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Evaluation of lumbosacral nerve root conduction in chickens by electrophysiological testing including high-resolution spinal magnetic stimulation

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ABSTRACT

The value of avian models in peripheral nerve research recently became substantiated by the immunobiological similarity of avian inflammatory demyelinating polyradiculoneuropathy to human Guillain–Barré syndrome providing an alternative animal model for experimental autoimmune neuritis. As electrophysiologic evaluation of nerve roots is essential part of the diagnosis of polyradiculoneuropathies in humans, it would be favourable to have similar research methods available for juvenile chickens. Hence, this study was performed (1) to establish a tool-set that allows for reproducible evaluation of the tibial/sciatic nerve and its nerve roots, (2) to achieve age-matched reference values, and (3) to trace the kinetics of peripheral nerve maturation within chickens.

Nine chickens underwent serial electrodiagnostic examinations between the age of 6 and 15 weeks. Several methods of sensory and motor nerve fiber stimulation of the tibial/sciatic nerve were tested and modified or established. Ultimately, scalp-recorded somatosensory evoked potentials, compound muscle action potentials elicited by tibial/sciatic nerve electrical as well as spinal magnetic stimulation and motor nerve conduction velocity were available for tibial/sciatic nerve and nerve root evaluation in chickens. Base values were obtained for all investigations and parameters. Results indicated that the maturation of the nerve fibers is incomplete up to the age of 15 weeks.

The methods tested here provide an excellent tool-set for quantitative tibial/sciatic nerve and nerve root assessment in avian polyradiculoneuropathies, especially within the scope of longitudinal monitoring of the disease course.

1. Introduction

Electrodiagnostic evaluation of spinal nerves and nerve roots, as aspired in diagnosis and monitoring of human polyradiculoneuropathies like Guillain–Barré syndrome (GBS) (Gupta et al., 2008; Kalita et al., 2008), is routinely performed to assess the function of proximal sensory and motor nerve fibers. Examination of the nerve roots hampers from the difficulty to access the particular anatomic structures since deep location and surrounding bones at the emergences complicate the controlled application of stimuli and render these areas less suitable for taking nerve biopsies for morphological disease monitoring.

Currently, the nerve root function may be assessed electrodiagnostically by H reflexes, F wave, and somatosensory evoked potentials (SSEPs) with varying feasibility (Aminoff, 2002). Furthermore, the status of the ventral nerve roots and the most proximal parts of the motor nerve fibers can be assessed directly without including parts of the CNS by high-voltage percutaneous electrical stimulation and paravertebral magnetic stimulation (Aminoff, 2002; Chokroverty et al., 1993).

Abbreviations: AvIDP, avian inflammatory demyelinating polyradiculoneuropathy; CDP, cord dorsum potential; CMAP, compound muscle action potential; CNS, central nervous system; GBS, Guillain–Barré syndrome; GM, gastrocnemic muscle; IDP, inflammatory demyelinating polyradiculoneuropathy; MNCV, motor nerve conduction velocity; PNS, peripheral nervous system; SNCV, sensory nerve conduction velocity; sMS, spinal magnetic stimulation; SSCV, somatosensory conduction velocity; SSEP, somatosensory evoked potential; TSN, tibial/sciatic nerve.

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The value of juvenile chickens in neurological research recently has been emphasized by the highly prevalent GBS-like avian inflammatory demyelinating polyradiculoneuropathy (AvIDP) (Bader et al., 2010) as well as in previous work on inflammatory polyneuropathies (Bacon et al., 2001). These potentially overlapping syndromes and the classical form of Marek's disease (Pepose et al., 1981; Stevens et al., 1981) provide naturally occurring alternatives to experimental animal models for human inflammatory demyelinating polyradiculoneuropathies (IDPs). Further benefits of chickens as compared to rodents are given by the bipedal gait and a body size that facilitates electrophysiological testing.

Unfortunately, there still is a considerable lack of electrophysiologic data on age and breed dependent variables in normal chickens even though some basic investigations for chickens and/or other avian species have been carried out, including establishment of electrodiagnostic techniques to measure motor nerve conduction velocity (MNCV) (Bagley et al., 1995; Bagley et al., 1992; Kornegay et al., 1983b; Maguire et al., 1998; Massicotte et al., 2001; Platt et al., 1999), F waves (Bagley et al., 1993), sensory nerve conduction velocity (SNCV) (Brenner et al., 2008), cortical SSEPs (Gregory and Wotton, 1989; Machida et al., 1994) and cord dorsum potentials (CDPs) (Brenner et al., 2008).

It was the aim of this study to develop an optimized and reproducible protocol and reference values for evaluation of lumbosacral nerve roots by needle electrodes in juvenile chickens. Furthermore, we used high-resolution magnetic coil stimulation using triple stimulation technique in order to evoke nerve currents in the most proximal aspects of the motor nerve roots.

