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Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system

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Vallbo, Åke B., Karl-Erik Hagbarth, and B. Gunnar Wallin. Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol* 96: 1262–1269, 2004; 10.1152/japplphysiol.00470.2003.—A historical review is given of the development of microneurography and its application for studies of sympathetic nerve activity in humans.

human nerve recordings; skin; muscle

MICRONEUROGRAPHY IS THE METHOD to record impulse traffic in peripheral nerves of human subjects. The purpose of the present paper is to give a short historical review. The first part deals with the background of microneurography and the methodological obstacles during the development of the technique. The second part illustrates the potential of the method as revealed by the exploration of the sympathetic nervous system. The description is made primarily from a Swedish perspective, and many later sympathetic studies from other countries are not covered. In addition, other research areas, such as motor control or cutaneous sensibility, in which microneurography has proved equally potent, will not be considered. Such findings have been dealt with in earlier reviews (e.g., Ref. 12).

DEVELOPMENT OF THE MICRONEUROGRAPHY TECHNIQUE

Date and Place of Birth

Microneurography was born in Uppsala, Sweden 1965–1966 within the department of clinical neurophysiology at the Academic Hospital. Karl-Erik Hagbarth was the head of the department, and Åke Vallbo joined him in 1965 shortly after taking his MD and PhD degrees. Both had their basic training in neurophysiology in Stockholm, at the Nobel Institute of Neurophysiology, headed by Ragnar Granit. Hagbarth wrote his PhD thesis on cutaneous reflexes and Vallbo on ionic currents in single nerve fibers. Both had experience of single-unit recordings from muscle spindle afferents in animals but neither had studied autonomic nervous activity.

The department of clinical neurophysiology was responsible for performing electroencephalography (EEG), electromyography (EMG), and a number of other diagnostic tests on neurological patients of the hospital. Hence the clinical demand was considerable. As head of department, Hagbarth had a wide range of responsibilities, whereas Vallbo worked full time in the research project.

The official date of birth of microneurography was June 17, 1966 when the first data were presented in a short communication at a meeting of the Scandinavian EEG society in Copenhagen (31).

The term microneurography was suggested by Yngve Zotterman, professor of physiology at the Royal Veterinary College in Stockholm, a leading profile in Swedish neurophysiology of the twentieth century.

Impetus and Concerns

It is probably true to say that the impetus behind microneurography was the muscle spindle. Hagbarth and Vallbo shared a great interest in motor control, no doubt inspired by the work and ideas of Granit and colleagues (8), Merton (25), and Matthews (25) on the fusimotor system. However, all experimental data available at that time came from reduced preparations, e.g., decerebrate or anesthetized animals. The primary prospect of microneurography was to record multiunit activity from the large spindle afferents and, on the basis of such recordings, define the role of muscle spindles and the intriguing gamma system in voluntary movements.

The idea to insert a needle in a human nerve was not unique but had been discussed in several laboratories. However, many scientists argued that it would be impossible to record nerve impulses with an extracellular needle, considering the small currents produced by the nerve fibers and the low resistance between intraneural and reference electrodes, because both would be located in the extracellular space. Other colleagues emphasized the risks of damage and argued that the nerves would suffer from the mechanical trauma, intraneural bleedings, and infections.

It should be pointed out that normal traffic of nerve impulses had been recorded in attending human subjects before microneurography appeared. Hensel and Boman (17) took the brave step to open the skin and scarify a small branch of a skin nerve under local anesthesia in young volunteers. The peripheral stump was then split until single afferents could be discriminated. They presented beautiful recordings of cutaneous mechanoreceptive units from the hairy skin and identified one unit as thermoreceptor responding to cooling alone. Using a noninvasive approach, Sears (26) managed to record compound action potentials with extracellular electrodes in response to a tap on a fingernail.

