A Prolongation of the Postspike Afterhyperpolarization Following Spike Trains Can Partly Explain the Lower Firing Rates at Derecruitment Than Those at Recruitment

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Wienecke J, Zhang M, Hultborn H. A prolongation of the postspike afterhyperpolarization following spike trains can partly explain the lower firing rates at derecruitment than those at recruitment. J Neurophysiol 102: 3698–3710, 2009. First published October 21, 2009; doi:10.1152/jn.90995.2008. The original motivation for this study was the observation in previous human experiments that the motor neuron firing frequency (recorded from the motor units in the EMG) was lower at derecruitment than that at recruitment, with slow linearly varying voluntary contractions. Are the lower firing rates at derecruitment correlated with a change in the postspike afterhyperpolarization (AHP) after preceding spike trains? This question was investigated by intracellular recordings from cat motor neurons in both unanesthetized and anesthetized preparations. The firing frequencies at recruitment and derecruitment were compared during slow triangular current injections mimicking the slow linearly varying voluntary contractions in humans. There was a lower frequency at derecruitment in almost all motor neurons (83 of 86 motor neurons; mean = 3.35 Hz). Thus intrinsic mechanisms play an important role for the lower frequencies at derecruitment. This was independent of whether the current injection had activated persistent inward current (PIC; plateau potentials, secondary range firing). It was found that a preceding spike train could prolong the AHP duration following a subsequent spike. The lower rate at derecruitment matches the prolongation of the AHP. However, a quantitative comparison between the lowest firing frequency and AHP duration for individual motor neurons reveals that the predicted lowest firing frequency does not match the absolute AHP duration; the lowest frequency is lower than that predicted from AHP duration in fast motoneurons and higher than expected in slow motoneurons. It is suggested that these deviations are explained by the presence of synaptic noise as well as recruitment of PICs below firing threshold. Thus synaptic noise may allow spike discharge even after the end of the AHP in “fast” motor neurons, whereas synaptic noise and PICs below spike threshold tend to give higher minimum firing frequencies in “slow” motor neurons than predicted from AHP duration.

I N T R O D U C T I O N

Observations from earlier experiments using human subjects—that motor neuron firing frequencies at the termination of a spike train (recorded from the motor units in the electromyography [EMG]) were lower than those at recruitment with slow linearly varying contractions (Christova and Kossev 1998; De Luca et al. 1982; Gorassini et al. 2002a; Romaiguere et al. 1993)—were the motivation behind the current study. From these former studies it could not be concluded whether the difference in firing rate at recruitment and derecruitment was due to differences in the synaptic input or to changes in intrinsic properties that determine spike frequency. This phenomenon has also been illustrated or described in acute experimental settings (Bennett et al. 2001a; Button et al. 2006; Hounsgaard et al. 1988; Lee and Heckman 1998). In these experiments, the “input” consisted of a slowly rising ramp of current injected into the motor neurons, followed by an equally slow falling ramp. As observed in the human studies, the frequency of discharge at derecruitment was often less than that at recruitment. Thus it appears obvious that the intrinsic properties in the motor neurons themselves must play an important role in this phenomenon.

The relationship between synaptic excitation (excitatory drive) and the firing frequency for motor neurons has been the topic of many investigations since the pioneering studies by Adrian in the late 1920s demonstrating a frequency modulation (“FM”) following recruitment (Adrian and Bronk 1929). They had postulated a “relative refractoriness” following the spike as the basis for shorter interspike intervals (ISIs) with stronger excitation and this was later to be visualized as the postspike afterhyperpolarization (AHP) (Brock et al. 1952) as Eccles and collaborators introduced the technique of intracellular recordings in the beginning of the 1950s. It was soon demonstrated that the AHP had a longer duration in motor neurons supplying slow muscles than that in motor neurons innervating fast muscles in accordance with the lower firing frequencies observed for slow motor units than for fast ones (Eccles et al. 1958). The input/output relation was further studied by Granit, Kerrnell, and colleagues (Granit et al. 1963; Kerrnell 1965a,b) using intracellularly injected currents (through the intracellular recording microelectrode) to mimic synaptic excitation. Kerrnell (1965b) described that the lowest firing rates (i.e., the longest ISIs) obtained with long-lasting rectangular current pulses were equivalent to the duration of the AHP. With increasing current intensities the firing frequency increased linearly (“primary range”), sometimes followed by a second steeper linear part (“secondary range”) (Kerrnell 1965a).

The AHP is caused by a K+ current [I_{K,Ca(SK)}] that is elicited by Ca2+ entry during the action potential (Hounsgaard and Mintz 1988; Krnjevic et al. 1978; Schwindt and Crill 1984). Increased influx of calcium with two or more action potentials with short interval results in a larger amplitude of the AHP (Gustafsson 1974; Ito and Oshima 1962; Sacchi et al. 1995). There are a number of conductances in addition to the AHP I_{K,Ca(SK)} that could affect the trajectory of the AHP and conse-
First, the sag current (I_sag) range (Hounsgaard et al. 1988; for reviews see Heckman et al. 2003, 2007) developing after a larger number of action potentials. Nevertheless, a progressive increase in the spike threshold (V_th) caused by partial Na⁺ conductance inactivation may also contribute to the adaptation (Bradley and Somjen 1961; Miles et al. 2005; Powers et al. 1999; for reviews see Kernell 2006; Rekling et al. 2000). In addition there is evidence for a Na⁺-induced potassium current (I_KNa) (Safronov and Vogel 1996; Wallén et al. 2002, 2003, 2007) developing after a larger number of action potentials. Following longer-lasting repetitive discharges a “late adaptation” is seen (Kernell and Monster 1982), but the mechanism behind this remains unknown (Kernell 2006).

