participant, the higher threshold test unit (*middle*) was derecruited at a much lower level of estimated synaptic input (i.e., firing rate of control unit, *bottom*) compared with when it was recruited, to give an estimated PIC amplitude ( $\Delta F$ ) of 3.8 imp/s. That is, to

Table 1. Motor unit firing rate and contraction force characteristics

	Control Mean Rate, imp/s	Test Mean Rate, imp/s	Rate-Rate $r^2$ (range)	Test Activation Time, s	Control-Test Recruit Diff, s	Peak Force, %MVC
NBruX	16.9 $\pm$ 3.3	13.5 $\pm$ 2.0	0.81 $\pm$ 0.1 (0.71–0.95)	3.7 $\pm$ 1.2	2.9 $\pm$ 1.3	12.6 $\pm$ 6.7
BruX	16.0 $\pm$ 4.2	11.8 $\pm$ 2.3	0.81 $\pm$ 0.1 (0.71–0.89)	2.9 $\pm$ 0.7	3.2 $\pm$ 1.2	17.2 $\pm$ 10.5

Values represent means  $\pm$  SD. Comparison of 1) firing rate of control and 2) test motor units during entire contraction; 3) coefficient of variation ( $r^2$ ) between the smoothed firing rate of the control and test motor unit (range in parenthesis); 4) duration of time the test unit was active for during the ascending phase of the contraction; 5) time difference between the recruitment of the control and test unit; and 6) peak force of the contraction [expressed as percent maximum voluntary contraction (%MVC)] for both nonbruxer (NBruX) and bruxer (BruX) groups. For mean  $r^2$ , the median  $r^2$  was calculated for each participant and this value was then averaged across participants in a group. Student's  $t$ -test was used to compare values between groups (all  $P > 0.05$ ).

Fig. 1. The amplitude of persistent inward currents [PICs ( $\Delta F$ )] in nonbruxer (NBrux) participants. **A**: instantaneous firing rate of lower threshold control (*bottom*) and higher threshold test (*middle*) motor unit during isometric contraction (bite force: *top*). Thick black line represents 5th order polynomial (smoothed rate) fit through the firing rates. Dotted vertical lines mark time of recruitment and derecruitment of the test unit. Solid horizontal lines indicate smoothed firing rate of control unit when test unit was recruited and derecruited, with the difference between the 2 rates ( $\Delta F$ ) marked by the arrow. *Insets*: overlain traces of control and test motor units. **B**: smoothed mean firing rate of control unit from **A** plotted against smoothed mean firing rate of test unit during contraction (black circles) and relaxation (open circles) phase of contraction. \*Beginning of test unit firing. **C**: control unit firing rate at time of recruitment of test unit plotted against control unit rate when test unit was derecruited for 45 contractions from the 9 NBrux participants (5 contractions per participant, different symbol for each participant). Solid line marks slope of 1 (parity line). Mean of data is shown by the large gray circle and error bars represent SD. **D**: mean  $\Delta F$ s ( $\pm$ SD) measured in biceps brachii, soleus, and tibialis anterior muscles (black bars) compared with mean  $\Delta F$  for masseter muscle (white bar).



counteract the added depolarization from the PIC to derecruit the test unit, the synaptic input to the test motoneuron had to be reduced by an amount that produced a decrease in the firing rate of the control unit by 3.8 imp/s. To determine if the firing rate of the test motor unit was modulated in a similar manner as the control motor unit, and thus, receiving the same synaptic drive as the control motor unit, the smoothed firing rate of the test unit was plotted against the smoothed firing rate of the control unit (Fig. 1B). The coefficient of determination for the rate-rate plot was high ( $r^2 = 0.96$ ), indicating that 96% of the modulation of the test unit could be accounted for by the modulation of the control unit and that the use of the control unit as a measure of synaptic input to the test unit was justified. The rate-rate plot also shows the hysteretic firing pattern of the test motor unit where, during the descending (relaxation) phase of the contraction (white circles), the test motor unit continued to fire at levels of synaptic input well below the level needed to recruit it (at asterisk), indicative of self-sustained firing due to the sustained depolarization provided by the PIC.