2. Material and methods

2.1. Animals

Twelve female White Leghorn chickens were obtained from a commercial breeder (LSL, Lohmann Tierzucht, Cuxhaven, Germany) at an age of 6 weeks. Physical and neurological examinations were performed on each chicken as described previously (Clippinger et al., 2007) to evaluate the general health status. All animal experiments were performed in accordance with the German Protection of Animals Act and approved by the government of Upper Bavaria, Germany (55.2.1.54-2531-91-08).

2.2. Study protocol

Nine chickens were used for sequential electrophysiologic testing at the ages of 6 weeks, 8 weeks, 10 weeks, and 15 weeks. Additionally, lidocaine nerve block and neurectomy of the sciatic nerve and rhizotomy of the associated nerve roots were performed as confirmatory explorations in three additional animals.

2.3. Anesthesia

All electrophysiologic measurements were performed under anesthesia. The chickens were manually restrained, while anesthesia was induced with 5% isoflurane (Isofluran CP, CP-Pharma, Germany) administered via a purpose built face mask. The feasibility of isoflurane anesthesia for magnetic stimulation has been demonstrated in a pilot investigation on chickens of the same age. Once relaxation of the muscles had occurred and the corneal reflexes were sluggish, the chickens were positioned in lateral recumbency and obtained 1.5–3% of isoflurane in oxygen via inhalation for the duration of the procedure. Body temperature was continually measured and maintained within the physiological range supported by an underlain hot gel pad. The depth of anesthesia was monitored using muscle tone, withdrawal reflexes, corneal reflexes, breath and heart rate, variations of which led to according changes in the concentration of isoflurane.

2.4. Technical equipment

Electric stimulation and all recordings were performed with a Viking Quest™ neurodiagnostic system, Version 11.0.0 (Viasys Healthcare Neurocare Group, Judex A/S). For spinal magnetic stimulation, a 2 T PowerMAG magnetic stimulator (MAG & More GmbH, Munich, Germany) was used.

2.5. Motor nerve conduction velocity (MNCV) and compound muscle action potentials (CMAPs)

Needle electrode stimulation of the motor parts of the tibial/sciatic nerve (TSN) was based on modified protocols (Bagley et al., 1992; Kornegay et al., 1983b; Maguire et al., 1998; Massicotte et al., 2001; Platt et al., 1999).

2.5.1. Stimulation

Percutaneously, TSN stimulation was performed with 2 monopolar Teflon-coated needle electrodes (diameter: 0.36 mm (28 G), length: 25 mm; Viasys Healthcare GmbH, Hoechberg, Germany) at a proximal and a distal stimulation site. Proximally, the cathode was inserted 1 cm caudal to the femoral trochanter. The anode was positioned subcutaneously 1 cm caudal to the cathode. Distally, the cathode was inserted in the popliteal fossa close to the tibial nerve and the anode was positioned subcutaneously 1 cm proximal to the cathode.

Stimulation was achieved by rectangular pulses of 0.2 ms duration, a frequency of 1 Hz, at an intensity 15–20% greater than that required for maximal CMAP amplitude.

2.5.2. Recording

The CMAP was recorded from the gastrocnemic muscle (GM) with two subdermal platinum needle electrodes (length of 12 mm; Cardinal Health, Hoechberg, Germany). One needle was placed percutaneously over the motor point area of the GM and one needle subcutaneously over the ankle tendon (belly-tendon-montage) connected to the inverting and non-inverting inputs of the preamplifier, respectively. The electrodes were positioned in this manner to achieve a CMAP having a sharp initial negative deflection, followed by a positive deflection. The ground electrode was placed subcutaneously between the stimulating and the recording electrodes, overlying the lateral epicondyle of the femur. Signals were amplified by filters set at a band pass of 20 Hz–10 kHz. Sampling rate was set at 20 kHz.

2.5.3. Parameters

The following parameters were measured from the CMAPs obtained after proximal and distal stimulation (Fig. 2A): onset latency (ms), peak-to-peak amplitude (mV), negative area (mV ms) and CMAP duration (ms). Onset latency was defined as the time between the stimulus artefact and the first negative deflection from the base line. The cursors of the amplitudes were set peak to peak. The negative area and the CMAP duration were measured between cursors positioned at the first negative deflection and the first baseline crossing. In order to obtain motor nerve conduction velocity (MNCV; m/s) the distance between proximal and distal stimulation site was measured manually with a flexible reference tape and fed into the computer program. Motor nerve conduction velocity was determined by the computer software following the standard formula:

$$\mathrm{MNCV} = \frac{\text{distance between proximal and distal stimulation site}}{(\text{latency}_{\text{prox}} - \text{latency}_{\text{dist}})}\ (\mathrm{m/s}).$$