Transcranial magnetic motor evoked potentials in Great Danes with and without clinical signs of cervical spondylomyelopathy: Association with neurological findings and magnetic resonance imaging

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ABSTRACT

Transcranial magnetic motor evoked potentials (TMMEPs) assess the functional integrity of the descending motor pathways, which are typically compromised in canine cervical spondylomyelopathy (CSM). The objective of this prospective study was to establish the reference ranges of TMMEP latency and amplitude in clinically normal (control) Great Danes (GDs), compare TMMEPs obtained in GDs with and without CSM, and determine whether there is any association between TMMEP data and severity of neurological signs or magnetic resonance imaging (MRI) findings. Twenty-nine client-owned GDs were enrolled (15 controls, 14 CSM-affected). All dogs underwent TMMEPs under sedation, and latencies and amplitudes were recorded from the extensor carpi radialis (ECR) and cranial tibial (CT) muscles. MRI of the cervical vertebral column was performed to evaluate the presence and severity of spinal cord (SC) compression, and the presence of SC signal changes.

ECR and CT latencies were significantly longer in CSM-affected than control GDs. No significant differences between groups were found for amplitudes or neuronal path lengths. For the CT TMMEPs, CSM-affected GDs with moderate and severe clinical signs had significantly longer latencies than those with mild clinical signs. Significantly longer CT latencies were found in dogs with moderate and severe SC compression compared with dogs with mild compression. CT TMMEPs could not be recorded in 7/9 CSM-affected GDs with SC signal changes. These results provide a reference range for TMMEPs of clinically normal GDs. The use of TMMEPs is a valid ancillary test to assess the integrity of motor pathways in GDs with CSM.

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Introduction

The use of transcranial magnetic motor evoked potentials (TMMEPs) was first described in humans in 1985 (Barker et al., 1985). To obtain TMMEPs, a magnetic stimulator and a coil are used to apply a brief magnetic field to the motor cortex, which generates a recordable motor evoked potential in the contralateral appendicular muscles (Nollet et al., 2003). This technique provides a non-invasive method for assessing descending motor pathway function (Barker et al., 1985; Di Lazzaro et al., 1999).

In humans, TMMEPs have been used to evaluate the functionality of the motor pathways in cervical spondylotic myelopathy, which is a common cause of chronic compressive cervical myelopathy similar to canine cervical spondylomyelopathy (CSM) (Di Lazzaro et al., 1999; Lo, 2007; da Costa, 2010). Magnetic resonance imaging (MRI) is typically used to diagnose this human disease and define the compressive sites, but it cannot provide information about spinal cord (SC) functionality (Capone et al., 2013). In this human condition, TMMEPs can be used to detect preclinical myelopathy, monitor disease progression by obtaining serial recordings, and monitor SC function during surgery (Travlos et al., 1992; Lo et al., 2004, 2006; Capone et al., 2013).

The use of TMMEPs has been reported in horses and dogs with cervical SC disease (Nollet et al., 2002; Poma et al., 2002; da Costa et al., 2006; De Decker et al., 2011). In CSM-affected Doberman Pinschers, TMMEP latencies were increased when compared with clinically normal Dobermans, and correlated with the severity of neurological signs and MRI findings in affected dogs (da Costa et al., 2006; De Decker et al., 2011). Great Danes (GDs) are also frequently affected by CSM (da Costa, 2010). However, no study has reported TMMEP values in clinically normal GDs or investigated its use in GDs with CSM. In humans and horses, TMMEP latencies are influenced by body size (Chu, 1989; Nollet et al., 2004). GDs are
larger than Doberman Pinschers; thus, TMMEPs reference ranges obtained in Doberman Pinschers may not apply to GDs.

