Quantitative assessment of nociceptive processes in conscious dogs by use of the nociceptive withdrawal reflex

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Objective—To investigate the feasibility of evoking the nociceptive withdrawal reflex (NWR) from fore- and hind limbs in conscious dogs, score stimulus-associated behavioral responses, and assess the canine NWR response to suprathreshold stimulations.

Animals—8 adult Beagles.

Procedure—Surface electromyograms evoked by transcutaneous electrical stimulation of ulnaris and digital plantar nerves were recorded from the deltoideus, cleidobrachialis, biceps femoris, and tibialis cranialis muscles. Train-of-five pulses (stimulus<sub>train</sub>) were used; reflex threshold (I<sub>1 train</sub>) was determined, and recruitment curves were obtained at 1.2, 1.5, and 2 X I<sub>1 train</sub>. Additionally, a single pulse (stimulus<sub>single</sub>) was given at 1, 1.2, 1.5, 2, and 3 X I<sub>1 train</sub>. Latency and amplitude of NWRs were analyzed. Severity of behavioral reactions was subjectively scored.

Results—Fore- and hind limb I<sub>1 train</sub> values (median; 25% to 75% interquartile range) were 2.5 mA (2.0 to 3.6 mA) and 2.1 mA (1.7 to 2.9 mA), respectively. At I<sub>1 train</sub>, NWR latencies in the deltoideus, cleidobrachialis, biceps femoris, and cranial tibialis muscles were not significantly different [19.6 milliseconds (17.1 to 20.5 milliseconds), 19.5 milliseconds (18.1 to 20.7 milliseconds), 20.5 milliseconds (14.7 to 26.4 milliseconds), and 24.4 milliseconds (17.1 to 40.5 milliseconds], respectively. Latencies obtained with stimulus<sub>train</sub> and stimulus<sub>single</sub> were similar. With increasing stimulation intensities, NWR amplitude increased and correlated positively with behavioral scores.

Conclusions and Clinical Relevance—In dogs, the NWR can be evoked from limbs and correlates with behavioral reactions. Results suggest that NWR evaluation may enable quantification of nociceptive system excitability and efficacy of analgesics in individual dogs. (Am J Vet Res 2006;67:882–889)

Investigations involving animal models of nociception are mainly used as transitional studies to provide better understanding of pain mechanisms and the effectiveness of analgesic drugs for subsequent administration to humans. The recent growth of veterinary interest in understanding the mechanisms underlying pain and its treatment has opened doors to studies of nociception in animals for the benefit of animals. Animal models of nociception and their pharmacologic modulation can provide information regarding the efficacy of new analgesic drugs to the veterinary pharmaceutical industry, and investigations of nociception in animals should represent the preliminary step before clinical studies are undertaken to pursue better treatment options in small companion animals.

Unlike cats (for which there is extensive literature), dogs are seldom used as experimental animals in nociception studies. There are some experimental and clinical studies in dogs in which mechanical, thermal, and electrical stimulations have been applied to evoke nocifensive reactions and evaluate their pharmacologic modulation. The end point of these models of acute nociception in dogs is determined by monitoring the evoked gross behavioral reaction or the thresholds at which the behavioral aversive response is elicited. The prolongation of the latency of the withdrawal response or an increase in the threshold is interpreted as analgesia.

A major drawback of all these models is evident when the drugs used exert a contemporaneous sedative effect that can clearly alter the pattern of the behavioral reaction observed and the interpretation of the analgesic efficacy. A more refined model consists of recording the EMG reflex response to a nociceptive (thermal or electrical) stimulus. Electromyographic recordings of NWRs of the limbs elicited by electrical stimuli have been investigated in rats, cats, and dogs that have been anesthetized or undergone spinal cord transection. All these models are of limited clinical interest because of their invasiveness and the influence of anesthetics on the NWR. Therefore, there is need for a new, noninvasive, technically simple, sensitive, specific, repeatable model to objectively quantify nociception in conscious dogs with intact spinal cords (ie, nontransected dogs). In conscious human volunteers, a close relationship between pain threshold and...
the NWR threshold has been identified, the NWR and its modulation have been widely used in experimental, clinical, and pharmacologic studies as a noninvasive neurophysiologic tool to objectively assess spinal nociceptive processing. The ability to investigate the nociception-related responses in humans objectively but noninvasively has fueled interest in applying similar experimental procedures in nontransected, unmedicated animals. Recently, results of a series of studies highlighted the feasibility of evoking the NWR via electrical stimulation of the digital nerves of the fore- and hind limbs in conscious horses and indicated that the NWR could be used as a noninvasive, objective method to measure nociceptive activity in this species. It was assumed that a similar investigation in dogs should be possible. The purpose of the study reported here was to demonstrate the feasibility of evoking the NWR from fore- and hind limbs in conscious unmedicated dogs, score behavioral responses to the electrical stimuli, and assess the response of the canine NWR to graded suprathreshold stimulations.

