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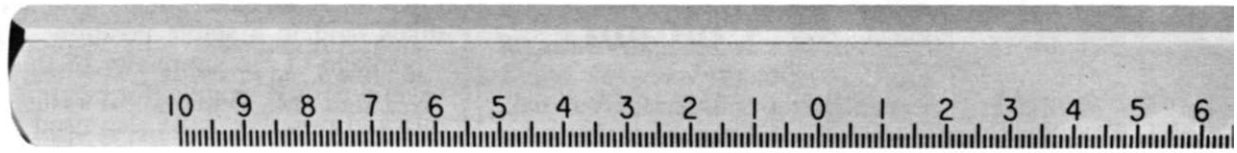

Spinal Evoked Potentials: Data and Experience

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Jan Galik recently returned to the Slovak Academy of Sciences from a two year Postdoctoral position with Tony Yaksh, Ph.D. Dr. Galik did his doctoral work with P.J. Safarik in Kosice, and is now engaged in research in his home Academy. Data presented here are from articles published in conjunction with his colleagues. He can be reached by e-mail; galik@linuxl. saske.ks, or by phone; +42-95-765064

INTRODUCTION

In the previous issue of the Carrier [1] we briefly described the principles of spinal evoked potential (EP) recording. In this article, we will provide data obtained by spinal EP recording in our laboratory, and share our practical experience with various electrode types.

Peripheral Recording

To monitor spinal cord function, peripheral input/output relationships _must be analyzed. Peripheral nerves are, therefore, important sites for stimulation and recording. As is typical for EPs, selection of electrodes and their use is essentially the same for stimulation and recording. There are several types of electrodes used for peripheral nerve stimulation or recording; 1) noninvasive - disc electrodes, cuff Velcro electrodes, subcutaneous needle electrodes, or 2) invasive - mono or multipolar hook or cuff style electrodes (fig.l). Using noninvasive superficial electrodes always requires that contact impedance between the electrodes and skin be kept as low as possible; usually by application of conductive paste or by moistening the skin. With acute invasive hook electrodes, it is important to protect the nerve against drying and hypothermia, usually by immersion in a warm mineral oil bath. Properly constructed cuff style electrodes are usually better than hook electrodes since they do not require an electrode holder, there is larger contact with the nerve, and the problems with nerve drying, hypothermia or mechanical damage are minimized since the wound is closed after implantation. In addition, cuff style electrodes are suitable for chronic experiments lasting for days or weeks. However, it is important to choose a cuff electrode with the proper internal diameter and to use minimal force to secure the electrode in order to prevent peripheral nerve compression and subsequent nerve ischemia.

EPs recorded from peripheral nerve can be evoked by stimulation of the distal parts of the nerve or its sensory field, or by stimulation of the proximal part of the nerve, spinal structures such as spinal ganglia, spinal roots, spinal tracts, or su-praspinal and cerebral structures (usually sensory or motor cortex) [2]. We have used peripheral EP recordings with simultaneous spinal somatosen-sory EP (SEP) recording to evaluate the function of rabbit spinal interneurons injured by spinal cord ischemia and treated by epidural cooling [3J. EPs were evoked by bipolar stimulation of a sciatic nerve. Identical bipolar recording J shaped hook electrodes were placed symmetrically on the contralateral sciatic nerve. Thus, sensory pathways were stimulated on one side and the EP recorded on the contralateral side. We found a clear correlation between neurological status, his-tological damage, recovery of ChAT and AChE activity, and recovery of peripherally recorded motor EPs with respect to the intraspinal temperature during spinal cord ischemia (fig.2).

Noninvasive Spinal EP Recording

Techniques for noninvasive spinal EP recording are, in principle, much the same as techniques used in elec-troencephalography (EEG). The same types of electrodes can be used, e.g., superficial disc or needle electrodes. However, there are frequently difficulties with disk electrodes with the quality of signal and stimulation artifact, which can be avoided by using subcutaneous needle electrodes. Needles can be inserted closer to the examined structure, even into the epidural space through intervertebral ligaments (fig.l). This method is optimal for acute experiments with anesthetized

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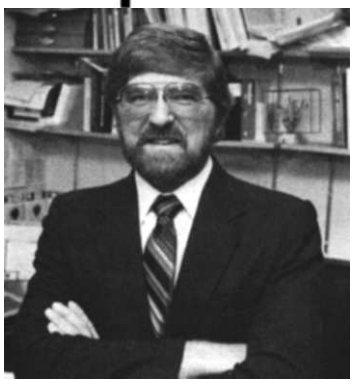

Editor's Column

This issue of the Carrier is the second by Jan Galik, and is noteworthy because Jan has returned to the Slovak Republic after a two year post-doc in the US. Jan sent me a brochure of his home town, Kospice, and it certainly looks like a beautiful town which would be a great place to visit. Many thanks to Jan for this article.