When plotting the smoothed firing rate of the control unit when the test unit was recruited against the smoothed firing rate of the control unit when the test unit was derecruited for all contraction trials ( $n = 45$ ; Fig. 1C, different symbol for each

NBrux participant), all data points fell below the parity line indicating that the test units were derecruited at lower levels of synaptic input than when they were first recruited. The mean  $\Delta F$  measured for masseter motoneurons was  $4.6 \pm 1.5$  imp/s (SD; Fig. 1D) and is in line with  $\Delta F$  values reported in different muscles of the upper and lower limbs [tibialis anterior:  $3.9 \pm 1.2$  imp/s, soleus:  $3.1 \pm 1.5$  imp/s (Gorassini et al. 2002; Udina et al. 2010); biceps brachii:  $3.8 \pm 1.7$  imp/s (Mottram et al. 2009)].

**Onion skin effect.** We also examined in the NBrux participants if masseter motor units display an onion skin effect, i.e., if the lower threshold control motor units had a higher mean firing rate compared with the higher threshold test (*test-1* and *test-2* units, see *Onion skin effect*) motor units. When plotting the firing rates of sequentially recruited control (black circles) and test units (*test-1*: open circles; *test-2*: grey circles; Fig. 2, A and B), the lower threshold control motor units typically had a faster mean firing rate compared with the higher threshold *test-1* or *test-2* motor units. A noticeable onion skin effect was observed in eight of the nine NBrux participants. When averaged across the NBrux group, the control, *test-1*, and *test-2* motor units had sequentially higher thresholds of recruitment

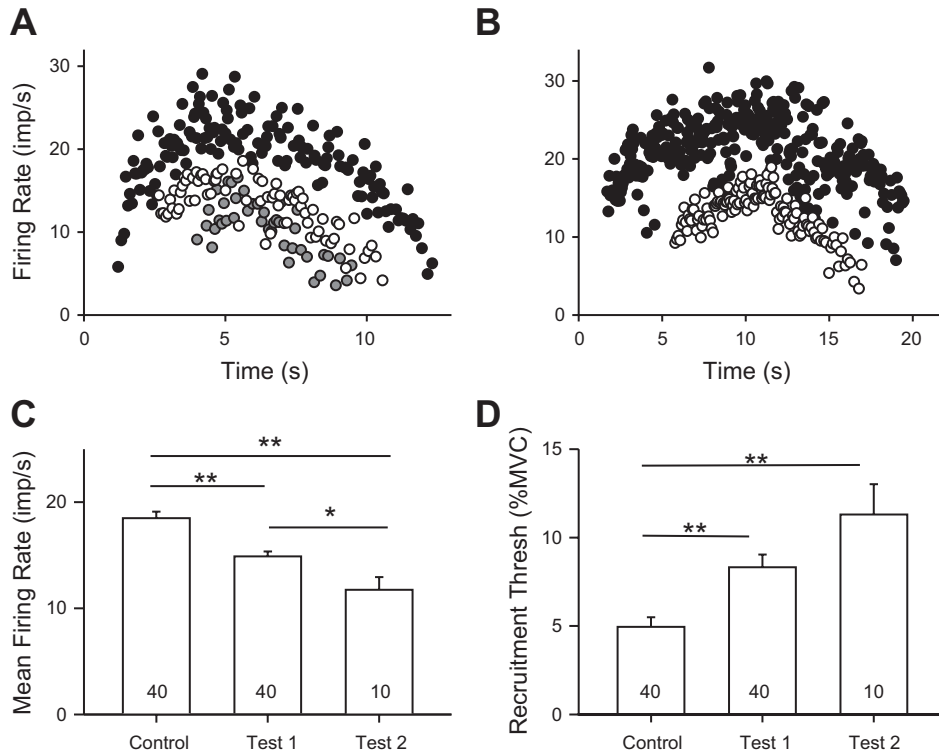


Fig. 2. Mean firing rate and recruitment threshold. *A*: firing rate profiles of three sequentially recruited motor units during an isometric, triangular contraction in a NBruX participant (control: black circles; *test-1*: white circles; *test-2*: gray circles). *B*: same as in *A* for a control and *test-1* unit pair in another NBruX participant. *C*: group mean firing rates of early-recruited control units ( $18.5 \pm 3.9$  imp/s) and later recruited *test-1* ( $14.9 \pm 3.0$  imp/s) and *test-2* ( $11.8 \pm 3.7$  imp/s) units from NBruX participants. Numbers of units analyzed are indicated in each bar graph, and error bars represent means  $\pm$  SE. A one-way ANOVA with post hoc Bonferroni *t*-tests were used. *D*: recruitment thresholds, expressed as a percent maximum voluntary contraction (%MVC) for control ( $5.0 \pm 3.4\%$ ), *test-1* ( $8.3 \pm 4.5\%$ ), and *test-2* ( $11.3 \pm 5.4\%$ ) units. A one-way ANOVA on ranks with post hoc Dunn's test was used. \* $P < 0.05$ , \*\* $P < 0.001$ .