Materials and Methods

Animals—Eight adult male purpose-bred Beagles (mean weight, 9.1 ± 1.7 kg) were included in the study. The dogs were 1.5 to 5 years old. They were judged to be healthy on the basis of findings of physical examination and clinico-pathological analyses. Dogs were housed together in runs (10 dogs/run), and food was withheld in the morning prior to the experimental session. The experiments were approved by the Committee for Animal Experimentation of the Canton Basel City, Switzerland (approval No. 2090).

Experimental procedure—All measurements were started in the morning and took place in a constant-temperature (22°C) room. Prior to the experiment, each dog underwent physical examination and the rectal temperature was measured. The stimulation and recording sites were clipped, shaved, and degreased. The dog was placed in right lateral recumbency in a comfortable, commercial dog bed (filled with corncob-balls) that took the shape of the body. The limbs were extended laterally in a natural position but not supported, without weight bearing or movement restriction of the nondependent limb (Figure 1). In general, the dogs accepted to lie still or had to be slightly restrained. The surface electrodes were then positioned, the nerves were transcutaneously stimulated by electrical stimuli, and the response was recorded by surface EMG. On completion of the trials, the electrodes were removed; to avoid local reactions, the skin was washed and a dermatological cream was applied.

Recording and stimulation equipment—The stimulation electrodes were placed over the dorsal branch of the ulnar nerve at the level of the left fifth metacarpal bone of the forelimb and over the lateral plantar digital nerve of the hind limb at the level of the fourth metatarsal bone, just distal to the base and proximal to the head of each bone. The electrodes were placed parallel to the nerve with the anode in the distal position, with an interelectrode distance of 0.8 cm. The distal portion of the limb was bandaged to prevent dislocation of the electrodes (Figure 1).

By means of pairs of self-adhesive electrodes, surface EMGs were recorded from the deltoideus and cleidobrachialis muscles of the forelimb and from the biceps femoris, caput pelvis and cranial tibial muscles of the hind limb. Special care was taken to place the electrodes over the muscle bellies at a distance of 1 cm to avoid multichannel cross-talk contamination from adjacent muscles and minimize common-mode noise and stimulus artefacts. Their position was marked with a pen, which allowed for exact repositioning in case the dog moved. The ground electrode was placed over the plantar side of the right foot and taped in place. Flexible leads were connected to the electrodes. The resistance of each electrode pair was checked and confirmed to be < 5 kΩ before starting and at the end of each experimental session.

Stimulation and recordings were performed by use of a specially designed computerized system. The final stage of the stimulator that received input from the computer was a battery-powered optoisolated constant-current device with a maximum voltage of 100 V and a maximal current of 40 mA. Electromyographic signals were amplified with an overall gain of 5,000 and a bandpass of 7 to 200 Hz (first-order active filters with 6 dB/octave slope). They were passed through a digital converter to a computer for further processing and storage. An interval of 400 milliseconds after stimulation was analyzed with 512 sampling points (sampling frequency of 1,280 Hz).