We hope to see many of you at the Society for Neuroscience Meeting in October. The Carrier has been a part of the Kopf Company for many years now. David and Carol Kopf have been very supportive of the publication, and hope it has been useful to many of you. We are thinking of putting some of the future issues of the publication on a Web page rather than sending it out in paper form. However, we need to know whether this would be useful or accessible to the Neuroscience community. I would appreciate it if you would let me know your feelings about whether and how you would like to see the Carrier continued, and your opinion of the value of it to you. You can call, FAX or e-mail me at the numbers and address below. We will also be seeking opinions at the Neuroscience Meetings, so stop by the booth to talk with us about these issues. With rising costs of production and mailing, the value of the publication and methods of distribution need to be assessed. I would appreciate your input.

The full moon is shining through my home office window as I type this and it is a wonderful sight. It is supposed to get cool this weekend, a sure sign that fall is coming. The changing seasons are wonderful, making us even more aware of the power and beauty of our world, wherever we are. Help it stay that way. Protect our earth and its beings.

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animals held in a stable position. We have taken advantage of the simplicity of this procedure in several animal models, for example in dogs, rabbits and rats. We have also used this method to record spinal SEPs in dogs before, during and after ischemia of the hypothermic spinal cord [4]. Ischemia was induced by occlusion of the aorta and left subclavian artery for 40 min. with partial exsanguination. The spinal cord was cooled during ischemia by epidural application of 5°C saline. EPs were recorded in response to left-side sciatic nerve stimulation, using cuff-style bipolar stimulating electrode (0.5mA, 0.3ms, 4. IHz). Recording needle electrodes were inserted to the LI and L4 vertebrae, so that the uninsulated tips rested on the vertebral laminae. We have used this technique for repeated EP recording over several days. Position of the inserted needle was always carefully marked to ensure same position when the needle was reinserted. Although this method is not optimal for repeated recordings, satisfactory results were obtained. Characteristic preischemic recordings consisted of three initial negative waves (Nl, N2, N3) and one late positive (P) wave. The Nl and N2 have been reported to reflect the presynaptic intramedullary potentials followed by a large N3 wave as a result of postsynaptic intramedullary interneuronal activation. The P wave has been related to evoked motoneuronal discharges [5]. In both groups of animals, postsynaptic waves N3 and P were highly sensitive to hypoxic changes, while presynaptic Nl and N2 were more resistant and disappeared much later. Almost complete recovery of the N3 wave in the hypothermic group after two days vs. partial recovery of the same wave in normothermic group was highly correlated with neurological outcomes, which were significantly better in hypothermic group. These results are, in principle, the same as the data shown in figure 3, which were obtained in the analogous experiment with rabbit spinal cord cooling.

Invasive Spinal EP Recording

Invasive implantation of stimulating or recording electrodes allows more specific placement of electrodes, increases signal to noise ratio, allows chronic recording, and makes EP interpretation simpler. On the other hand it requires surgical preparation. We have used several types of invasive spinal EP recording techniques - from the bone above recorded structure (vertebral body or skull), from epidural or intrathecal space, and directly from the internal structures. These techniques can also be used for stimulation. We have recorded SEPs evoked by direct sciatic nerve stimulation in rabbit (fig.3) [3, 6]. Recording silver ball electrodes