Returning to our original question, we thus ask whether the lower firing rate at derecruitment than that at recruitment is a consequence of changes in intrinsic properties regulating the firing? We will systematically investigate the lower frequency at derecruitment with intracellular current injections in different preparations (unanesthetized vs. anesthetized) with and without activating PICs during the spike train. We will also demonstrate that the AHP is prolonged following preceding trains of action potentials. Nevertheless, a quantitative comparison between AHP duration and lowest frequencies at derecruitment for individual motor neurons demonstrates that AHP duration alone cannot fully explain the difference in firing rate at derecruitment. In addition to the AHP duration, synaptic noise may allow spike discharge even after the end of the AHP without activating PICs during the spike train. We will also discuss the findings in relation to experiments for estimating plateau potentials in human subjects using the firing profile of simultaneously recorded motor units. Some of the results have been previously published in abstract form (Wienecke et al. 2005a,b).

METHODS

The results of this study were obtained from 20 cats (2.5–4 kg). In all cats anesthesia was induced using isoflurane and for 16 cats anesthesia was maintained with isoflurane and nitrous oxide (N₂O) until they were decerebrated; the anesthesia was terminated after the surgical procedures 5–6 h after the ligation of the vessels (referred to as “unanesthetized” preparation). The 4 remaining cats were intraperitoneally administered Mebumal (i.e., pentobarbital, 40 mg/kg) subsequent to the initial induction with isoflurane) and this initial dose was supplemented with 2 mg·kg⁻¹·h⁻¹ (intravenous [iv]) throughout the experiment (referred to as “unanesthetized preparations”). The experimental protocol was approved by the Danish Animal Experimentation Inspectorate.

Further information regarding the decerebrate preparation

During surgery the animals were kept under deep anesthesia with isoflurane (2–2.5%) mixed with N₂O (50%) and oxygen (50%). During anesthesia the cats were anemically decerebrated by ligating the basilar and both common carotid arteries, a procedure that has been shown to produce a decerebration that involves all cortical tissue above the pons, where the basilar ligature is placed (Crone et al. 1988). Anesthetics used in the lastcommon carotid was often used for an additional 5 min at the end of the surgical procedure (5–6 h), immediately prior to the onset of general anesthesia. The decerebration was then confirmed to be clinically complete by the development of tonic extensor muscle tone (alpha rigidity), lack of spontaneous movements, and large nonreactive pupils (Crone et al. 1988). In some cats stereotyped stepping movements developed and, in these cases, anesthesia was immediately reinstated and the brain removed rostral to an intercollicular section. In these cases the brain was found to be necrotic and it was concluded that the stepping movements must have originated from the output brainstem centers. Following these procedures and tests, pancuronium bromide (0.6 mg·h⁻¹) was given to block neuromuscular transmission and artificial respiration was initiated.

General maintenance of the preparation

After induction of anesthesia the animals were intubated and cannulas were inserted in one of the carotid arteries for monitoring blood pressure and one of the jugular veins for the administration of fluid and drugs. A urine catheter was inserted to prevent bladder overfilling. A rectal thermometer was inserted and used for an automatic temperature control of the preparation. Atropine (0.1 mg·kg⁻¹, subcutaneous) and dexamethasone (1 mg·kg⁻¹, iv) were administered at the beginning of the experiment, whereas a buffer solution (10% dextrose and 1.7% NaHCO₃) was infused continuously (4.5 ml·h⁻¹).

The expired CO₂ was maintained between 3.0 and 5.0% during artificial ventilation and the blood pressure maintained by infusion of dextran when needed. The temperature of the animal was kept at about 38°C by a servo-controlled heating system. At the end of the experiments the animals were killed by an overdose of Mebumal (i.e., pentobarbital).

Nerve preparation, laminectomy, stimulation, and recording

Nerve dissection, laminectomy, stimulation, and recording followed routine procedures (see Bennett et al. 1998 for details). The following nerves from the left hindlimb were cut and dissected for stimulation: anterior biceps and semimembranosus, posterior biceps and semitendinosus, medial gastrocnemius, lateral gastrocnemius plus soleus (in a few experiments soleus was dissected separately), flexor digitorum and hallucis longus, plantaris, and the deep peroneal nerve. To expose the lumbar spinal cord (L6–S1 spinal cord segments) a laminectomy was performed and the animals were transferred to a rigid frame, which secured and stabilized the spinal column. In the rigid (recording) frame the skin flaps around the exposed areas of the spinal cord and the hindlimb were sewn and retracted to form pools that were filled with warm paraffin oil. The hindlimb nerves were mounted on silver electrodes. Pneumothorax was performed bilaterally prior to recording to reduce movement artifacts. J

N. Physiol. • VOL 102 • DECEMBER 2009 • www.jn.org

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After removing the dura the L6, L7, and S1 dorsal root were cut to allow better access to the motor neurons.