Electrical stimulations—The dogs initially received 4 test stimuli at different intensities to make them familiar with the experimental method prior to formal threshold measurement. In session I, the experiment was started by applying a standard stimulus (ie, stimulus, delivered at a frequency of 200 Hz (total duration, 25 milliseconds). The initial current intensity was 1 mA; if no reflex response could be elicited, the current was gradually increased in steps of 0.2 mA until the I1 train value was defined. The I1 train was defined as the minimum stimulus intensity that evoked EMG activity from the deltoideus muscle (forelimb) and the biceps femoris (hind limb) in the 20- to 100-millisecond epoch with an amplitude > 10 times the EMG background activity, duration > 10 milliseconds, and a behavioral reaction score of 1 or 2. To assess reproducibility of the response and confirm I1 train, the detected I1 train intensity was repeated 3 times; if not reproducible, the current intensity was increased by 0.2 mA and the threshold assessment repeated.

Care was taken to perform the stimulations when the dog had relaxed extended limbs, and no EMG background activity was evident on the computer. To avoid stimulus habituation, the time that elapsed between successive stimulations was > 60 seconds. Once I1 train was defined, stimuli

Figure 1—Photograph of a Beagle prepared for recording of surface EMGs from the deltoideus, cleidobrachialis, biceps femoris, and tibialis cranialis muscles evoked by transcutaneous electrical stimulation of ulnaris and digital plantar nerves. The dog was lying without restraint on a bed filled with corncob-balls. Notice the ground electrode on the dependent hind foot and the recording electrodes over the deltoideus and cleidobrachialis muscles. The stimulation electrodes are covered by a bandage.
were applied at suprathreshold intensities of 1.2, 1.5, and 2 × \( I_{\text{train}} \) in a stepped manner.

In session II, the effect of stimulus configuration on the canine NWR was evaluated by use of a stimulus single-stimulus single was 1 pulse (duration, 1 millisecond), and stimulustrain was train-of-five pulses (duration, 25 milliseconds; 200 Hz).

Each experiment started with the hind limb (sessions I and II), and the whole procedure (sessions I and II) was repeated for the forelimb. Between hind limb and forelimb trials, the dogs could walk and rest for 30 minutes. A total of 44 EMG responses were analyzed for each dog.

### Behavioral reactions

The same investigator (AB) observed and scored the behavioral reactions of the dogs to each electrical stimulus. The investigator was unaware of the timing and intensity of the stimulus. The behavioral score was assigned as follows: 0 = no movement; 1 = slight flexion of carpus or tarsus; 2 = flexion of elbow or stifle joint; 3 = brisk flexion of elbow or stifle joint; 4 = brisk flexion of the forelimb or hind limb and flexion maintained; 5 = brisk flexion of the forelimb or hind limb and general awareness (ie, turning the head toward the stimulated limb or attempts to stand from a lying position); and 6 = brisk flexion of the forelimb or hind, general awareness, and vocalization.

### Time analysis and response quantification

To separate reflex components of various origins, the 400-millisecond poststimulation interval corresponding to the EMG recording time was divided into 3 epochs: 0 to 20 milliseconds, > 20 to 100 milliseconds, and > 100 to 400 milliseconds. These epochs were defined on the basis of the conduction velocities of the nerve fibers\(^{33,34,38,39}\) and the conduction pathway lengths of Beagles. The 0- to 20-millisecond epoch should contain spinal, non-nociceptive components resulting from the activation of Aβ afferent nerve fibers. The conduction velocity of the sensory afferent fibers in dogs is 69.4 ± 6.9 m/s for the ulnar nerve and 63.4 ± 5.3 m/s for the tibial nerve.\(^{35}\) The mean length of the afferent pathway is 38.5 ± 2.4 cm, so an afferent latency > 5 milliseconds should be expected. Adding a mean efferent time of 2.5 milliseconds for the motor component over a mean distance of 18 cm from between the scapulae or the sacrum and the recording electrodes and an overall 5-millisecond period\(^{33,34,38,39}\) for the afferent component, after adding a mean efferent time of 2.5 milliseconds and an overall time of 5 milliseconds for spinal and motor endplate delay, the NWR in dogs should occur in the > 20- to 100-millisecond poststimulation epoch.