The Process of Technique Development

The very first step toward developing the microneurography technique was taken by Hagbarth, who inserted a needle into

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his own ulnar nerve. With maximal amplifier gain he was then able to perceive a barely audible modulation of the noise when he tapped lightly on the skin within the innervation zone of the nerve. The signal-to-noise ratio was so low that the signals could not be seen on the oscilloscope screen. Still, this was a great starting point, evoking an enormous excitement when demonstrated to his younger coworker (Vallbo) who had just started working in his laboratory.

To increase the signal-to-noise ratio, a large series of trial-and-error tests were carried out, using electrodes of various metals and designs on surgically exposed animal nerves. It was found that platinum-iridium, which has excellent electrical properties, was all too soft, steel was too brittle, whereas tungsten had the appropriate mechanical properties, being both hard and nonbrittle.

Tungsten electrodes used in animal studies could not be used because the delicate electrode tip was invariably bent when forced through human skin and connective tissue. Attempts to minimize the mechanical strain by inserting the electrode through a hypodermic needle rather than directly through the skin did not work. In the end, it turned out that a substantially larger electrode tip (5–10 μm) than anticipated provided acceptable records of nerve activity.

The electrode coating was a major problem. The high mechanical strain on the electrode tip tore off the coating when common brands of lacquer were used. Finally, an epoxy-based varnish provided acceptable coating.

Initially, the progress in the process to optimize electrode design was hampered by a variation in signal quality that seemed random and difficult to explain. In some sessions, very faint or no neural activity was detected despite the electrode tip having attained an intraneural position. In other sessions much better signal-to-noise ratio was achieved. A main factor behind these variations became clear when it was found that the electrode tip could pass through the whole nerve without penetrating into a nerve fascicle.

The electronic equipment in the laboratory included amplifiers, oscilloscopes, Grass cameras to register events displayed on an oscilloscope and an analog tape recorder. Computers were, of course, not available at that time, and hence the nerve signals had to be documented on light-sensitive paper. Filming was a semiblind procedure because a tube was mounted between the oscilloscope screen and the camera lens to exclude daylight. Adjusting the intensity of the oscilloscope beam was critical to capture the fast events without overexposing the slowly running film. Because the experimenter was denied a direct view of the screen while the film was running, it was difficult to judge to what extent test procedures and data capturing were successful until the film was developed. Therefore, the most exciting moments often occurred while developing the long strips of film in the dark room, where, in the red light, one was eagerly looking for nerve impulses slowly emerging on the white paper film.

Exciting Surprises

As the electrode design was modified in small steps, the signal-to-noise ratio gradually improved. It was truly exciting when it became apparent that afferent multiunit activity from skin and muscle could be recorded with sufficient quality to reveal modulations of the activity related to mechanical skin stimuli and voluntary movements (Fig. 1*A*).

Single unit recordings of myelinated afferents. The excitement was even greater when it was discovered in one of the early experiments that a strip of film displayed a series of uniform deflections standing out above the background in a pattern that clearly indicated action potentials from a single nerve fiber (Fig. 1*B*). Even in retrospect, the excitement seems justified because, in a flash, it became obvious that the microneurography technique might allow analysis of impulses in individual identified nerve fibers. This implied that an approach that had been extremely fruitful in animal experiments was now applicable to attending human subjects, whose perceptual and voluntary activities might be related to exact measures of activity in single nerve fibers.

Spontaneous activity: artifact or gamma efference? In many early experiments, a very puzzling phenomenon was encountered. When carefully listening to the amplifier output, we could sometimes hear faint modulations of the noise. These modulations appeared intermittently and had a rhythmic frequency of ~1 Hz. Initially these noise modulations were noticed in the sound from the loudspeaker alone, whereas they were not visible on the screen. The sound was reminiscent of waves approaching a distant shore or the mooing of large animals far away.