The intracellular recordings were made with potassium acetate-filled glass microelectrodes with an Axoclamp 2B amplifier (Axon Instruments) in either standard bridge mode or in discontinuous current-clamp (DCC) mode. The DCC mode allowed for more accurate measurements of membrane potential despite changes in electrode resistance with injected current. For comparison of synaptic noise we performed a fast Fourier transformation (FFT) of recordings made in standard bridge mode. We used a function in the Spike2 software (Cambridge Electronic Design, Cambridge, UK) for calculation of the variance of the membrane potential and the FFT. The data were collected onto two computer systems, a Canadian software-based QNX-system [developed by the Winnipeg Spinal Cord Research Center to run under real-time Unix (Concurrent 5450) computer] and a PC with Spike2 software.

**Experimental protocol**

Following penetration of a motor neuron, the basic properties of the cell were measured, including antidromic identification, resting membrane potential, spike height, afterhyperpolarization (AHP) duration, input resistance ($R_{in}$), rheobase, and the $f$–$I$ slopes for primary and secondary ranges (Granit et al. 1963; Kernell 1965a,b). The AHP was measured to the peak of the late afterdepolarization (ADP, if it was present) as described by Kernell (1965b; see arrow in Fig. 2A). The sag (Gustafsson and Pinter 1985; Ito and Oshima 1965) was measured by injecting a 5-nA hyperpolarizing pulse of 300-ms duration. Cells with resting membrane potentials more negative than −60 mV and a spike height of >65 mV (thus with a positive overshoot) were included in the results. Plateau properties were inferred from 1) self-sustained firing following depolarizing current pulses, 2) frequency acceleration during constant-current injection, and 3) a counterclockwise $f$–$I$ hysteresis in response to triangular current pulses (Hounsgaard et al. 1988).

**Testing firing rates at recruitment and derecruitment**

As illustrated in Fig. 1 the firing frequencies at recruitment and derecruitment were compared during slow triangular current injections. The slope of the current injection was usually in the range of 3–8 nA/s; for some motor neurons it was systematically varied to compare fast and slow slopes (see following text). The total period of current injection was usually 6–12 s (mainly dependent on rheobase). The peak firing frequency was different since the ascending phase of current injection was manually terminated (and switched to the descending phase) as soon as the spike showed signs of inactivation. In some cases the firing reached a secondary range (and thus the spike train contained a large number of spikes); in other cases the peak frequency was low (and the total number of spikes was small). In several cases we compared the effect of short-lasting low-frequency spike trains with long-lasting high-frequency discharges in the same motor neuron.

**Testing the AHP duration and the sag amplitude and their dependence on preceding spike trains**

The experimental protocol is illustrated in Fig. 2. AHP durations were measured from the end of the spike to the peak of the late afterdepolarization (ADP; Fig. 2A) and to the half-amplitude AHP decay (measured as in Zenegel et al. 1985). Test AHPs were elicited by spikes triggered by brief (1-ms) depolarizing pulses, under control conditions they were evoked at 1 Hz ($n > 50$; Fig. 2B, red). The conditioning discharge was obtained by injecting increasingly more current for 5–8 s. It was then abruptly terminated and the pulse-evoked spike of the test AHP triggered after a delay of 200–300 ms, depending on when the membrane potential had returned to a stable level after the end of the conditioning ramp current ($n = 6–20$; with intervals of 10–20 s; Fig. 2B, black). The duration of the conditioning spike train could be varied (longer duration to obtain more conditioning spikes at higher frequency and elicit “plateau currents” if possible; shorter duration to avoid activating “plateau currents”). Finally, control test AHPs were again evoked to confirm that they were the same as before the conditioning ramps (to ensure the stability of recording conditions; Fig. 2B, green). The procedure for testing the sag process was performed in the same way as that for the AHP, but using the aforementioned hyperpolarizing current pulse instead of the 1-ms depolarizing pulse evoking the spikes. The sag is defined as the difference between the early peak response and the following plateau (during the hyperpolarizing pulse) divided by the size of the peak response. We measured the sag 200 ms after the peak response as did Gustafsson and Pinter (1984).
Each point represents averaged values (the trial was repeated 3–10 times) for individual motor neurons. The lower frequencies at derecruitment were seen in almost all motor neurons (83 of 86 motor neurons). On average, the derecruitment rate was 3.34 Hz lower than the recruitment rate. There was no difference between unanesthetized and anesthetized preparations. When the current slope was reduced (by 2.5-fold) the end frequency at derecruitment was reduced (in 46 of 53 motor neurons; generally around 10–20%, although some motor neurons decreased derecruitment rate by 50%). As can be intuitively understood from Fig. 1, a slow reduction of injected current would optimize the chance of reaching firing threshold, “once more” with a long ISI, before finally reaching a membrane potential below spike threshold. The number of spikes (short- vs. long-lasting trains) did not appear to be of importance for the amount of rate difference between recruitment and derecruitment (data from 61 motor neurons). We were thus able to observe remarkably similar differences between recruitment and derecruitment rates in individual motor neurons independent of the number of spikes and peak frequencies in response to the triangular current injection. This is illustrated in Fig. 3B for a motor neuron from an unanesthetized preparation. Here the difference between the recruitment and derecruitment rates with a short- and long-lasting triangular current injection (with the same slope) is illustrated. For the ascending part of the long-lasting triangular current injection (E) it can be seen that the f–I slope becomes much steeper at a current injection of about 12 nA (corresponding to a firing rate of 35 Hz); this we interpret as the recruitment of a substantial PIC. The frequency reaches about 70 Hz before the injected current is reversed and it is seen that the firing frequency is maintained at higher level during the descending phase of the current injection (F). There is a counterclockwise hysteresis reflecting the recruitment of PIC. With a shorter-lasting current injection (ascending phase ♦; descending phase ◆) the firing frequency stayed <25 Hz, well within the primary range, and there was no sign of a hysteresis. In both cases the firing rates were lower at derecruitment than those at recruitment.