### Signal analysis

The latency of the NWR was defined as the time elapsed from the stimulus onset to the reflex

### Table 1—Amplitude ratio (median; 25% to 75% interquartile range) of EMG activity in 3 poststimulation epochs at \( I_{\text{train}} \) for the muscles of fore- and hind limbs of 8 dogs. Stimulussingle was 1 pulse (duration, 1 millisecond), and stimulustrain was train-of-five pulses (duration, 25 milliseconds; 200 Hz).

<table>
<thead>
<tr>
<th>Epoch</th>
<th>0 to 20 ms</th>
<th>&gt; 20 to 100 ms</th>
<th>&gt; 100 to 400 ms</th>
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<tbody>
<tr>
<td>STIMULUS</td>
<td>STIMULUS</td>
<td>STIMULUS</td>
<td>STIMULUS</td>
</tr>
<tr>
<td>SINGLE</td>
<td>RATIO</td>
<td>RATIO</td>
<td>RATIO</td>
</tr>
<tr>
<td>D</td>
<td>1.165 (0.825–1.995)</td>
<td>3.860 (1.835–6.140)</td>
<td>1.210 (0.950–1.922)</td>
</tr>
<tr>
<td>CB</td>
<td>1.510 (0.765–1.810)</td>
<td>7.580 (4.465–10.110)</td>
<td>2.350 (1.315–4.755)</td>
</tr>
<tr>
<td>CT</td>
<td>3.995 (2.195–4.130)</td>
<td>23.980 (15.44–34.625)*</td>
<td>2.025 (1.720–2.585)</td>
</tr>
<tr>
<td>D</td>
<td>1.810 (1.520–2.970)</td>
<td>14.205 (8.750–21.690)*</td>
<td>2.185 (1.750–3.330)</td>
</tr>
</tbody>
</table>

*Value associated with stimulustrain significantly (\( P < 0.001 \)) different than the value associated with stimulussingle for the same muscle.

D = Deltoid muscle; CB = Cleidobrachialis muscle; BF = Biceps femoris muscle. CT = Cranial tibial muscle.

### Table 2—Amplitude ratio (median; 25% to 75% interquartile range) of EMG activity evoked by the stimulus train at suprathreshold stimulation intensities recorded in the 3 poststimulation epochs from the muscles of the fore- and hind limbs of 8 dogs.

<table>
<thead>
<tr>
<th>Epoch</th>
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<tr>
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</tr>
<tr>
<td>SINGLE</td>
<td>RATIO</td>
<td>RATIO</td>
<td>RATIO</td>
</tr>
<tr>
<td>D</td>
<td>2.470 (1.280–4.350)</td>
<td>7.730 (1.700–23.38)</td>
<td>17.815 (5.210–36.28)</td>
</tr>
<tr>
<td>CB</td>
<td>1.805 (1.045–3.195)</td>
<td>2.875 (1.370–8.02)</td>
<td>7.235 (&lt; 0.001)</td>
</tr>
<tr>
<td>BF</td>
<td>4.880 (2.008–17.407)</td>
<td>11.585 (5.730–44.31)</td>
<td>12.400 (0.116)</td>
</tr>
<tr>
<td>CT</td>
<td>13.600 (4.495–16.075)</td>
<td>10.320 (5.380–42.190)</td>
<td>33.840 (7.690–98.71)</td>
</tr>
</tbody>
</table>

*Value derived via a Friedman repeated-measure ANOVA (significance set at a value of \( P < 0.05 \)).

See Table 1 for remainder of key.
onset (EMG deflection) as determined by visual inspection of the records. The root-mean-square amplitude of the reflex was calculated for the 0- to 20-millisecond, 20- to 100-millisecond, and 100- to 400-millisecond poststimulation epochs. Resting background EMG amplitude was calculated as the root-mean-square amplitude of the 100-millisecond interval prior to stimulation. To minimize interindividual variability, the ratio of the root-mean-square amplitude of the reflex for each epoch to the background EMG amplitude was calculated.

Analysis of data—Data were analyzed for normality. Nonparametric tests were chosen because of the small sample size. Results are expressed as median and range (25% to 75% interquartile range).