We considered many interpretations to account for this capricious phenomenon but soon focused on two main possibilities. It seemed that the strange noise modulations either were an electronic artifact, possibly due to voltage variations in the mains or in the grid current, or they were of neural origin. If of neural origin, it seemed likely that they should represent activity in efferents rather than in afferents, because all at-

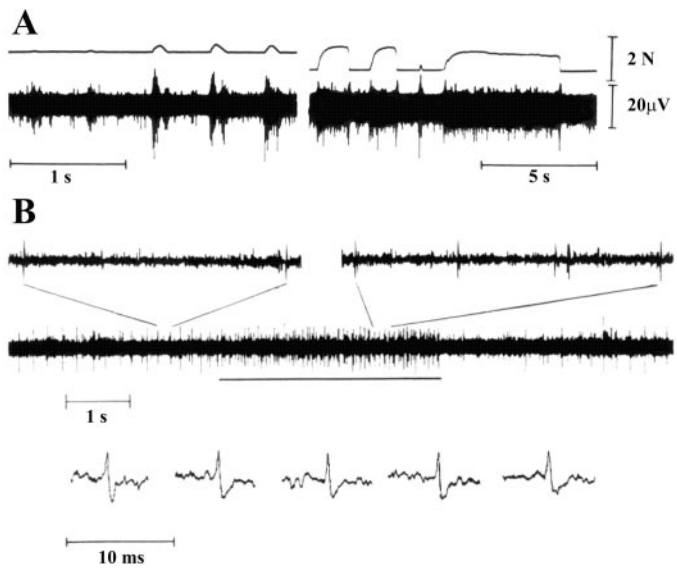

Fig. 1. *A*: one of the first published microneurography recordings. Multiunit responses of cutaneous mechanoreceptor afferents to taps and sustained pressure are shown. Electrode was in a cutaneous nerve bundle in the popliteal fossa. Stimuli were applied to the back of the lower leg. [Reprinted from Ref. 32, p. 277, with permission from Elsevier.] *B*: the first microneurography recording of single-unit activity. Electrode was in the superficial branch of the peroneal nerve. The unit responded to deep pressure on the lateral side of the lower leg. [Reprinted from Ref. 13, with permission from Blackwell Publishing.]

tempts to modify the activity by peripheral stimuli failed. Moreover, sensory activity of similar characteristics had not been described in animal experiments.

If the modulations represented natural activity of an intact efferent system, we were, for long, convinced that any neural activity that our coarse electrodes could pick up must originate from myelinated fibers. Thus the only reasonable candidate was the gamma fibers. Because the gamma efferents are smaller than the large spindle afferents but still myelinated, it seemed reasonable that they should produce small deflections, barely discernible against the background noise. This interpretation fitted with the observation that the rhythmic modulations of the noise were seen in muscle nerves but not in skin nerves. It should be pointed out that the characteristics and temporal patterns of the gamma activity in intact and behaving organisms were largely a terra incognita and any pattern of activity seemed reasonable to consider. Hence, in the laboratory jargon this type of noise modulation was called "the mooing of the gamma system."

The true nature of the phenomenon became obvious, however, as soon as we opened our minds to an idea that seemed unduly brave at that time, i.e., that our relatively crude electrodes allowed the recording of impulses in unmyelinated nerve fibers. In fact, the very first check of this hypothesis, i.e., a simple inspection of the coincidence between the spontaneous modulations of the noise amplitude, on the one hand, and the phases of the cardiac and respiratory cycles, on the other, revealed the true nature of the phenomenon (Fig. 2).

The efferent nature of the activity was soon confirmed when dual recordings were obtained from a nerve, the electrodes being inserted ~100 mm apart. When the two records were inspected, two important features emerged. First, it was clear that the temporal modulation of the signal as analyzed after low-pass filtering was grossly similar in the two recordings, indicating that the two electrodes were seeing activity from the same central generator. Second, the direction and magnitude of time shift between the deflections in the two records proved that the activity was efferent and had a conduction velocity of ~1 m/s.