(Fig. 2*D*) and correspondingly, lower mean firing rates (Fig. 2*C*, see values in legend).

To determine if the pairs of motor units with larger differences in recruitment thresholds also had larger differences in mean firing rates, the difference in recruitment threshold ( $\Delta RT$ ) between sequentially recruited units in a pair (e.g., *test-1* minus control, *test-2* minus control, or *test-2* minus *test-1*, Fig. 3*A*) was plotted against the corresponding difference in mean firing rate between the two units in a pair (Fig. 3*B*). There was a significant, linear relationship between the difference in mean rate between units in a pair and the

difference in their recruitment thresholds ( $r = 0.43$ ,  $P = 0.0007$ ).

$\Delta F$ : *Brux participants*.  $\Delta F$  measurements were obtained from Brux participants to determine if the involuntary chewing and teeth clenching present in this group during sleep were associated with larger estimates of PICs compared with control participants, even during awake conditions. The  $\Delta F$  values obtained from the Brux participants were all within the range of values obtained in the control NBruX group, as shown for the two example Brux-2 and Brux-4 participants in Figs. 4, *A* and *B* (Brux-2 = 4.1 imp/s; Brux-4 = 5.8 imp/s). The mean  $\Delta F$

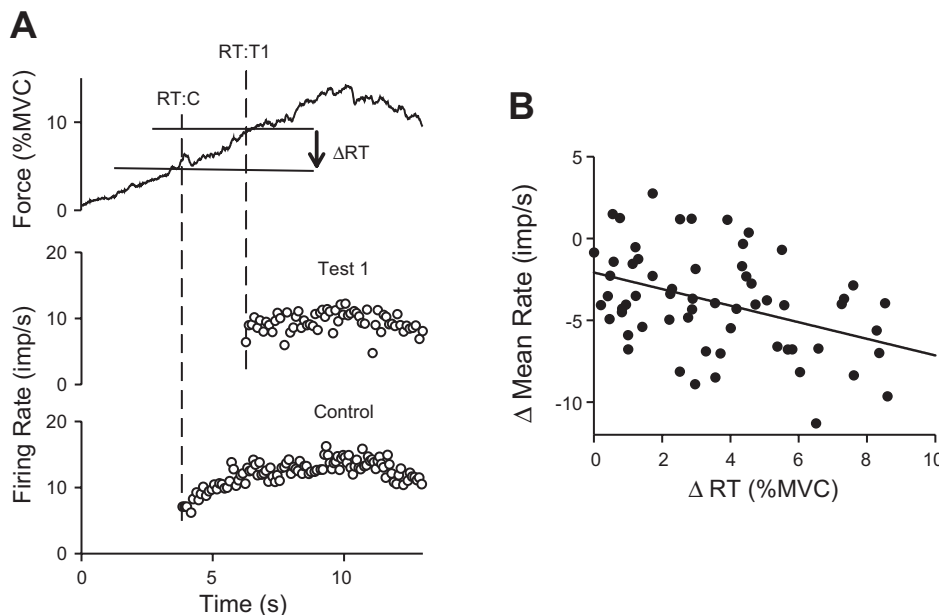


Fig. 3. Differences in recruitment thresholds and mean firing rates. *A*: calculation of recruitment threshold difference between control (*bottom*) and *test-1* (*middle*) motor units during a voluntary contraction (force expressed as %MVC, *top*). Dashed vertical lines mark start of firing of control and *test-1* units and corresponding recruitment forces for the control (RT:C) and *test-1* (RT:T1) units. Arrow marks the difference in recruitment force ( $\Delta RT$ ) between the 2 units. *B*: difference in mean rate between a control and *test-1*, control and *test-2*, or *test-1* and *test-2* motor unit pair plotted against the corresponding difference between their recruitment thresholds ( $n = 60$  unit pairs). A linear regression is fit through the data points ( $r = 0.43$ ,  $P = 0.0007$ ).