From Fig. 3A it appears that the largest difference between recruitment and derecruitment is seen for motor neurons with a high initial frequency at recruitment. When the material was divided into “slow” and “fast” units (divided on the basis of

FIG. 2. Experimental protocol for testing the prolongation of afterhyperpolarization (AHP) duration by preceding spike trains. A: the postspike AHP and late afterdepolarization (ADP; an overshoot). The AHP duration is measured from the end of the spike to the peak of the ADP; see arrow (method similar to that reported by Kernell 1965b). The illustrated AHP is the average of 50 pulse-evoked spikes. B: the experimental paradigm for investigating the prolongation of AHP duration following preceding spike trains. Top traces are recordings of the membrane potential (and action potentials). Bottom records monitor the amount of current injected through the recording microelectrode. Further description is in the text. C: averaged records of the control test AHPs before (red; >50 AHPs averaged) and after (at 1 Hz; >50 AHPs averaged; green) testing the effect of conditioning spike trains. The conditioned test AHPs (black) is more noisy because it is the average of only 10 responses. Intracellular recording was done in DCC mode (see METHODS).
differences in AHP duration at rest) it was seen that motor neurons with short AHPs show a larger difference between recruitment and derecruitment rates than motor neurons with long AHPs. This relationship appears to be comparable for the unanesthetized and anesthetized preparations (see regression lines in Fig. 3A), although the difference between “slow” and “fast” motor neurons was significant only for the anesthetized preparation [Fig. 3C: unanesthetized; no significant difference; Fig. 3D: anesthetized; \( P < 0.01 \) only between “fast” (AHP <75 ms) and “slow” (AHP >120 ms) motor neurons]. The lack of a significant difference in the unanesthetized group may depend on a too small number of motor neurons with an AHP >120 ms (thus all motor neurons with >75 ms were pooled together). Altogether, the lack of statistical significance for the amount of difference in recruitment/derecruitment frequencies in the unanesthetized material (Fig. 3C) leaves the relation to motor unit type open for further experimental evaluation.

**Relationship between AHP duration and minimum firing frequency during 2-s current pulses**

Kernell (1965b) demonstrated that the minimal repetitive firing frequency is determined by the duration of the AHP. Since this relationship is central to the present study we decided to collect our own independent sample. This relationship was tested for 24 motor neurons under barbiturate anesthesia. Figure 4A illustrates the results with the AHP duration on the abscissa (converted to Hz) and the minimal firing frequency on the ordinate (also in Hz). The slope of the regression line is 0.85 (\( R^2 = 0.79 \)); with a slope of 1.0 the minimal frequency would be fully explained by the AHP duration. It is obvious that motor neurons with AHPs of long duration had higher minimal firing frequencies than would be expected given by the AHP duration. When the same data are replotted with the AHP duration expressed in milliseconds (abscissa) against the minimal frequency (expressed as an ISI in milliseconds; ordinate) (Fig. 4B) it becomes increasingly obvious that the relation deviates from the unity line; the slope of the regression line is now 0.55 (\( R^2 = 0.93 \)). Why is the minimal firing frequency higher than would be expected from the AHP duration for “slow” motor neurons? In Fig. 4C we have superimposed the AHP trajectory seen following the averaged pulse-evoked single spike (red trace) onto two AHP trajectories observed during rhythmic discharge at the lowest possible discharge frequency. It can then be seen clearly that the trajectory during repetitive discharge is suddenly accelerating (indicated by black arrow) to reach firing threshold at a time point long before the end of the AHP (indicated by red arrow) following a single spike. This suggests that PICs evoked below spike threshold (and before the end of the previous AHP) are of importance for the initiation of spikes (see Discussion for further details). Note that the trajectories of the “minimal firing” (black trace) and the pulse-evoked spike AHP (red) are superimposed at the end of the spikes to facilitate comparison. In reality the membrane potential during the averaging of the AHPs following the pulse-evoked spikes was slightly more hyperpolarized to avoid spontaneous spiking.
The AHP durations used in Fig. 4 were measured at resting membrane potential. Would it be possible that the duration of the AHP changed with membrane potential? To avoid complications as a result of (uncontrolled) voltage-dependent ion conductances the most relevant membrane potential for the comparisons with minimal firing frequencies would of course be close to (although below) firing threshold. We therefore estimated the AHP duration for the same 24 motor neurons both at the spontaneous “resting” membrane potential and additionally “just below” firing threshold. The change in the measured AHP durations is indeed dependent on depolarization, as illustrated in Fig. 5A. Somewhat surprisingly we observed that the late ADP increased in amplitude with more depolarized levels (Fig. 5B). After dividing the motor neurons into two groups (“fast” with AHP <75 ms; “slow” with AHP >120 ms) it was evident that the change in ADP was twofold more sensitive (in μV/mV depolarization of the membrane potential) for “slow” motor neurons than that for “fast” ones (P < 0.05).