The purpose of this study was to establish the reference ranges of TMMEP latency and amplitude in clinically normal GDs, compare TMMEPs obtained in GDs with and without clinical signs of CSM, and determine whether there is any association between TMMEP data, severity of neurological signs, and MRI findings. We hypothesized that differences would be identified in the TMMEP latencies between clinically normal and CSM-affected GDs, but no amplitude differences would be identified between groups, similar to what has been previously reported in a TMMEP study performed in Dobermans with and without CSM (da Costa et al., 2006). We also hypothesized that TMMEP latencies would be longer in the CSM-affected GDs with more severe clinical signs and SC compression.

**Materials and methods**

**Animals**

The study was conducted in accordance with the guidelines and with the approval of The Ohio State University Clinical Research Advisory Committee and the Institutional Animal Care and Use Committee (2011A00000027). Written owner consent was obtained prior to study enrollment. Two groups of client-owned GDs were prospectively enrolled between April 2011 and October 2012. The first group included 15 clinically normal (control) GDs based on a normal neurological examination and no history of neurological disease. Only GDs ≥ 1 year of age were eligible for enrollment as control dogs. The second group included 14 GDs with clinical signs and neurological examination consistent with CSM and diagnostic confirmation via MRI. The time of onset of signs was recorded. A video of the gait of all CSM-affected dogs was obtained at the time of enrollment. All GDs were examined by the two investigators, and underwent TMMEPs and MRI of the cervical vertebral column.

**Gait grading**

The video material was reviewed at a later time by one investigator (PMV) to assign a neurological grade to each CSM-affected GD. At least 2 min of video material were available for all dogs. The grade was graded from 0 to 3 for each thoracic and pelvic limb as follows: grade 0, normal limb; grade 1, abnormal use of the limb <40% of the steps; grade 2, abnormal use of the limb between 40% and 70% of the steps, and grade 3, abnormal use of the limb >70% of the steps. Signs of both paresis/weakness (i.e., knuckling, scuffing, dragging) and/or ataxia/incoordination (inconsistent limb/foot placement) were considered as an abnormal use of the limb. If the grade was given to each limb, the most severe type of compression was used for statistical analysis in the same dog, the most severe type of compression was used for statistical analysis. Sites of SC signal changes, defined as SC hyperintensity on T2-weighted images, were also recorded.

**Statistical methods**

For all TMMEP variables (latency, amplitude, and neuronal path length for the ECR and CT muscles), values recorded in each dog for left and right limbs were averaged to obtain a single value for each variable and dog. A random-effects linear regression model was used to compare the TMMEP variables between control and CSM-affected GDs, and to investigate associations between TMMEP latencies and amplitudes with the neurological status and MRI findings in CSM-affected GDs. Adjustments were made for age, gender, and weight. The P values were adjusted by Holm’s procedure to conserve the type I error at 0.05. Significance was set at P < 0.05.

**Results**

**Clinical data and MRI findings**

The clinically normal GDs included seven females (six spayed, one intact) and eight males (seven neutered, one intact). Their median age at the time of enrollment was 2.3 years (range, 1–6.4 years). The median weight was 52 kg (range, 40.5–73 kg). All clinically normal GDs had a normal neurological examination. The CSM-affected GDs included two spayed females, 11 neutered males, and one intact male. Their median age at the time of enrollment was 4.2 years (range, 1–7.2 years). The median weight was 57.5 kg (range, 45–79.3 kg). The reported median age at the onset of signs of CSM was 1.6 years (range, 0.4–4.2 years). The clinical signs had been present for a mean time of 1.6 years (range, 0.2–5 years) before enrollment. Thirteen out of the 14 CSM-affected dogs showed ambulatory tetraparesis with proprioceptive ataxia of all limbs, and one showed a hypertonic thoracic limb gait with ambulatory paraparesis and proprioceptive ataxia of the pelvic limbs. All CSM-affected GDs had delayed postural reactions involving all limbs, and five had mild neck pain.