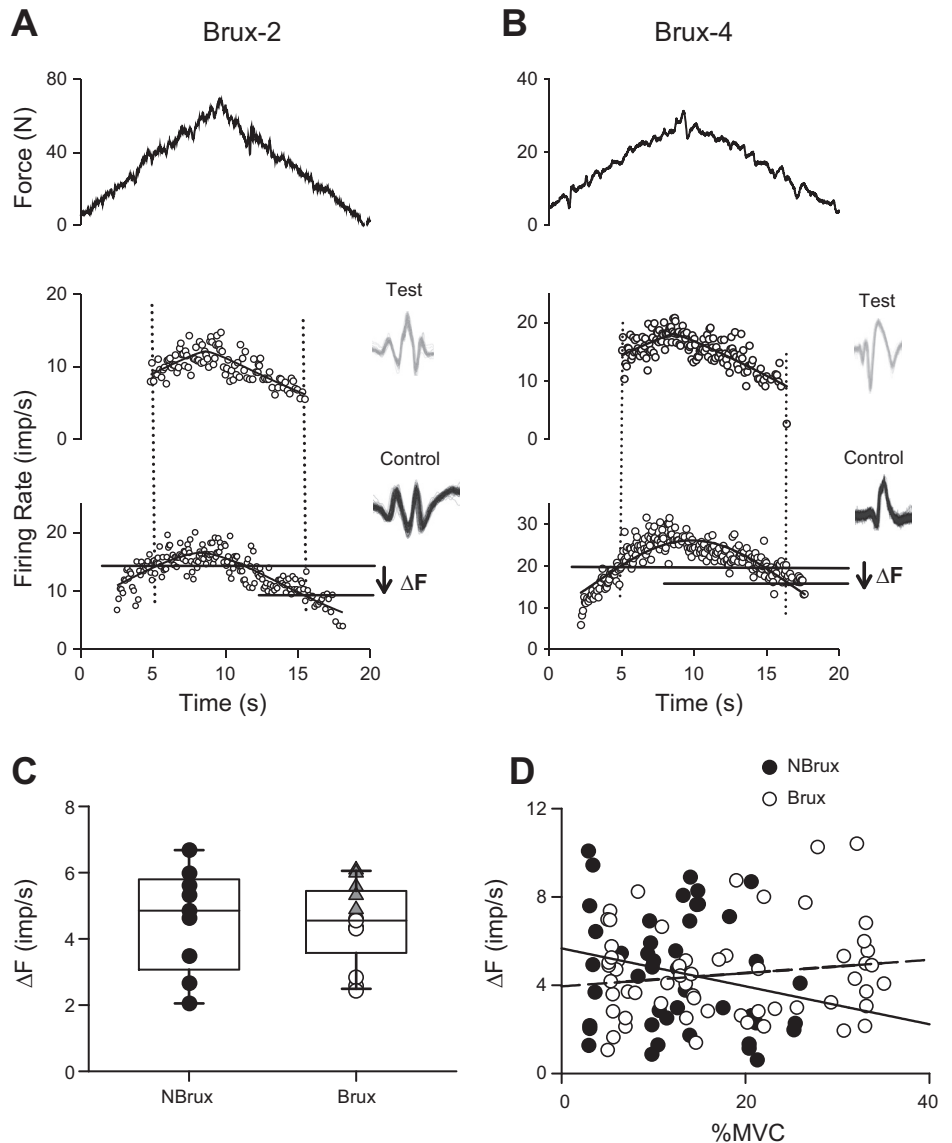


Fig. 4.  $\Delta F$  in Brux participants. *A*: instantaneous firing rate of a lower threshold control (*bottom*) and higher threshold test (*middle*) motor units during isometric contraction in Brux-2 (*A*) and Brux-4 (*B*) participants. Same format as Fig. 1. Note different scales in *A* and *B*. *C*: group mean  $\Delta F$  from NBruX (black bar:  $4.6 \pm 1.6$  imp/s) and Brux (white bar:  $4.5 \pm 1.2$  imp/s) participants (Student's *t*-test,  $P = 0.83$ ). *D*:  $\Delta F$  plotted against peak force (%MVC) reached during each contraction for the 9 NBruX (black circles, solid line,  $n = 45$  contractions) and 13 Brux participants (open circles, dashed line,  $n = 65$  contractions).