**Prolongation of the AHP duration following preceding spike trains**

The possible change in duration of the AHP after a single spike (test) induced by a conditioning train of impulses to a ramp depolarization was tested in 66 motor neurons (unanes-
For all motor neurons the amplitude and duration of the control (unconditioned) test AHP were the same before and after the series of conditioning ramps. This is shown for one single motor neuron in Fig. 2, which illustrates the experimental protocol. The results are summarized in Fig. 6A (unanesthetized; ○) and Fig. 6B (anesthetized; △). A prolongation of the conditioned test AHP duration was seen in 58 of 66 motor neurons when measured to the peak of the ADP. Zengel et al. (1985) demonstrated that the half-amplitude AHP duration gives a more consistent measure (i.e., it is easier to determine than the peak of the ADP, which is more affected by the synaptic noise). To support our data we also measured the duration of the half-amplitude AHP as described earlier and, consequently, 65 of 66 motor neurons then showed a prolongation. In Fig. 6 the average AHP prolongation for all 66 motor neurons was 11.5%. The average AHP prolongation for motor neurons (n = 46) from unanesthetized preparations (○) is plotted against the AHP duration when conditioned by a spike train evoked by the ramp-current injection (abscissa). See Fig. 2 for an illustration of the experimental protocol. If the control AHP had the same duration as that of the conditioned AHP, the data points would fall on the interrupted diagonal line. The slope of the regression line is 0.69 (R² = 0.88). The average prolongation of the AHP is 15.3%. B: relation for motor neurons (n = 20) for anesthetized preparations (△). The slope of the (solid) regression line is 0.926 (R² = 0.93). The average prolongation of the AHP is 7.61%. The ✖ signs refer to those 9 motor neurons where conditioning protocol were performed at depolarized membrane potentials close to firing threshold. This did not change the slope of the (dashed) regression line (0.923, R² = 0.83), but the average AHP prolongation by the conditioning spike train was larger (average prolongation by 21.4%). C: an illustration from the motor neuron with the most affected AHP trajectory (from B, tibial motor neuron in anesthetized preparations) when conditioned at a membrane potential just below spike threshold. There are 2 green traces: one is the control AHP at resting membrane potential (bottom) and the other is the control AHP at elevated membrane potential just subthreshold to spike initiation (the top green trace, abbreviated m.p. subthreshold). The blue trace is the conditioned AHP at resting membrane potentials without background current injection. The black trace is the conditioned AHP at membrane potentials just subthreshold to the spike initiation (abbreviated: conditioned AHP m.p. subthreshold). Red traces are the SD. Scale bars are the same for all traces. Color-coded arrows indicate the peak of the late afterdepolarization to where the AHP is measured. In this illustration both the AHP duration and the half-amplitude AHP duration are prolonged in the conditioned situation (blue trace) and both measures are even further prolonged in the depolarized situation (black trace). Further it is illustrated that the control AHP becomes prolonged just by elevation of the membrane potential (compare the 2 green traces; this is also shown in Fig. 5A).
for the unanesthetized preparation was 15.3% and for the anesthetized preparation 7.61%. In Fig. 6 each point represents one motor neuron and they are plotted with the duration of the unconditioned (control) AHP on the ordinate and the duration of the conditioned AHP on the abscissa. Slopes of the regression lines were 0.69 and 0.93 for unanesthetized (Fig. 6A) and anesthetized (Fig. 6B) preparations, respectively. There was no difference in the lengthening of the AHP between the groups of motor neurons for which the conditioning ramp caused only primary range firing or reached into secondary range (tested in 17 motor neurons from unanesthetized preparation).

The test AHPs (control as well as conditioned) described herein were elicited at resting membrane potentials (no bias current). In nine motor neurons (from the anesthetized preparation) it was possible to additionally perform the whole sequence of control test and conditioning test when the membrane potential was kept close to the firing threshold using a biasing depolarizing current. In all these motor neurons (X in Fig. 6B) the conditioned AHP was more prolonged (about threefold, from 7.61 to 21.4% on average; range 3.3–46%) when the membrane potential was kept close to threshold, compared with resting membrane potential. The most dramatic example of this is illustrated in Fig. 6C. There were no significant differences between “fast” and “slow” motor neurons.

Why is the AHP duration prolonged by a preceding spike train? As mentioned earlier Gustafsson and Pinter (1984) suggested that the sag seen during hyperpolarizing current train? As mentioned earlier Gustafsson and Pinter (1984) suggested that the sag seen during hyperpolarizing current pulses (activation of $I_h$) may contribute to a shortening of the AHP trajectory. Therefore it would be interesting to test whether the sag seen with a hyperpolarizing current pulse would change with a conditioning protocol similar to that used for the test AHP. Only 11 motor neurons were tested with this protocol. In 10 of the 11 motor neurons the sag increased when conditioned (by 15%; range, 6–37%; $P < 0.05$). It was also seen that the postinhibitory rebound (the overshoot) increased in 9 of 11 motor neurons (by 16%; range, 5–43%; $P < 0.05$) as a result of the conditioning spike train.

**Comparison of AHP duration following a spike train with firing frequency at derecruitment**

The results of Figs. 1 and 3A demonstrate the robust finding that the firing frequency is lower at derecruitment than that at recruitment. Could the prolongation of the AHP duration following a preceding spike train (Fig. 6) explain this difference? In Fig. 7 the duration of the conditioned (prolonged) AHP (abscissa) is plotted against the last ISI (derecruitment; ordinate) for the same motor neurons illustrated in Fig. 6. From this it is obvious that the regression slopes are far from the 1.0 that would be expected if the conditioned AHP duration would fully determine the last ISI at derecruitment. In Fig. 7A (unanesthetized preparation) the regression slope is 0.53 ($R^2 = 0.30$) and in Fig. 7B (anesthetized) the slope is 0.44 ($R^2 = 0.61$). Note that the values for the duration of the conditioned AHPs for the nine motor neurons investigated close to firing threshold are included in the graph (X); it does not appear to change the regression line (compare continuous and interrupted lines in Fig. 7B).