Gait grading yielded the following results: grade 1, n = 1; grade 2, n = 3; grade 3, n = 1; grade 4, n = 3; grade 5, n = 1; grade 6, n = 5. For statistical analysis, four dogs were considered to have mild signs (grades 1–2), four had moderate signs (grades 3–4), and six had severe signs (grades 5–6). Overall, 43 sites of SC compression were determined.
Fig. 1. Transcranial magnetic motor evoked potential (TMMEP) recorded from the left extensor carpi radialis muscle (upper trace) and left cranial tibial muscle (lower trace) from a clinically normal Great Dane. Vertical bars indicate distance from the stimulus artifact to the onset of the response (onset latency). Horizontal bars indicate peak-to-peak amplitude. Sensitivity: 1000 μV/division. Distance between dotted lines: 10 ms.

Recorded in the CSM-affected G.Ds. Based on the severity of the SC compression, one dog was classified as having mild compression, three had moderate compression, and ten had severe compression. Fourteen sites of SC hyperintensity were recorded in 9 CSM-affected G.Ds.

**TMMEPs and associations with neurological signs and MRI findings**

The TMMEPs waveform appeared polyphasic in all G.Ds, regardless of their clinical status (Figs. 1 and 2). In 8/14 CSM-affected G.Ds, TMMEPs could not be recorded in either pelvic limb. In an additional three CSM-affected G.Ds, TMMEPs were recordable only in one pelvic limb (Fig. 3). In the remaining three affected G.Ds, TMMEPs were recordable in both pelvic limbs. The TMMEPs were recordable in the thoracic limbs for all CSM-affected G.Ds and in all four limbs for all control G.Ds. Mean values for latencies, amplitudes, and neuronal path lengths for control and CSM-affected G.Ds were calculated (Table 1). Extensor carpi radialis and CT latencies were significantly longer for CSM-affected G.Ds when compared with control G.Ds. No significant differences between groups were found for amplitudes or neuronal path lengths.

In the CSM-affected G.Ds, ECR mean latencies progressively increased with severity of neurological signs (Table 2). However, only the comparison of ECR latencies between CSM-affected G.Ds with mild and severe signs yielded significant differences. Extensor carpi radialis amplitudes were significantly different in dogs with moderate signs when compared with those that had mild signs. For the CT TMMEPs, CSM-affected G.Ds with moderate and severe clinical signs had significantly longer latencies that those with mild signs (Table 2).

For the ECR TMMEPs, there were no significant associations between latencies or amplitudes and severity of SC compression recorded on MRI (Table 3). The degree of SC compression did show a significant association with CT latencies, with longer latencies in affected G.Ds with moderate and severe SC compression, compared with dogs that had mild SC compression (Table 3). Out of the nine CSM-affected G.Ds with SC signal changes, two had recordable TMMEPs in one pelvic limb, and 7/9 had no recordable TMMEPs on either pelvic limb. No SC signal changes were present in 3/3 CSM-affected G.Ds with recordable TMMEPs from one pelvic limb, and in 1/8 CSM-affected G.D with no recordable TMMEPs from either pelvic limb. No significant associations were found between ECR latencies (P = 0.463) or CT latencies (P = 0.999), and the presence of SC signal changes in the CSM-affected G.Ds.

**Discussion**

The present study provides reference ranges for TMMEP latencies and amplitudes in clinically normal G.Ds. We also found that CSM-affected G.Ds had significantly longer TMMEP latencies for the ECR and CT muscles when compared with control G.Ds. Moreover, CT latencies were significantly associated with the severity of neurological signs and SC compression in CSM-affected G.Ds. The results are in agreement with two previous studies performed on Doberman Pinschers with and without signs of CSM, which also reported that CT latencies were significantly different between groups, and correlated with the degree of neurological signs and severity of SC compression (Da Costa et al., 2006; De Decker et al., 2011).