in the Brux group ( $4.5 \pm 1.2$  imp/s) was not significantly different than the mean  $\Delta F$  in the control NBruX group ( $4.6 \pm 1.6$  imp/s,  $P = 0.83$ ) with a similar range of  $\Delta F$  values in each group (Fig. 4C). However, the Brux-4 group, who have severe joint pain and tooth abrasion, had  $\Delta F$  values that were all higher than the mean  $\Delta F$  of the NBruX controls ( $5.6 \pm 0.5$  imp/s, gray triangles in Fig. 4C), but this difference was not significant ( $P = 0.19$ ), likely owing to a small number of participants in this group ( $n = 5$ ).

In all, the firing rate profiles of the motor units in the Brux and NBruX groups were similar during the isometric contractions with no differences in mean rates of the control and test motor units measured throughout the contraction (Table 1). In addition, the control and test motor units were modulated in a similar manner in both groups, with a mean  $r^2$  value of  $\sim 0.81$  in the smoothed rate-rate plots. There was at least 3 s of separation between the recruitment time of the control and test motor units in both groups and the test unit was active for at least 3 s during the ascending phase of the contraction (Table 1), two important requisites when estimating PIC amplitude with paired motor unit analysis, as outlined in the DISCUSSION.

On average, the Brux group reached higher peak forces in terms of %MVC during the isometric contraction compared with the NBruX group (Table 1), although the difference was not significant. Moreover, when plotting the size of the  $\Delta F$  against the peak force reached during a contraction (Fig. 4D), there was no relationship between the two for either group (NBruX:  $r = 0.22$ ,  $P = 0.15$ ; Brux:  $r = 0.15$ ,  $P = 0.27$ ).

## DISCUSSION

Similar to animal studies following the application of serotonin or serotonin receptor agonists (Hsiao et al. 2005), PICs are activated in human trigeminal motoneurons as estimated by recording pairs of motor units in the masseter muscle. Unlike the animal experiments recorded *in vitro*, there is likely sufficient endogenous levels of serotonin and/or norepinephrine in the awake human to allow for activation of PICs during voluntary contractions. Excessive monoaminergic drive to trigeminal motoneurons was likely not present in the awake Brux participants, who present with involuntary chewing and teeth clenching during sleep, as indicated by estimates of PIC

amplitudes that were similar to the NBrux controls. Lastly, similar to motoneurons in limb muscles, trigeminal motoneurons display a consistent onion effect where, during moderately sized contractions of 20% MVC or less, lower threshold motor units discharge at higher rates (by 4 to 7 imp/s) compared with higher threshold units.

**Validity of  $\Delta F$  measurements.** Estimating the amplitude of a PIC via paired motor unit analysis relies on how well the control motor unit reflects the level of synaptic input onto the test motor unit since any discharge of the test unit occurring at levels of synaptic input below that needed for recruitment can be attributed to PIC activation. We assume that a PIC is also activated in the control unit but this should not affect its ability to monitor synaptic drive. For instance, after recruitment of a PIC, the firing rate of a motoneuron is linearly related to the injected or synaptic current it receives (Bennett et al. 2001a,b; Gorassini et al. 2004; Hsiao et al. 1997, 1998). Because of this, the firing rate of one motoneuron (control) that receives the same input as another (test) can be used as a measure of input to both motoneurons. The contractions employed in this study were designed to maximize the possibility that the firing rate of the control motor unit indeed reflected the degree of synaptic input to its motoneuron and to the test motoneuron as well. For example, we only used trials where the control motor unit was active for at least 3 s before the test unit (see Table 1) to ensure that the PIC in the control unit was fully or nearly fully activated given that it can take  $\sim 500$  ms for the slow calcium component of the PIC to activate (Li et al. 2004, 2007; Moritz et al. 2007). After PIC activation, any changes in the firing rate of the control motor unit (motoneuron) should mainly reflect changes to its synaptic input and not from an added depolarization during PIC activation, which can occur near the time of recruitment (Li et al. 2004; Udina et al. 2010). Likewise, we only chose trials where the test unit was active for at least 3 s during the ascending phase of the contraction (Table 1), again to ensure that the PIC was fully and securely activated.

