Arh. hig. rada toksikol., **30** *(1979) suppl. pp. 239–243.*

AN ELECTROPHYSIOLOGICAL METHOD FOR EXAMINATION IN VIVO OF PERIPHERAL NEUROPATHY DUE TO ORGANIC MERCURY POISONING IN RATS

J. MISUMI and S. NOMURA

Department of Public Health, Kumamoto University Medical School, Kumamoto City, Kumamoto, Japan

ABSTRACT

This report describes an electrophysiological method by which action potentials in the tail nerve of rats can be recorded and the conduction velocity of sensory fibres (SCV) and the maximum conduction velocity of motor fibres (MCV) estimated. Also, it explains the usefulness of the method as a tool for detecting dysfunction of peripheral nerves in animals treated for a long time with methylmercuric chloride. By use of the technique, the amplitudes of action potentials were found to decrease significantly in the early stages of treatment with the chemical. In the later stage the SCV and MCV of the tail nerve decreased as well.

Recently, the conduction velocity of sensory or mixed nerves involving GIa fibers has frequently been used as a diagnostic tool for sensory neuropathy in human subjects[2,3]. However, no reliable method *in vivo* by which peripheral neuropathy can be examined from an electrophysiological point of view has yet been established in small experimental animals.

This report describes an electrophysiological method by which compound action potentials (APs) *in vivo* can be recorded in the tail nerve of rats. Also, it explains the usefulness of the method as a tool for detecting dysfunction of peripheral nerves in animals treated with methylmercury chloride, which is well known as a chemical substance causing peripheral neuropathy.

MATERIALS AND METHODS

Male Donryu strain rats aged 80 to 360 days were used for the study. The rats were lightly anesthetized with an intraperitoneal injection of amobarbital-sodium (70–80 mg/kg), and their backs fixed on a supporter. Room temperature was conditioned at 28 ± 1 °C.

Twenty male rats 9 and 10 weeks old were used in the experiment. One group (12 rats) was treated with CH_3HgCl, while the other (8 rats) was not

treated and served as control. The treatment schedule consisted of a daily subcutaneous injection of the substance (0.5 mg per rat, 6 days a week). The treatment was continued for 5 to 7 weeks until the animals developed severe ataxia and abnormal reflexes of the hind limbs. The measurement of conduction velocity was undertaken on the 23rd, 35th and 55th day of injection, the total accumulated doses of CH_3HgCl reaching 10 mg, 15 mg and 15–22 mg, respectively. The room temperature was kept at 27.5 ± 0.5 °C.

For stimulation of the tail nerve, a pair of bare steel needles with a diameter of 0.25 mm was inserted under the skin at the ventral site of the tail. The needles were located about 2.5 cm from the tail end (S of Fig. 1). The nerve was supramaximally stimulated with a single pulse of 0.3 msec duration at a frequency of 1 Hertz, delivered by an electric stimulator (MNS-1101, Nihon Kohden).

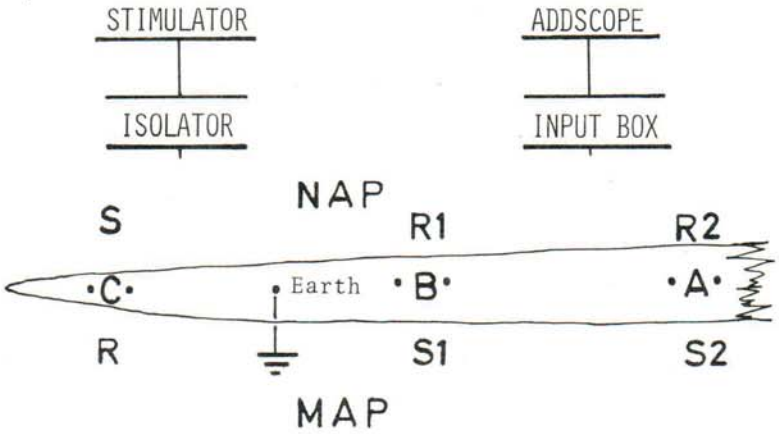

FIG. 1 – Arrangement of the stimulating and recording electrodes in measurement of the SCV and MCV of the rat's tail nerve. Further explanation in the text.