The ECR latencies yielded conflicting results in Doberman Pinschers with and without signs of CSM. One study reported no differences between clinically normal and CSM-affected Dobermans Pinschers, whereas another study did report significant differences between the two groups (Da Costa et al., 2006; De Decker et al., 2011). Our study did show significantly longer ECR latencies in CSM-affected G.Ds when compared with control G.Ds. However, the onset latencies for the ECR and CT muscles obtained in both clinically normal and CSM-affected G.Ds were longer than the equivalent latencies in normal and CSM-affected Doberman Pinschers.

G.Ds are larger and taller than Doberman Pinschers, with a mean neuronal path length of 106.7 cm for the ECR muscle and 167.5 cm for the CT muscle in normal G.Ds, when compared to a mean neuronal path length of 78.4 cm and 124.9 cm in normal Dobermans,
respectively (da Costa et al., 2006). A longer neuronal path length means that the impulse will need to travel a larger distance before reaching the muscle and generating a motor evoked potential; thus, yielding longer latencies. The influence of body size on TMMEP latencies has also been reported in humans and horses (Chu, 1989; Nollet et al., 2004). These results indicate that reference ranges for TMMEP latency will likely be different across dog breeds with different body sizes.

Overall, TMMEP amplitudes were markedly variable for both control and CSM-affected GDs, showing a high degree of overlap between groups and no significant differences. In humans, TMMEP amplitudes are reported to be extremely variable even within the same individual (Eisen and Shytbel, 1990). Amplitudes also showed more variability and appeared less reliable than TMMEP latencies in dogs and horses (Nollet et al., 2002; da Costa et al., 2006).

In this study, no TMMEPs could be elicited from the pelvic limbs in several of the CSM-affected GDs, whereas pelvic limb TMMEPs were recordable from all control GDs. Similar findings have been reported in human cervical spondylotic myelopathy (Tavy et al., 1994; Lo et al., 2004, 2007). One study reported absent TMMEPs in 34/141 (24.1%) people with cervical spondylotic myelopathy of varying severities (Lo et al., 2004). Similarly, absent pelvic limb TMMEPs have been reported in CSM-affected Doberman Pinschers and horses with cervical myelopathy (Nollet et al., 2002; De Decker et al., 2011). The reason behind this phenomenon is not well understood; however, experimental studies of SC injury in humans and animals have suggested that the propagating impulse, albeit present in the SC distal to the lesion, may not be strong enough to increase the postsynaptic membrane potential of the motor neuron to its threshold; thus, no impulse will be present in the peripheral nerve and muscle from

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Muscle</th>
<th>Control×</th>
<th>CSM-affected×</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency (ms)</td>
<td>ECRM</td>
<td>18.1 (14.4–21.8)</td>
<td>25.3 (22.4–28.3)</td>
<td>0.006×</td>
</tr>
<tr>
<td></td>
<td>CTM</td>
<td>29.6 (25.9–33.3)</td>
<td>46.4 (42.4–50.3)</td>
<td>&lt;0.001×</td>
</tr>
<tr>
<td>Amplitude (µV)</td>
<td>ECRM</td>
<td>2910 (1978–3842)</td>
<td>2504 (1745–3263)</td>
<td>0.760</td>
</tr>
<tr>
<td></td>
<td>CTM</td>
<td>1617 (685–2549)</td>
<td>599 (583–1782)</td>
<td>0.331</td>
</tr>
<tr>
<td>Neuronal path length (cm)</td>
<td>ECRM</td>
<td>106.7 (102.8–110.5)</td>
<td>101.7 (98.6–104.9)</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>CTM</td>
<td>167.5 (163.6–171.4)</td>
<td>170.2 (165.2–175.2)</td>
<td>0.629</td>
</tr>
</tbody>
</table>

Data are presented as means (95% confidence interval).
× TMMEPs were recordable from all four limbs in all 15 clinically normal GDs enrolled.
× TMMEPs were recordable from the ECRM in all 14 CSM-affected GDs enrolled. In 8/14 affected GDs, TMMEPs could not be recorded from either pelvic limb. TMMEPs were recordable from the CTM of both pelvic limbs and one pelvic limb in 3/14 and 3/14 of affected GDs, respectively.
× Indicates statistical significance, p<0.05.