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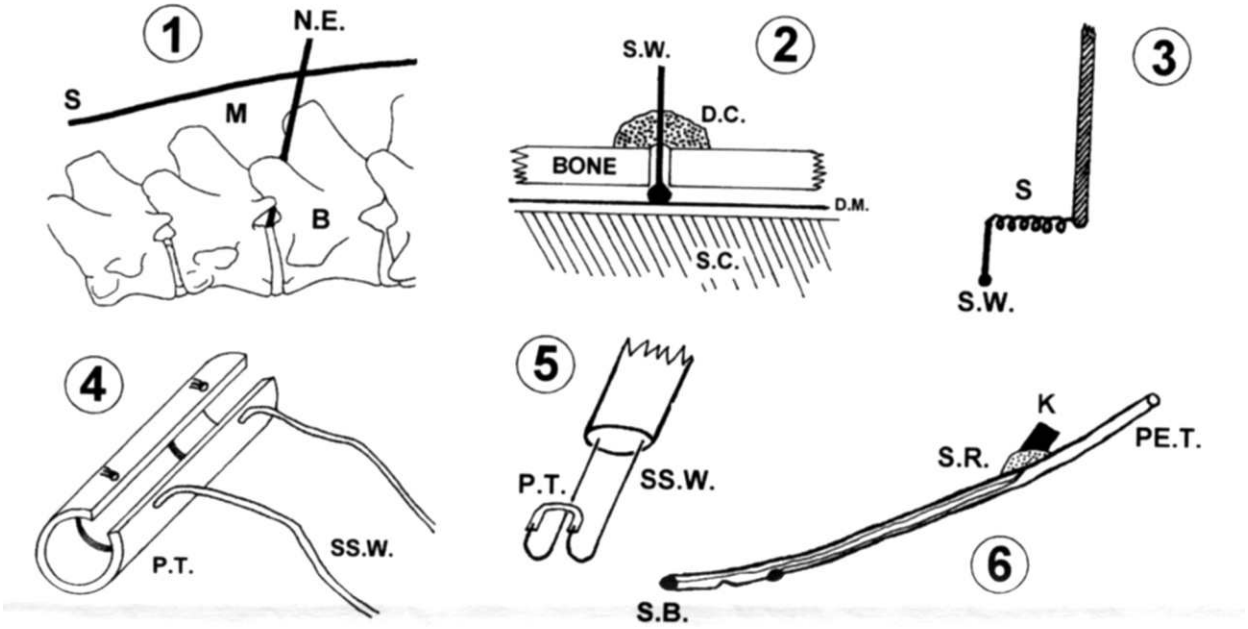

Figure 1. Several types of electrodes used for EPs recording and stimulation (scale differs for each electrode): 1) Needle electrode inserted to the intervertebral space (B-bone, M-paravertebral muscles, S-skin, N.E.-needle electrode), 2) Silver ball electrodes implanted epidurally (D.C.-dental cement, D.M.-dura mater, S.W.-silver wire, S.C.-spinal cord), 3) Silver ball movable electrode connected to a spring to reduce pressure on dura surface (S.W.-silver wire, S-spring), 4) Cuff style electrode for acute or chronic implantation around the peripheral nerve (P.T.- plastic tubing, SS.W.- stainless steel wire), 5) wire hook bipolar electrode for peripheral nerve or spinal roots (P.T.-plastic tubing bridge enhancing manipulation, SS.W.-stainless steel or silver wire, 6) bipolar flexible intrathecal electrode with catheter for drug injection (S.B.-silver ball, S.R.-silicone rubber, K-electrical plug, PET.-polyethylene tubing).

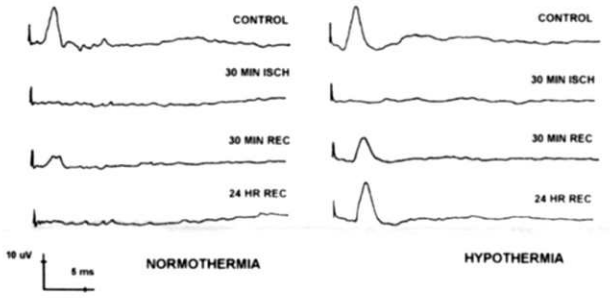

Figure 2. Motor EPs recorded from the right sciatic nerve as a response to stimulation of the left contralateral nerve under normothermic and hypothermic conditions, before, during and after 40 min of spinal cord ischemia (ISCH-ischemia, REC-recirculation).