In order to record the APs evoked in the tail nerve, two pairs of bare needles were inserted at two points in the tail, one in the middle part (R1 of Fig. 1) and the other in the proximal part about 1.5 cm from the anus (R2 of Fig. 1). Distances between the stimulating and recording electrodes, or between two recording electrodes, were measured on the skin, and used for calculating the conduction velocities (CVs) of the tail nerve. Muscle action potentials were also evoked by stimulation at S1 or S2 of Figure 1, and recorded at R of Figure 1, for the measurement of maximum motor fibers conduction velocity[4] (MCV). APs were amplified with a time constant of 0.01 sec, and displayed on the screen of an Addscope (ATAC-250, Nihon Kohden) and photographed on polaroid films.

RESULTS

The APs recorded both in the proximal and in the middle part of the tail showed negative deflections or diphasic ones as illustrated in A of Figure 2. The

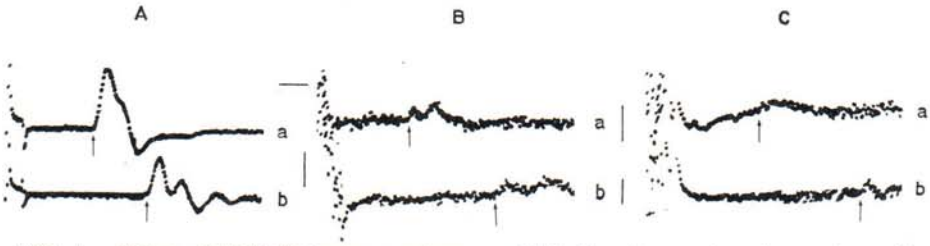

FIG. 2 – Effects of CH_3HgCl *in vivo* on action potentials in the tail nerve. In each record, *a* and *b* were recorded in the middle part and the proximal part, respectively, when stimulated at the distal end of the tail. A, control; B and C, recorded from the two different rats treated with CH_3HgCl of 15–22 mg each. The arrows indicate the onset of the first deflection in each compound action potential. Calibration, 50 μV in A and 5 μV in B and C, and 1 msec for all the records.

mean amplitude of the APs recorded in the proximal part of the tails of 67 healthy rats was 22 ± 7.6 μV ($\pm$S.D.). The mean value for CVs of the most rapidly conducting impulse in the tail nerve was found from measurements on 76 healthy rats over 150 days old to be 46.3 ± 4.1 m/sec. In the proximal part of the tail nerve the values of the fastest SCVs were significantly greater (53.6 ± 6.5 m/sec, $n = 10$) than MCVs (39.9 ± 4.7 m/sec, $n = 10$). Therefore, the fastest conduction velocity in an afferent direction (SCV) was considered to derive from GIa fibers. The values for either SCV or MCV increased with increasing age of the rat.

TABLE 1

Conduction velocities in the tail nerve of rats at various stages after subcutaneous injection of CH_3HgCl (0.5 mg/rat/day).

	Conduction part	Group of rats	Days of dosing			
			initial	23rd	35th	55th
Sensory fibres (SCV)	whole (C → A)	control	34.8 ± 4.2	38.6 ± 2.0	43.9 ± 2.8	44.1 ± 2.1
		treated	35.8 ± 2.8	38.2 ± 2.6	35.2 ± 4.1*	–
	distal (C → B)	control	32.3 ± 3.6	35.5 ± 2.5	40.5 ± 3.6	39.6 ± 5.3
		treated	32.3 ± 3.7	37.0 ± 2.9	33.9 ± 2.8*	–
	proximal (B → A)	control	38.3 ± 5.9	42.5 ± 2.6	49.7 ± 4.3	49.4 ± 4.6
		treated	40.1 ± 7.2	39.8 ± 3.1	37.2 ± 6.0*	–
Motor fibres (MCV)	proximal (A ← B)	control	28.7 ± 4.7	32.6 ± 4.5	34.6 ± 4.0	39.1 ± 4.3
		treated	28.2 ± 2.6	30.6 ± 3.3	30.3 ± 3.1 (7)	28.1 ± 5.2 (4)*