### Table 2

<table>
<thead>
<tr>
<th>Severity of signs</th>
<th>ECRM latency</th>
<th>ECRM amplitude</th>
<th>CTM latency</th>
<th>CTM amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (n = 4, n = 2)×</td>
<td>21.9 (17.7–26.1)</td>
<td>3939 (2716–5162)</td>
<td>37.6 (33.1–42.2)</td>
<td>2046 (360–3733)</td>
</tr>
<tr>
<td>Moderate (n = 4, n = 2)×</td>
<td>24.1 (19.8–28.4)</td>
<td>1245 (1.3–2490)</td>
<td>50.2 (44.9–55.5)</td>
<td>970 (1.419–3360)</td>
</tr>
<tr>
<td>Severe (n = 6, n = 2)×</td>
<td>28.5 (25.1–31.8)</td>
<td>2299 (1314–3263)</td>
<td>51.9 (48.5–55.8)</td>
<td>284 (2053–1484)</td>
</tr>
<tr>
<td>Mild vs. moderate×</td>
<td>0.489</td>
<td>0.010†</td>
<td>0.001†</td>
<td>0.478</td>
</tr>
<tr>
<td>Mild vs. severe×</td>
<td>0.042</td>
<td>0.069</td>
<td>&lt;0.001†</td>
<td>0.193</td>
</tr>
<tr>
<td>Moderate vs. severe×</td>
<td>0.253</td>
<td>0.026</td>
<td>0.018</td>
<td>0.808</td>
</tr>
</tbody>
</table>

Data are presented as mean (95% confidence interval).
× n number of affected dogs categorized as having mild, moderate, and severe signs. For a given severity of neurological signs, the first n indicates the number of recordable TMMEPs from the ECRM, and the second n indicates the number of recordable TMMEPs from the CTM in the affected dogs.
† P values based on a linear regression model adjusted for age, gender, and weight.
× Indicates statistical significance, p<0.05.

### Table 3

<table>
<thead>
<tr>
<th>Severity of signs</th>
<th>ECRM latency</th>
<th>ECRM amplitude</th>
<th>CTM latency</th>
<th>CTM amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (n = 1, n = 1)×</td>
<td>18.8 (9.4–28.2)</td>
<td>5114 (2766–7462)</td>
<td>31.9 (22.4–41.3)</td>
<td>3502 (1155–5849)</td>
</tr>
<tr>
<td>Moderate (n = 3, n = 1)×</td>
<td>24.6 (18.9–30.2)</td>
<td>2161 (752–3570)</td>
<td>49 (42.5–55.6)</td>
<td>1665 (852–421)</td>
</tr>
<tr>
<td>Severe (n = 10, n = 4)×</td>
<td>26.6 (23.4–29.7)</td>
<td>2154 (1366–2941)</td>
<td>48.8 (45.2–52.5)</td>
<td>320 (10–169–1710)</td>
</tr>
<tr>
<td>Mild vs. moderate×</td>
<td>0.622</td>
<td>0.073</td>
<td>0.007†</td>
<td>0.570</td>
</tr>
<tr>
<td>Mild vs. severe×</td>
<td>0.387</td>
<td>0.061</td>
<td>0.003†</td>
<td>0.064</td>
</tr>
<tr>
<td>Moderate vs. severe×</td>
<td>0.563</td>
<td>0.993</td>
<td>0.956</td>
<td>0.353</td>
</tr>
</tbody>
</table>

Data are presented as means (95% confidence interval).
× n number of affected dogs categorized as having mild, moderate, and severe spinal cord compression. For a given severity of spinal cord compression, the first n indicates the number of recordable TMMEPs from the ECRM, and the second n indicates the number of recordable TMMEPs from the CTM in the affected dogs. When n = 1, the mean and 95% confidence interval presented is the result of the eight TMMEPs (four from the right side, four from the left side) recorded for that given dog from either the ECRM or the CTM.
† P values based on a linear regression model adjusted for age, gender, and weight.
× Indicates statistical significance, p<0.05.
which the TMMEP recordings are obtained (Konrad et al., 1987; Owen et al., 1989; Kraus et al., 1990; Nollet et al., 2002).