Intensity threshold values and behavioral scores obtained by stimulating the forelimb and hind limb were compared by use of a Wilcoxon signed rank test. The same statistical test was used to compare NWR latencies and amplitude ratios between muscles of the same limb and between forelimb and hind limb and to analyze the effect of the different stimulus types (stimulus\textsubscript{train} and stimulus\textsubscript{single}). Latencies, amplitude ratio, and behavioral scores after stimulation at suprathereshold intensities were compared by use of a Friedman test followed by a Tukey test, if required.

Correlations among behavioral scores, stimulation intensities, and amplitude ratios were calculated with a Spearman rank test. Overall significance was set at a value of $P < 0.05$. Data were analyzed by use of commercially available computer programs.\textsuperscript{37}

Results

No abnormalities were detected on physical examination of the dogs, and rectal temperatures were within reference limits. All dogs tolerated the experiments well; throughout the study period, they were relaxed and remained in lateral recumbency. Even when suprathereshold intensities were used for stimulation, the behavioral reactions stopped immediately on stimulus cessation. With the current intensities used to elicit the NWR in the present study, none of the dogs vocalized or appeared distressed. Some erythema of the skin over the lateral aspects of the treated metacarpal and metatarsal regions was present for 2 days after the experiments but healed fully. No skin or hair changes were present at a 6-month follow-up examination.

Poststimulation interval from 0 to 20 milliseconds—The EMG activity was minimal at $I_{\text{train}}$ for both muscles of the forelimb and hind limb. When suprathereshold intensities were applied, the EMG activity in this epoch increased significantly as a result of a decrease in latency of the NWR itself (Tables 1 and 2; Figures 2 and 3).

Poststimulation interval from $>20$ to 100 milliseconds—It was possible to define an $I_{\text{train}}$ value in all dogs (Figures 4 and 5). The $I_{\text{train}}$ of the forelimb
had a median value of 2.5 mA (range [25% to 75% interquartile interval], 2.0 to 3.6 mA). The \( I_{\text{train}} \) of the hind limb had a median value of 2.1 mA (1.7 to 2.9 mA). Forelimb and hind limb \( I_{\text{train}} \) values did not differ significantly \((P = 0.563)\). In 5 of the 8 dogs, the \( I_{\text{train}} \) was redefined at the end of the trial and remained identical for both the forelimb and hind limb.

The median latencies at \( I_{\text{train}} \) for the deltoideus and cleidobrachialis muscles of the forelimb were 19.6 milliseconds (17.1 to 20.5 milliseconds) and 19.5 milliseconds (18.1 to 20.7 milliseconds), respectively. The median latencies at \( I_{\text{train}} \) for the biceps femoris and cranial tibial muscles of the hind limb were 20.5 milliseconds (14.7 to 26.4 milliseconds) and 24.4 milliseconds (17.1 to 40.5 milliseconds), respectively. There were no significant differences in the latencies between the 2 muscles of the same limb or between forelimb and hind limb values, indicating that the NWR could be elicited and recorded for both muscles of the forelimb and both muscles of the hind limb. When the \( I_{\text{single}} \) was used, no significant difference in the NWR latencies was found. When suprathreshold stimulation intensities of the \( I_{\text{train}} \) or \( I_{\text{single}} \) were applied, the latency of the NWR for all muscles was decreased significantly, compared with the latency at \( I_{\text{train}} \) (Figures 2 and 3).

At \( I_{\text{train}} \), the amplitude ratios of the NWR between muscles of the same limb or between muscles of the forelimb and hind limb were not significantly different. These findings indicated that the muscles chosen for use in our study provided adequate recordings of the forelimb and hind limb NWRs. For both forelimb and hind limb, the amplitude ratio was significantly \((P < 0.001)\) greater when the \( I_{\text{train}} \) was used, compared with the amplitude ratio calculated when \( I_{\text{single}} \) was used (Table 1).

When stimuli at suprathreshold intensities were applied, the amplitude ratio for the biceps femoris \((P = 0.005)\) and cranial tibial \((P < 0.001)\) muscles of the hind limb were significantly increased, compared with the respective amplitude ratio at \( I_{\text{train}} \). For the forelimb, the amplitude ratio recorded for the cleidobrachialis muscle was significantly \((P = 0.04)\) increased, compared with the amplitude ratio at \( I_{\text{train}} \), whereas the change in the amplitude ratios recorded for the deltoideus muscle was not significantly different (Table 2). At suprathreshold intensities, the amplitude ratio associated with \( I_{\text{single}} \) was not significantly different from that associated with \( I_{\text{train}} \).