Conduction part: C → A, for example, represents conduction velocity of the impulse conduction from point C to point A in Figure 1 of reference 2. Values are means ± S.D. (m/sec) ($n = 8$, except those values with parentheses in which numbers of rats used are indicated). The control group was not treated with CH_3HgCl. The treated group was given 10 mg, 15 mg and 15–22 mg of CH_3HgCl in total amounts on the 23rd, 35th and 55th day after the beginning of the experiment, respectively.
* statistical significance in the difference from control rats at a level of $p < 0.01$.

Using the technique described above, changes in the amplitudes of APs and both SCVs and MCVs of the tail were investigated on the animals during a continuous administration of methylmercuric chloride. In the early stage of administering the chemical (the total amount given being 10 mg), the amplitudes of APs decreased significantly as compared with those in the controls (Table 1). The SCVs were also found to decrease significantly when the total amount reached 15 mg, as shown in Table 2. At the later stage of administration, when the total amount reached 15–22 mg, the APs in four out of six surviving rats could not be recorded so clearly as in the control group, probably because of the severe peripheral neuropathy (compare B and C of Figure 2 with A). The MCVs were also found to decrease at later stages (see Table 2).

TABLE 2
Changes produced by treatment with CH_3HgCl in the amplitudes of action potentials conducting in the afferent direction.

Conduction part	Group of rats	Days of dosing			
		initial	23rd	35th	55th
whole (C → A)	control	18 ± 9.1	22 ± 5.5	21 ± 12.5	21 ± 4.7
	treated	13 ± 5.6	10 ± 4.4**	7 ± 6.4*	–
middle (C → B)	control	47 ± 12.8	45 ± 14.1	60 ± 23.3	51 ± 17.7
	treated	36 ± 11.9	34 ± 11.6	20 ± 15.0**	–

C → A: stimulated at the distal end (at point C in Figure 1 of reference 2) and recorded in the proximal part (at point A) of tail. C → B: stimulated at the same point as above and recorded in the middle part (at point B). Values are means ± S.D. in μV (n = 8). The control group was not treated.
The treated group was given 10 mg, 15 mg and 15–22 mg of CH_3HgCl in total amount on the 23rd, 35th and 55th day after the beginning of the experiment, respectively.
*and** statistical significance in the difference from the controls at a level of $p < 0.05$ and $p < 0.01$, respectively.

DISCUSSION

The decreased SCVs found in the present study by administering CH_3HgCl represent a dysfunction of GIa fibers which come out of the muscle spindles of the tail and have the largest diameter. As to the function of superficial sensation, the measurement of SCVs is not informative. However, measurement of the amplitudes of the APs seems to be useful in detecting dysfunction of the superficial sensation, because the APs involve those derived from the nerve fibers supplying cutaneous receptor organs.

In carrying out experiments applying this electrophysiological technique, one should pay attention to the environmental temperature (27–28 °C), the age of the animals used (150–360 days)[1] and the amount of anesthetic (60–80 mg/kg, if it is amobarbital-Na, now we are adopting 60 mg/kg).

REFERENCES

1. *Birren, J. E. and Wall, P. D.* Age changes in conduction velocity, refractory period, number of fibers, connective tissue space and blood vessels in sciatic nerve of rats. J. Comp. Neurol., **104** (1956) 1–16.
2. *Buchthal, F., Rosenfalck, A. and Trojaborg, W.* Electrophysiological findings in entrapment of the median nerve at wrist and elbow. J. Neurol. Neurosurg. Psychiatry, **37** (1974) 340–360.
3. *Dawson, G. D. and Scott, J. W.* The recording of nerve action potentials through skin in man. J. Neurol. Neurosurg. Psychiatry, **12** (1949) 259–267.
4. *Fullerton, P. M.* Chronic peripheral neuropathy produced by lead poisoning in guinea-pigs. J. Neuropathol. Exp. Neurol., **25** (1966) 214–236.