Moreover, no pelvic limb TMMEPs could be recorded in 7/9 CSM-affected GDs showing SC signal changes. Even if no statistical significant associations were found between the presence of SC signal changes and TMMEP latencies, the difficulty in recording TMMEPs from this subset of CSM-affected GDs is consistent with studies investigating the use of TMMEPs in people with cervical spondylotic myelopathy and SC signal changes (Lo et al., 2004). In one study, no TMMEPs could be recorded in 24/36 (67%) people with cervical spondylotic myelopathy and hyperintense T2-weighted SC signal changes, whereas absent TMMEPs were only found in 10/77 (13%) subjects with cervical SC compression but without SC signal changes (Lo et al., 2004). In this study, CSM-affected dogs with more severe SC compression and dogs with the presence of SC signal changes showed lack of recordable pelvic limb TMMEPs more often than those dogs with milder SC compression and normal SC signal. Hence, the lack of recordable pelvic limb TMMEPs suggests more severe SC involvement.

A limitation of our study is its small sample size. However, this was a prospective study with limited funds and time for subject enrollment. The prospective nature of this investigation allowed us to use a constant sedation protocol and TMMEP technique for all dogs enrolled. Various sedation protocols have been used to obtain TMMEPs in dogs (Sylvestre et al., 1992; Van Ham et al., 1994; Van Soens et al., 2009). We used a combination of an α₂-adrenergic agonist (dexmedetomidine) and an opioid (hydromorphone). Combined sedation protocols achieve better levels of sedation to obtain TMMEPs in dogs, and α₂-adrenergic agonists originate more profound sedation than other drugs (Van Ham et al., 1994; Van Soens et al., 2009). One study reported that a combination of medetomidine and methadone yielded longer TMMEP latencies and smaller amplitudes in dogs (Van Ham et al., 1994). In contrast, a more recent study compared the effects of medetomidine and acepromazine/methadone on canine TMMEPs and found no differences in latencies and/or amplitudes between the two protocols, concluding that both sedation protocols were equally valid and that the previously reported effects of α₂-adrenergic agonist on TMMEPs were probably due to the higher doses used in the first study (Van Soens et al., 2009). The dose of dexmedetomidine used in our study was similar to the medetomidine dose administered in this latter study by Van Soens et al. (2009).

While MRI of the cervical vertebral column is necessary to confirm the presence, extent, and location of SC compressive sites in CSM-affected GDs, the significant TMMEP latency differences found in this study between clinically normal and CSM-affected GDs, suggest that TMMEPs could be used as an initial screening test in GDs with clinical signs consistent with CSM before MRI is pursued. The use of TMMEPs can offer functional information about the status of the SC motor pathways, which cannot be obtained from the advanced imaging modalities routinely used to diagnose CSM. In CSM-affected dogs treated surgically, TMMEPs could be used as an objective outcome measure of the functionality of the SC motor pathways before and after surgery is pursued.

Conclusions

We report reference ranges for TMMEP latencies and amplitudes for clinically normal GDs. Both ECR and CT TMMEP latencies helped differentiate between control and CSM-affected GDs. In CSM-affected GDs, CT TMMEP latencies were significantly associated with the degree of neurological signs and the severity of SC compression on MRI. The use of TMMEPs is a valid ancillary test to assess the integrity of motor pathways in CSM-affected GDs.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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