As mentioned above, if two motoneurons receive the same synaptic input, then the firing rate of one motoneuron (or motor unit) can be used as a measure of input to the other. One indication that both motoneurons are receiving common inputs is that their firing rates are modulated in a similar manner during a muscle contraction. For this reason, we plotted the relationship between the smoothed firing rates of the control and test motor units and on average 80% of the modulation in firing of the test motor unit could be accounted for by the modulation in firing rate of the control motor unit ( $r^2 = 0.8$  on average, Table 1). The firing rates of the control and test motor units also closely followed the trajectory of the force profiles (see Figs. 1, 3, and 4), indicating that the units were firing within a sensitive range of their input-output properties. In trigeminal motoneurons, the relationship between firing rate and injected current remains linear up to  $\sim 50$  imp/s (Hsiao et al. 1997) when recorded *in vitro*, whereas the firing rate of trigeminal motoneurons or motor units continues to increase with increasing force up until  $\sim 30$  imp/s when recorded *in vivo* (Lund et al. 1979). The peak firing rates of the control units during the  $\leq 20\%$  MVC contractions performed in the current study were  $\leq 30$  imp/s, indicating that the control motoneurons fired in a range that was sensitive to changes in synaptic input. In summary, the firing behavior of the control motor unit in relation to the test motor unit and bite force

suggests that it was a good approximation of synaptic input to the test motor unit, enabling a reasonable estimation of PIC amplitude and its presence in trigeminal motoneurons, similar to that found in animal studies.

**Role of PICs in masseter motoneuron activity.** During motoneuron discharge, CaPICs require long periods (e.g.,  $>500$  ms) of depolarization to fully activate because the afterhyperpolarization (AHP) that follows the spike of the action potential effectively holds the membrane potential below firing threshold to slow down full activation of the voltage-dependent CaPIC (Li and Bennett 2007). Although NaPICs are deactivated and reactivated quickly enough to aide in repetitive firing of the motoneuron (Li et al. 2004), the contribution of the CaPIC is likely more pronounced during prolonged activation of the masseter motoneuron due to its slow activation properties. Examples of prolonged activation of the masseter muscle include involuntary teeth clenching and grinding during sleep in bruxers (Yoshimi et al. 2009) and voluntary teeth clenching and yawning in nonbruxers (Farella et al. 2008). However, the masseter muscle is most active during eating and nonnutritive chewing (Farella et al. 2008) where burst durations are  $\sim 500$  ms (Kato et al. 2011; Po et al. 2013). Even though the CaPIC may not be fully activated during these brief periods of activation, it still may facilitate motoneuron activity. For example, CaPICs have been proposed to mediate the voltage-dependent facilitation of short ( $\sim 800$  ms) locomotor drive potentials in the decerebrate cat (Brownstone et al. 1994). Additionally, because the CaPIC is activated subthreshold to firing, the acceleration in membrane potential produced during CaPIC activation helps to produce high discharge rates at the onset of firing, resulting in the potentiation of force production in the muscle (i.e., catch property: Burke et al. 1970). Thus the NaPIC and CaPIC likely aide in the recruitment, discharge, and synaptic amplification of masseter motoneurons during both brief and prolonged motor activity.

**Onion skin effect.** In eight of the nine NBrux participants, the firing rates of the lower threshold control motor units were faster than the firing rates of the higher threshold test motor units, even near the peak of the  $\sim 20\%$  MVC contraction. The discrepancy in firing rates between motor units of different thresholds could be mediated by differences in how a given synaptic input is transduced in the motoneuron. For example, in the higher threshold motoneurons with lower input resistance (higher conductance), only the top of the synaptic input profile may have reached the axon hillock to produce lower firing rates compared with the lower threshold motoneurons where a larger portion of the synaptic current reaches the axon hillock to produce faster firing. This of course assumes that the motoneuron pool receives equal amounts of a given synaptic input, which may be the case for descending inputs that drive voluntary contractions but can vary for activation of the motoneuron pool by primary spindle afferents (Heckman and Binder 1993a,b; Powers and Binder 1995). If the synaptic drive to the higher threshold motor units increased beyond that used for the  $\sim 20\%$  MVC contractions, the firing rates of these units would likely increase further, potentially matching or even exceeding that of the lower threshold units, especially at very high levels of contraction effort (Bigland and Lippold 1954; Grimby and Hannerz 1976; Heckman and Binder 1993a,b; Kanosue et al. 1979; Kuo et al. 2006; Manuel et al. 2009; Tansey and Botterman 1996).