Poststimulation interval from 100 to 400 milliseconds—In this epoch, the EMG activity recorded at \( I_{\text{train}} \) was significantly \((P = 0.024)\) higher for the hind limb than for the forelimb. With suprathreshold stimulation intensities, the EMG activity was increased significantly for biceps femoris \((P = 0.029)\) and cranial tibial \((P < 0.001)\) muscles, compared with activity at \( I_{\text{train}} \).

Behavioral reactions—At \( I_{\text{train}} \) values, the median behavioral score was 1 (range [25% to 75% interquartile interval], 1 to 2) for the forelimb and 1 (0 to 2) for the hind limb \((P = 0.75)\) and there was slow flexion of the carpus or tarsus joint without any general body reaction in all dogs. When suprathreshold stimulation intensities were used, the behavioral reaction scores were increased significantly (Figure 6), compared with the scores at \( I_{\text{train}} \) reaching a median value of 3 (2 to 5) at \( 2 \times I_{\text{train}} \) \((P < 0.001)\) for the forelimb and 4 (1 to 5) at \( 2 \times I_{\text{train}} \) for the hind limb \((P < 0.001)\). The dogs had a brisk flexion of the foot followed by a slower but persistent flexion of the entire limb, accompanied by lifting of the head or attempts to stand from the lying position. None of the dogs vocalized at any stimulation intensity.

When the \( I_{\text{single}} \) was applied to the forelimb, the median behavioral scores were significantly lower, compared with the behavioral scores obtained with the \( I_{\text{train}} \) at 1.5 \( I_{\text{train}} \) \((P = 0.031)\) and 2 \( I_{\text{train}} \) \((P = 0.023)\). For the hind limb, no significant

![Figure 5](image-url)  
Figure 5—Electromyograms evoked by the \( I_{\text{single}} \) at 1, 1.2, and 2 \( I_{\text{train}} \) recorded from the tibialis cranialis muscle in a dog. At \( I_{\text{train}} \), the NWR can be recognized in the >20- to 100-millisecond epoch. With increasing stimulation intensities (ie, 1.2, 1.5, and 2 \( I_{\text{train}} \)), the latency of the NWR decreases, whereas its amplitude increases significantly. See Figure 4 for key.

![Figure 6](image-url)  
Figure 6—Median behavioral scores ± range (25% to 75% interquartile interval) associated with \( I_{\text{single}} \) (triangles) or \( I_{\text{train}} \) (circles) of the forelimbs (A) and hind limbs (B) in 8 dogs. The x-axis represents the intensity of stimulation relative to \( I_{\text{train}} \) (ie, 1, 1.2, 1.5, 2, and 3 \( I_{\text{train}} \)), and the y-axis represents the behavioral score (0 to 6 scale of increasing severity). With increasing stimulation intensities of \( I_{\text{single}} \) and \( I_{\text{train}} \), the behavioral scores increased significantly \((P < 0.05)\) [Friedman repeated-measures ANOVA] for the forelimb and hind limb. * Score associated with \( I_{\text{single}} \) significantly \((P < 0.05)\) (Wilcoxon signed rank test) different from score associated with \( I_{\text{train}} \).
difference in behavioral scores was detected between stimulus\textsubscript{single} or stimulus\textsubscript{train} (Figure 6).

There was a significant positive correlation between behavioral reaction score and stimulus intensity of the forelimb ($r = 0.409; P = 0.018$) and between behavioral reaction score and the stimulus intensity of the hind limb ($r = 0.334; P = 0.05$). The behavioral score also correlated significantly with the amplitude ratio of the deltoideus ($r = 0.451; P = 0.018$) and cleidobrachialis ($r = 0.449; P = 0.009$) muscles of the forelimb and with the amplitude ratio of the biceps femoris ($r = 0.39; P = 0.022$) and cranial tibial ($r = 0.757; P < 0.001$) muscles of the hind limb.