There does appear to be a surprising difference between the unanesthetized and anesthetized preparations, although the slopes of the regression lines look similar. In the unanesthetized preparation the “fast” motor neurons appear to have a longer last ISI than would be expected from the AHP duration, whereas it is the “slow” motor neurons that have a shorter last ISI than would be expected from the AHP duration in the anesthetized preparation. It would appear as if there is a parallel shift that separates the relations in the unanesthetized and anesthetized preparations. For the same duration of (conditioned) AHP the last ISI is longer in the unanesthetized than that in the anesthetized preparation.

The significance of the prolongation of the AHP for the lower derecruitment rates is also illustrated in Fig. 8 for a

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**FIG. 7.** Comparison between the last interspike interval (ISI) (derecruitment) with the duration of the conditioned AHP. The interrupted diagonal lines in A and B show the expected relation if the last ISI was fully determined by the duration of the conditioned (prolonged) AHP. A: data from unanesthetized decerebrate preparations (○) (including the 4 motor neurons that did not have a prolongation of the AHP duration). The slope of the regression line is 0.53 and $R^2 = 0.30$. B: data from anesthetized preparations (including the 4 motor neurons that did not have a prolongation of the AHP duration). The ○ symbols refer to the AHP durations obtained and measured at resting membrane potential. The slope of the (solid) regression line is 0.436 and $R^2 = 0.61$. The × symbols refer to 9 motor neurons with depolarized membrane potential during the conditioning and measurement of the AHP duration (same as in Fig. 6B). The slope of the (dashed) regression line is 0.477 and $R^2 = 0.86$. 

*J Neurophysiol* • VOL 102 • DECEMBER 2009 • www.jn.org
"fast" motor neuron from an unanesthetized preparation. The recordings of firing frequency at recruitment (Fig. 8, top traces) and derecruitment (Fig. 8, bottom traces) during slow triangular current injections originate from the same recordings used for Fig. 1. In Fig. 8 the beginning of the ascending and the end of the descending slopes are amplified to illustrate the interspike trajectories and to compare them with the shape of AHP following pulse-evoked spikes. Note that the trajectories are superimposed at the end of the spike and the voltage amplification increased for the blue trace (see voltage calibration bars; note that the AHPs following pulse-evoked spikes were recorded at more hyperpolarized levels and therefore the amplitude was smaller). Bottom: derecruitment at the descending current slope (green). The red trace superimposed onto the last ISI is the averaged conditioned AHP. To facilitate the comparison of the conditioned AHP time course with the last interspike trajectory the traces were superimposed at the end of the spikes and the voltage amplification increased for the red trace.

**DISCUSSION**

One major observation of this study is that nearly all the motor neurons had a lower firing rate at derecruitment than at recruitment— with a difference of around 3 Hz— when subjected to triangular current pulses independent of whether the preparation was unanesthetized or anesthetized. Recently, Button et al. (2006) demonstrated similar results from motor neurons recorded in vivo, in both the unanesthetized rat and the anesthetized rat. These differences are very similar to those reported for human motor units by De Luca et al. (1982) for the deltoid muscle (3.8 Hz difference) and by Gorassini et al. (2002a) for the tibialis anterior and soleus muscles (3 Hz...
difference) for slowly varying voluntary contractions. This suggests that the underlying mechanism may also be similar. Previous observations (Bennett et al. 2001a; Hounsgaard et al. 1988; Lee and Heckman 1998), the further analysis by Button et al. (2006), and the present investigation would therefore strongly suggest that the lower firing rates at derecruitment are dependent, to a large extent, on the intrinsic properties of the motor neuron itself.

On the basis of our present results we will now discuss the possible contribution of three different mechanisms to the lower firing rates at derecruitment: 1) a prolongation of the AHP after the preceding spike train during the triangular current injection; 2) a contribution of voltage-dependent PICs to the higher firing rates at recruitment, and 3) a contribution by synaptic noise in triggering additional spikes either before or shortly after the end of the AHP.

**AHP prolongation**

Because the AHP duration has been directly implicated in determining the minimal firing frequency (Kernell 1965b) we decided to investigate whether a preceding, conditioning spike train could cause a prolongation of the AHP to an extent that could quantitatively account for the difference in recruitment/derecruitment rates. Indeed, a burst of firing in response to a conditioning ramp-current injection did prolong the AHP duration (Figs. 2 and 6). Although the AHP prolongation is in the order of magnitude to partly explain the decreased firing rate at derecruitment, the quantitative relation illustrated in Fig. 7, A and B certainly demonstrates that the duration of the conditioned AHP alone cannot fully predict the duration of the last ISI before derecruitment. In the unanesthetized preparation the last ISI in “fast” motor neurons is too long to be explained by the AHP duration alone and, in the anesthetized preparation, the last ISI is shorter than would be expected from the AHP duration. We argue that these deviations may be explained by PICs and synaptic noise (see following text).