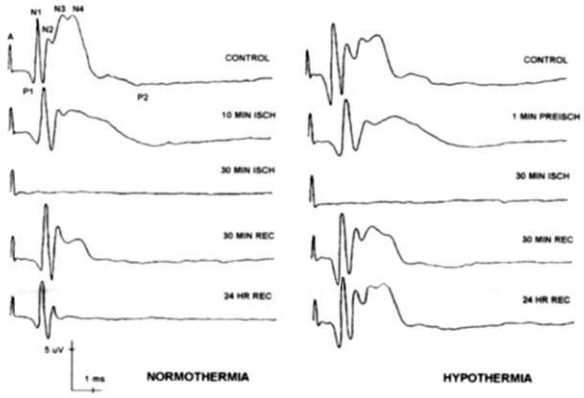

Figure 3. Spinal somatosensory EPs recorded from the rabbit spinal cord before, during and after 40 min of ischemia of normothermic and hy-perthermia in the cord. EPs were from spinal levels L5-6 in response to left scitatic nerve stimulation. Note the selective sensitivity o fpost-synaptic vs. presynaptic waves and the protective effect of hypothermia, with almost complete recovery of postsyaptic waves after 24 hrs in the hypothermic group and almost no recovery in the normothermic group.

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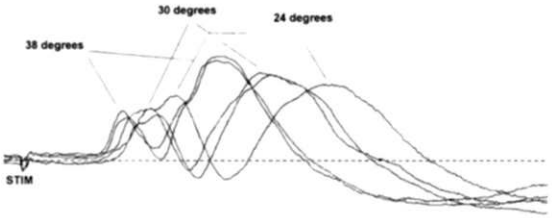

Figure 4. SEPs recorded by flexible electrodes implanted into the intrathecal space of the rat spinal cord at the level of L4 spinal segment. SEPs were recorded before during and after subcutaneous spinal cord cooling. Temperature values are of paravertebral muscle temperature (PMT) recorded by the needle thermocouple inserted to the muscle. Thermal regime: control (38°C), cooling to 30°C PMT (30 min), cooling to 24°C PMT (30 min), passive rewarming back to 30°C PMT (30 min) and active rewarming back to control PMT (38°C). Note that latency shifts and almost the same value of latency with the same PMT

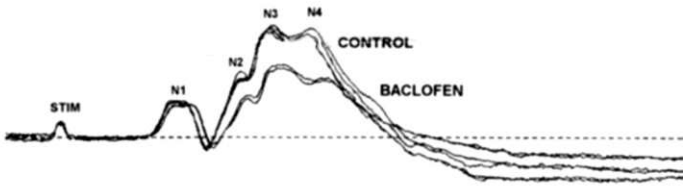

Figure 5. SEPs recorded intrathecally with simultaneous I.T. injection of Baclofen. Note significant changes of postsynaptic EP components, while presynaptic wave remains intact.

in bipolar configuration were fixed by dental cement inside holes drilled into, but not through, the L5 and L6 vertebrae. To avoid "insulation" of

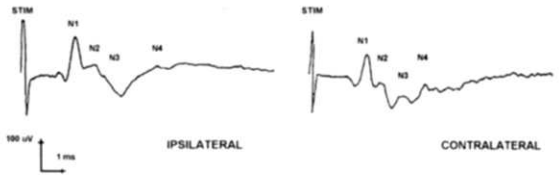

Figure 6. Intraspinal EPs evoked by the left sciatic nerve stimulation, recorded from dorsal horns on both sides of L4.

the electrode ball by liquid cement, the holes were filled before implantation with conductive ECG gel. A reference needle electrode was inserted into the paravertebral muscles. We have used this technique to record EPs repeatedly for 24 [3] and 48 hours [6], and up to one week without any problems. Similar techniques can be used for epidural (spinal or cortical) electrodes implantation. In this case the hole is drilled completely through the bone, so the silver ball electrodes can be inserted into the epidural space. This method has proven to be a reliable and still relatively simple technique. In some experiments, the epidural space can be opened by lami-nectomy or trephanation and moveable spring type silver ball recording electrodes can be used (fig.l). Here, however, the animal must be fixed in a stereotaxic apparatus.