**Discussion**

In the present study, we have demonstrated the feasibility of evoking the NWR from the forelimbs and hind limbs of conscious dogs and established a relationship between intensity of stimulation and nociception via EMG and by use of behavioral reaction scores. This new model of nociception in conscious dogs could be used to assess the excitability of the nociceptive system in individual dogs and evaluate the effect of analgesic drugs in this species.

For a thorough description of the NWRs elicited from the forelimb and hind limb of dogs, the EMG activities of 2 flexor muscles for each limb were studied. The principal functions of the deltoideus and cleidobrachialis muscles are flexion and protraction of the shoulder joint and flexion of the elbow joint, respectively. Results of a previous study in cats have indicated that the cleidobrachialis muscle has a burst of EMG activity that coincides with the evoked forelimb withdrawal response. We assumed that the response of the limb evoked by electrical stimulation could be compared with the withdrawal movement to overcome an obstacle during deambulation. The initial movement is a flexion of the shoulder joint together with a locking of the elbow joint and dorsiflexion of the carpus, which activates the aforementioned muscles. The tibialis cranialis muscle dorsiflexes and supinates the hind foot, whereas the caput pelvis of the biceps femoris muscle flexes the stifle joint and acts to withdraw the foot independently of whether the foot is in contact with the ground. In humans, the biceps femoris muscle has the earliest reflex activity and the cranial tibial muscle has been found to be most representative in the measurement of responses of the NWR. Therefore, it seemed appropriate to record NWRs of the forelimb and hind limb in dogs from these flexor muscles. Furthermore, those muscles are relatively superficial and easy to localize. These anatomic characteristics allowed for standardized positioning of the EMG electrodes, which is important because EMG variables are known to be affected by electrode location.

Knowledge of the conduction velocity range of Aβ fibers allows investigators to identify the epoch in which the NWR occurs during the 400-millisecond poststimulation interval. In humans and horses, this epoch is between 70 and 200 milliseconds and between 80 to 250 milliseconds respectively. Conduction velocities of mammalian nerve fibers are species specific, and the conduction velocity of Aβ fibers decreases with decreasing animal size. Extrapolation of conduction velocities among species could lead to misinterpretation of results; however, to our knowledge, no study has specifically addressed the conduction velocity of Aβ fibers in dogs. To calculate the epoch in which the NWR ensues, it was assumed that the conduction velocity of canine Aβ fibers could be similar to that reported for cats, for which the Aδ-Aβ shift is set at 30 m/s. Taking into account spinal delay and the mean afferent and efferent pathway distances of the Beagles used in the present study, the lower latency limit for the Aβ fibers would be 19.5 milliseconds. Therefore, the 20- to 100-millisecond epoch should include the NWR response in these dogs. The median latency of 19.5 milliseconds of the reflexes recorded at $t_{train}$ confirms the involvement of Aβ fibers in the observed reflexes.

In the published literature, a train-of-five pulses delivered at high frequency (which humans perceive as a single stimulus) are described as a standard stimulus to elicit the NWR. Along with other factors, the number of pulses and stimulus duration can influence the NWR. In the present study, 2 types of stimuli (stimulus\textsubscript{train} and stimulus\textsubscript{single}) were compared; the stimulus type did not influence the latency of the canine NWR.

Compared with the latency at $t_{train}$, the latency of the NWR decreased significantly when suprathreshold intensities were used for stimulation. This decrease in latency can be attributed to the recruitment of a greater number of afferent fibers with different conduction velocities, which results in the spatial summation of the input stimulus at the spinal level.

The EMG activity recorded in the > 100- to 400-millisecond epoch was probably a result of muscular activation that had mixed spinal and supraspinal origins. This activity was significantly higher for the hind limb than the forelimb at $t_{train}$ and increased significantly with suprathreshold stimulations (both stimulus\textsubscript{train} and stimulus\textsubscript{single}) only for the hind limb. The reason for this remains unclear.