How could the AHP prolongation by preceding spikes be explained? The AHP current, $I_{\text{Ca}^{2+}/\text{SK}}$, is thought to be directly related to the $\text{Ca}^{2+}$ concentration at the site of the channels. With several spikes at short intervals (shorter than the AHP duration) there is an AHP summation, likely reflected by a short-term accumulation of $\text{Ca}^{2+}$ (Baldissera and Gustafsson 1974; Ito and Oshima 1962). Could a $\text{Ca}^{2+}$ accumulation with a long train of action potentials (perhaps in combination with $\text{Ca}^{2+}$ PICs) cause a slowing of $\text{Ca}^{2+}$ clearance, thereby contributing to a longer AHP? To our knowledge there are no $\text{Ca}^{2+}$ imaging studies that have addressed this question. In addition to the $\text{Ca}^{2+}$-evoked AHP there is more recent evidence for an additional $\text{Na}^{+}$-induced potassium current (Safonov and Vogel 1996) developing after a larger number of action potentials. This has been described as having a time course of longer duration than that of the $\text{Ca}^{2+}$-evoked AHP (Wallén et al. 2002, 2003, 2007) and this may explain the prolongation we recorded.

The sag current (the $I_{\text{ADP}}$) (Ito and Oshima 1965) is activated by hyperpolarizing pulses and it will also be activated during the AHP. It would tend to depolarize the membrane as a result of the hyperpolarization during the AHP and consequently affect the AHP trajectory. Gustafsson and Pinter (1985) described that the sag was more pronounced in “fast” than in “slow” motor neurons and argued that this may actually explain the shorter AHPs in “fast” motor neurons as compared with the “slow” motor neurons. Therefore we investigated whether the conditioning spike train could possibly decrease the sag, thus explaining the AHP prolongation. This was not the case; on the contrary, we found that the sag (and the overshoot at termination of the hyperpolarizing pulse) was increased in almost all tested motor neurons. This increase could possibly be explained by increased levels of the second-messenger cyclic AMP (DiFrancesco and Tortora 1991), but it certainly does not help to explain the increased AHP duration. The sag was investigated only at resting membrane potential. Because the $I_{\text{Ca}^{2+}}$ is very potential dependent it is questionable whether the results seen at resting membrane potential will also apply at the membrane potentials during repetitive firing; thus more experiments with a different experimental protocol are needed for further conclusions about $I_{\text{Ca}^{2+}}$. To verify that the key observation of the prolongation of the AHP at resting membrane potential would also reflect a similar prolongation at more depolarized levels (closer to the membrane potential at firing) we also investigated the AHP duration following single pulse-evoked spikes as close to the firing threshold level as possible. The prolongation was not only present at this “more relevant” membrane potential, but was actually more prolonged and the ADP enhanced.

A prolongation of the AHP on the order of 20% when estimated at a membrane potential close to firing threshold is sizeable. In the case of a “slow” motor neuron with an AHP of 200 ms (and an expected minimal firing frequency of 5 Hz) the prolongation to 240 ms would reduce the expected minimal frequency to 4 Hz, or by about 1 Hz. For a “fast” motor neuron with an AHP of 50 ms (with an expected minimal frequency of 20 Hz) the AHP prolongation by 20% to 60 ms would reduce the expected minimal frequency to around 16 Hz and thus a reduction by about 4 Hz. This calculation may thus explain the difference between “fast” and “slow” motor neurons illustrated in Fig. 3D.

**Contribution of PICs**

Figure 3B illustrates how the PIC is boosting the injected current, leading to the steeper f-I relation in the secondary range and the maintained higher frequencies at the descending phase of the triangular current injection (see also Bennett et al. 2002; Conway et al. 1988; Hounsgaard et al. 1988). Clearly this PIC can explain why the motor neuron keeps firing at lower current levels at the end of the triangular current injection, but it does not directly explain the lower frequencies at derecruitment. However, part of the PIC may be activated below firing threshold and thus contribute to a stable and secure recruitment. That would imply that the frequency at recruitment does not equal the minimum firing rate (and the AHP duration), but indeed a frequency that is already boosted by the PIC. Recordings from motor neurons in decerebrate cats have directly demonstrated the development of a plateau potential below firing threshold (see Fig. 7 in Bennett et al. 1998) and the same is regularly observed in rat motor neurons in vitro (Bennett et al. 2001b). Recordings from single motor units in freely moving rats have also indicated that the initial frequency at recruitment is much higher than could be explained by the AHP duration; the high initial frequencies must be explained...
either by the contribution by PICs or by a very abrupt and strong synaptic excitation that would be unlikely with slow movements (Gorassini et al. 1999, 2000). If the initial firing frequency indeed reflects the recruitment of PIC, then the lower rate at derecruitment would include a measure of this PIC component (see the following text).

We have developed several lines of reasoning arguing against the PICs developing after recruitment being responsible for the difference in recruitment and derecruitment rates in this study. As illustrated in Fig. 3 the lower rates at derecruitment were seen both in the unanesthetized and in the anesthetized (barbiturate) preparations. It has been described that the Ca2+ plateau potentials are significantly reduced with barbiturate anesthesia. Crone et al. (1988) suggested that this effect was indirect by reducing the activity in the descending monoaminergic systems, but later Guertin and Houngsaard (1999) demonstrated a direct effect on the sensitivity of the L-Ca2+ channels (however, see Li et al. 2004). In Fig. 3B it is also shown that the reduced firing rate at derecruitment is seen independently of whether the triangular current injection causes firing reaching the secondary range (caused by the PIC) or only in the primary range (without PICs activated above spike threshold). It shall be noted that there is no information on the action of barbiturates on NaPICs. It has been demonstrated that it is impossible to evoke repetitive firing in response to long-lasting current pulses without the NaPIC (Kuo et al. 2006). Since repetitive firing can be easily evoked in barbiturate-anesthetized preparations it is likely that the NaPICs are not blocked by barbiturates.