Another implantation method allows flexible electrodes to be implanted into the epidural or in-trathecal space (fig.l). In comparison with the electrodes implanted through the bone, implantation of flexible electrodes produce less surgical trauma. However, the position of the electrode tip during recording is less certain. This limitation can be partly eliminated by implantation of two monopolar, instead of one bipolar electrode, or by using a type of loop guide for the flexible electrode tip, thus preventing the tip from moving around on the cord. We have used the technique of intrathecally implanted flexible electrodes in our experiments with subcutaneous spinal cord cooling in rats [7]. The heat exchanger was implanted on the back, just over the site of EP recording. EPs were recorded in the upper lumbar part of the spinal cord (~L4 segment) as the response

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to sciatic nerve stimulation (bipolar cuff style electrode, 10V, O.lms, 5Hz). Latencies of individual waves were measured and analyzed. The data were surprisingly consistent, despite the fact that some control EPs were slightly different in shape as a consequence of different electrode tip locations. We detected three negative waves, Nl, N2, N3, and one late positive wave P in each recording. Control latencies were around 1.4, 2.2, 3 and 6.4 ms, respectively. Analysis of latencies changes revealed that spinal EPs are extremely sensitive to spinal cord temperature changes. The latency value corresponding to each defined spinal temperature was constant, even after 5 hrs of intensive spinal cord cooling (fig.4).

We have constructed several types of intrathe-cal electrodes, including monopolar, bipolar, and bipolar with catheter for drugs injections. The last one (fig.l) was used to record EPs simultaneously with intrathecal application of the GABA receptor agonist Baclofen. Shortly after an intrathecal application of Baclofen, a pronounced decrease in amplitude of postsynaptic waves was detected (fig.5).

EP recording directly from the active structure provides the best defined data, but it is also the most traumatic recording technique. Electrodes are usually thin, insulated wires with an uninsulated tip, configured as monopolar, bipolar or multipolar electrodes. The wire material has to be nontoxic and commonly used materials are silver, tungsten, platinum and stainless steel. In acute experiments, glass capillary electrodes can be used. Recording or implantation procedures require stereotaxic electrode placement for precise results. The metal electrodes can also be used with some limitation in chronic experiments. They are usually inserted into the target structure through a hole in the bone and fixed with dental cement. To eliminate effect of spinal cord movements, flexible and very thin wires should be used, and they can be implanted with the help of a solid, removable guide. In [8] we recorded spinal SEPs at the L3-L4 level in the rabbit to show the effect of the unilateral peripheral electrical overstimulation on spinal postis-chemic neuronal degeneration. Two glass capillary pipettes (3M KC1, 20-40 (jm) were inserted to the left and right dorsal horns, respectively, and EPs recorded monopolarly in response to left sciatic nerve stimulation. The nerve was stimulated continuously (4Hz, 0.05mA, O.lms) for 1hr before ischemia, during 10 minutes of ischemia and 1 hr during reperfusion. Surprisingly, no significant differences in EP amplitude were found between stimulated and non-stimulated sides of spinal cord, during and 1 hr after ischemia. This corresponded well with bilateral histo-pathological changes which were noted (fig.6).

Technical Data

Cuff-style electrode fabrication. Our cuff style electrodes for rat sciatic nerve are made from a piece (-5-6 mm for bipolar electrode) of TYGON "B" flexible plastic tubing. Internal diameter of the tubing is optimal and the wall is hard enough to resist the pressure of a fixing suture. The tubing is cut longitudinally, and through holes made close to cut edges is inserted uninsulated 30G fluorocarbon coated stainless steel cable (Cooner Wire, part#AS814). Excessive uninsulated wire is cut, and both ends of the inserted wire are fixed in the holes by flexible silicon rubber. In case of multipolar electrodes the wires are inserted at appropriate spacing by the same method For convenience, it is possible to glue fixing sutures to the outer wall for ease of implantation around the nerve. Chronic cuff-style electrodes are well tolerated for days or weeks. To prevent the animals from biting external wires and connectors, we use longer cables (-10 cm), which are subcutaneously externalized on the upper part of the animal's back through a long needle before connectors are attached.

In fabricating the flexible electrodes for intrathecal implantation in rat, we use 40G Teflon coated silver wire (Cooner Wire, part # AS766-40) with a small ball, made by flame melting the uninsulated end. In monopolar electrodes the wire is inserted into PE5 flexible polyethylene tubing (OD - 0.014") so that silver ball gently plugs the end of the tubing. In the bipolar version, two wires are inserted to PE10 flexible polyethylene tubing (OD - 0.024"), one from the end and the second through a hole in the wall. Both silver balls just plug the holes. To make this type of electrode suitable for simultaneous application of drugs, an additional hole is made between the silver balls, the unplugged end of the tubing left open and connected to a microinjec-tor. At the end of electrode preparation, small connectors are fastened to the silver wires so that after implantation they can be fixed to the skin of the head to prevent electrode displacement.

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