Unlike horses, there was no significant difference in threshold stimulation intensities between fore- and hind limbs in dogs. In humans, the reflex threshold varies among body regions depending on the biological function of the reflex. In the present study, the dogs were lying in lateral recumbency, which is a physiologic, species-specific sleeping position, whereas recordings were performed on standing horses. The dogs were non-weight bearing, compared with fully weight-bearing horses. There is evidence of a significant inverse correlation between the load to which the limb is subjected and the size of the reflex response. Therefore, a direct comparison between dogs and horses should not be performed because the amplitude ratio of the reflex (which is taken into account for the determination of the $t_{train}$) is likely to be greater in dogs than in horses.

At $t_{train}$, the amplitude ratios of the EMG activities of the deltoideus and cleidobrachialis muscles of the forelimb and the biceps femoris and cranial tibial muscles of the hind limb in the > 20- to 100-millisecond epoch were of similar magnitude in the study dogs.
This confirmed our hypothesis that the muscles chosen for evaluation allowed adequate recording of the NWR. At I_\text{train}, the amplitude ratio was significantly higher for stimulus_\text{train} compared with stimulus_\text{single}, most likely as a result of the temporal summation of sensory inputs given repeatedly at a high frequency.

When stimuli of suprathreshold intensity were applied, the amplitude ratio increased significantly as more peripheral afferent fibers and more motor units were recruited, which agrees with findings in humans and horses. Overall, the increase in amplitude ratios of the muscles of the hind limb was more consistent, compared with the change in amplitude ratios of the muscles of the forelimb, and there was a strong positive correlation between the amplitude ratio of the cranial tibial muscle and the stimulation intensity. With suprathreshold intensities, the stimulus_\text{rain} did not evoke higher amplitude ratios than the stimulus_\text{single}; it is likely that the effect of the stimulus configuration on the amplitude ratio (which was significant at I_\text{train}) was masked by the cofactors already discussed.

In humans, the value of the reflex amplitude is generally related to that of subjective pain intensity; therefore, the NWR model is an interesting tool for correlation of an electrophysiologic measure with pain in experimental studies. To quantify the subjective pain sensation in humans, a visual analogue scale is generally used. Use of such a scale is obviously not possible in animals, and a 0-point behavioral scoring system was applied in the present study. The behavioral reaction score assigned to the dogs correlated positively with increasing stimulus intensity and with the amplitude ratio of the reflex. This could support the hypothesis that as in humans and horses, the NWR in dogs correlates with the subjective assessment of pain perception as evaluated by behavioral scoring.

In the present study to describe the NWR in conscious dogs, attention was paid to standardize and control for possible cofactors that could have influenced the results. Results of clinical studies in humans have indicated that NWR thresholds are often lower in individuals with pain disorders, compared with healthy persons, and none of the dogs in the present study had a painful condition (as assessed by physical examination). All experiments were performed at the same time of the day to minimize interindividual circadian variations in NWR thresholds. After instrumentation, the dogs received 4 test stimuli at different intensities to make them familiar with the experimental method prior to formal threshold measurement. It was noticed during pilot work in dogs that the reflex thresholds increased and then stabilized over time; this can be explained by high levels of anxiety, which may increase central excitability as indexed by lowering of NWR thresholds. As assessed in 5 of the dogs used in the present study, the I_\text{train} did not change over time, indicating a lack of habituation to the stimuli in the study dogs.

Results of the present study have indicated that it is feasible to evoke the NWR from the fore- and hind limbs in dogs. The positive correlations between the intensity of stimulation and amplitude ratio of the reflex and between the intensity of stimulation and behavioral reaction score appear to confirm the nociceptive origin of the NWR. The stimulus_\text{train} can be used as a standard stimulus. Thus, assessment of the NWR is proposed as a noninvasive neurophysiologic tool for quantifying nociception in dogs. It would be highly interesting to apply this method in pharmacokinetic-pharmacodynamic studies as an objective tool to evaluate the efficacy of analgesic drugs in this species. Future studies in larger and multibreed populations of dogs may develop the methodology to allow its use in daily clinical practice; thereby enabling evaluation of the degree of nociception amplification in chronic pain conditions and the efficacy of analgesic treatments in individual canine patients.

References