Contribution of synaptic noise

It has been proposed that the low firing rates at derecruitment may have been generated by a sustained subthreshold synaptic excitation with fluctuations in background synaptic noise occasionally driving the cell to fire (Kudina 1999; Mathews 1996; Powers and Binder 2000), rather than being determined strictly by the AHP duration. In actual fact our present results support this proposal. In Fig. 7A the rate at derecruitment was far below that which would be expected from the AHP duration for “fast” motor neurons. The direct superimposition of the trajectories of a single AHP following a pulse-evoked spike onto the interspike trajectories at minimal firing rate (Fig. 8) certainly suggests that the spikes at the lowest firing rates may be initiated by synaptic noise long after the end of the AHP. This is further supported by the finding that the last ISI for the fast units correlates well with the AHP duration in the anesthetized preparation in which the synaptic noise is much reduced (Fig. 7B). This reduction in synaptic noise in the anesthetized preparations is illustrated in Fig. 9A and was also estimated from differences in the variance of the resting membrane potentials and by lower values in the power spectrum seen following a fast Fourier transformation (Fig. 9B).

Matthews (1999) discovered, during a modeling study on the so-called subprimary range, that the synaptic noise in most cases will trigger the next spike before the full recovery of the AHP trajectory. The reason for slow motor neurons being more affected than fast motor neurons could be found in their different decay time constants of the AHP (and of the ADP). Since their time constants are much larger in slow motor neurons there is a relatively increased chance (around spike threshold) for the synaptic noise to trigger a spike before the full recovery of the AHP (Eccles et al. 1958; Matthews 1996). This could possibly explain the faster firing rates at minimal firing frequency (Fig. 4A) at derecruitment (Fig. 7B) than can be explained by the AHP duration. However, for the “slow” motor neurons there is a direct indication that subthreshold PICs also contribute to a spike discharge before the end of the AHP (Fig. 4C). That PICs, which develop below the spike threshold, are indeed part of the recruitment mechanism has been described both with intracellular recording from cat motor neurons (Bennett et al. 1998) and from motor unit recording from intact rats (Gorassini et al. 1999, 2000).

On the interpretation of PIC amplitudes in human experiments with the “paired motor unit” analysis

Gorassini and colleagues (2002a,b) introduced a technique of paired motor unit recordings to give a more quantitative estimation of the PIC in humans. To validate the method they used data from both intact and spinalized rats in which the “paired motor unit” paradigm could be compared with direct intracellular measurements (Bennett et al. 2001a,b). Briefly, pairs of motor units were recorded during voluntarily linearly varying contractions (triangular waveform); an early recruited unit was used as a “control unit” (often referred to as “reporter unit” or “reference unit”), whereas another “test unit” was recruited at significantly higher threshold. The FM of the reporter unit was then used to reflect changes in the synaptic excitatory drive during the “triangular contraction,” and it was assumed that the drive of this unit reflected a common drive onto all motor neurons of the muscle. It was a consistent finding (Gorassini et al. 2002a) that the test unit was recruited and derecruited at very different firing frequencies of the reporter unit; the firing frequency of the reporter unit was significantly lower at test unit derecruitment than at test unit recruitment. The reasoning is now that the synaptic excitation corresponding to this difference in firing frequency (ΔF) of the control unit would be an estimate of the persistent inward current (ePIC) in the test unit. This ePIC would keep the test unit firing despite lower common synaptic drive, corresponding to the “counterclockwise hysteresis” in the intracellular recordings shown in Fig. 3. Note that the ΔF in Gorassini’s paradigm refers to the reporter unit and thus is totally different from the frequency difference measured in the present work, where it refers to the difference between recruitment and derecruitment frequencies in individual units (no comparisons with other units).

The “paired motor unit” paradigm rests on at least three assumptions: 1) the difference in firing frequency of the reporter unit at which the test unit is recruited and derecruited shall only reflect differences in synaptic excitation of the control unit; thus the PIC current must stay the same, therefore requiring that the PIC of the control unit is activated in an all-or-none manner; 2) the firing rate of the reporter unit must be a sensitive and linear indicator of the net excitatory synaptic drive; and 3) the synaptic input to both recorded motor units must be the same during the voluntary activation. As discussed earlier (Contribution of PICs) the difference in recruitment and derecruitment rates in individual units may partly reflect the amount of subthreshold PIC when the initial rates at recruitment are higher than could be expected from the AHP duration. The
major result from the present investigation is that a prolongation of the AHP alone may explain a frequency difference between recruitment and derecruitment of about 1–3 Hz. To what extent can that affect the estimation of ePIC from the ΔF (difference in firing frequency of the reporter unit at recruitment/derecruitment of the test unit)? There is presently no answer to this question. This depends on whether the AHP prolongation develops in parallel in both reporter and test units, and how that would affect the f–I relation in these units. Since the frequency differences (between recruitment and derecruitment) in individual units that can be explained partly by the AHP prolongation in the same order of magnitude as the ΔF used to estimate ePIC, it is apparent that the changes in AHP duration warrant further experimental attention.

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