The strategy of “onion skin” recruitment proposed by De Luca and colleagues helps to relieve the central nervous system of having to modulate the input/output properties of each motoneuron separately (De Luca and Erim 1994; De Luca 1985). It allows for a common synaptic drive to recruit the motoneuron pool in an orderly fashion, whereby fatigue-resistant small motoneurons are recruited first and fire at faster rates, and fatigue-prone larger motoneurons are recruited later and fire at slower rates (De Luca and Hostage 2010). This motor control strategy helps to prevent fatigue, which is relevant to the masticatory system that must produce sustained motor activities such as chewing and talking.

*Amplitude of PICs activated during voluntary contractions is normal in Brux participants.* The presence of involuntary chewing and teeth clenching that occur during sleep in the Brux participants is not associated with abnormally large PICs activated during voluntary contractions under awake conditions. It may be that large PICs are only present during periods of involuntary chewing and teeth clenching given that these involuntary motor behaviors occur during periods of microarousals when monoaminergic drive to the trigeminal motoneuron pool is high (Leung and Mason 1999; Sakai and Crochet 2001; Takahashi et al. 2010). In line with this, drugs such as amphetamine and serotonin reuptake inhibitors, which increase levels of norepinephrine and serotonin, respectively, increase episodes of involuntary activity in Brux participants (Lavigne et al. 2003; See and Tan 2003) and the amplitude of PICs in limb motoneurons (D’Amico et al. 2013; Udina et al. 2010). Thus the amplitude of PICs should, in future studies, be estimated during sleep when involuntary muscle activity is present. The use of noninvasive surface EMG and motor unit action potential decomposition techniques (De Luca et al. 2006; Farina et al. 2004) could facilitate recordings of motor unit activity during microarousals without disrupting sleeping patterns.

*Large PICs in chronic pain?* The Brux-4 group, who are characterized as having chronic pain and tooth abrasion, displayed the highest  $\Delta F$  values that were consistently above the mean  $\Delta F$  measured in the other Brux-2 and Brux-3 participants and in the control NBrux group. Although there was only a trend for the  $\Delta F$  measured in the Brux-4 group to be higher than the  $\Delta F$  measured in controls ( $P = 0.19$ , likely due to the small numbers in this group), it does suggest that the presence of chronic pain may increase the excitability of motoneuron PICs. The experimental induction of pain in the masseter muscle can induce changes in the firing behaviour of motor units and increase the number of motor units recruited during a contraction (Minami et al. 2013; Sohn et al. 2004; Tucker and Hodges 2009). In addition, there is a reduction in both the duration and amplitude of inhibitory reflex responses evoked in the masseter muscle during tonic painful stimulation (Svensson et al. 1999). These findings, including our own, suggest that chronic pain may increase the excitability of trigeminal motoneurons to maintain muscle force, potentially by increasing the amplitude of PICs. Further studies in more Brux-4 participants or during periods of experimentally induced pain are needed to resolve this issue.

*Conclusions.* Similar to animal studies, PICs are activated in trigeminal motoneurons during voluntary contractions in the human. Both the onion skin effect and the activation of PICs likely facilitate the sustained activation of the masseter muscle

which is required during motor activities such as eating, nonnutritive chewing, clenching and yawning.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

#### AUTHOR CONTRIBUTIONS

Author contributions: J.M.D. analyzed data; J.M.D., S.U.Y., M.A.G., and K.S.T. interpreted results of experiments; J.M.D. and M.A.G. prepared figures; J.M.D. and M.A.G. drafted manuscript; J.M.D., S.U.Y., M.A.G., and K.S.T. edited and revised manuscript; J.M.D., S.U.Y., E.S.A., M.A.G., and K.S.T. approved final version of manuscript; S.U.Y., A.S., E.S.A., M.A.G., and K.S.T. performed experiments; M.A.G. and K.S.T. conception and design of research.

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