In the light of present-day knowledge, our speculations about the mooing of the gamma system may appear incredibly stubborn. However, our conviction that any neural activity seen by our electrodes must be traveling in myelinated fibers was not all that unreasonable considering that C-fiber activity in animal experiments had been identified only 10 years earlier (6). In fact, in 1957 it caused quite a stir among neurophysiologists when Iggo (19) presented single-unit recordings from C-afferents in the cat. Therefore, it seemed presumptuous to assume that C-fiber activity could be recorded in waking human subjects with tungsten needles having a tip size 5–10 times larger than the diameter of the fibers themselves.

As pointed out by Granit in an autobiography (10), it is interesting to note how difficult it is, in retrospect, to reestablish the state of ignorance that was present long ago and to understand its implications. In this context it is also comforting to read a comment in a recent survey by Andrew Huxley (18) on his and Alan Hodgkin's analyses of ionic current in the excitable nerve membrane: "It is easy to fail to think of an idea that with hindsight seems very obvious."

Single-unit impulses in unmyelinated nerve fibers. In the multiunit recordings of sympathetic efferent activity we sometimes observed larger and very short-lasting deflections, suggesting single-unit impulses. However, the issue was difficult to explore because the impulses appeared as singles alone and not in trains. Later Torebjörk and Hallin (30), working in the same laboratory, demonstrated beyond doubt that the microneurography method allowed single-unit recordings from unmyelinated nerve fibers in humans. Since then, a large number of important studies on unmyelinated afferents have been produced in various laboratories.

Early Series of Experiments and Risk Evaluation

The microneurography technique was developed during the years 1965–1966 when Karl-Erik Hagbarth and Åke Vallbo consistently explored their own nerves to collect a solid appreciation of risks of injuring the nerve. Careful observations of symptoms and signs were complemented by EMG tests to check for signs of denervation. After this initial period it seemed safe to proceed to recruit young volunteers in future experiments.

On the other hand, it must not be forgotten that microneurography is an invasive method that involves the exploration of very delicate structures that may suffer from improper procedures. Minor aftereffects have been documented (7), and it cannot be too strongly emphasized that it is essential to have a proper education in a laboratory with adequate experience of the technique before microneurography is adopted in a new laboratory.

Three Main Research Areas

During the initial period when the microneurography method was developed and refined to a powerful research tool, Hagbarth and Vallbo collected data from three neuronal systems that became the main research areas of microneurography, i.e., cutaneous sensation, proprioceptive control of voluntary movements, and sympathetic efferent activity. The results were presented in a number of short notes and four full papers,

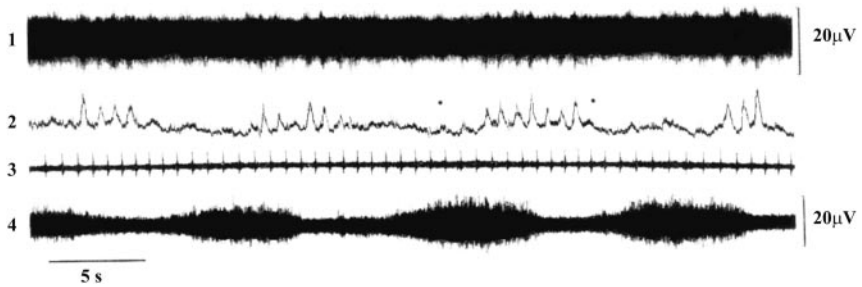

Fig. 2. Illustration from the first publication of microneurography recording of sympathetic activity in humans. Traces from above, original nerve recording from the deep peroneal nerve (*1*), mean voltage neurogram (*2*), ECG (*3*), and electrical activity of inspiratory muscles (*4*). [Reprinted from Ref. 14, with permission from Blackwell Publishing.]

two dealing with muscular afference (15, 16), one with cutaneous afference (32), and one with sympathetic efferent activity (14).

EXPLORATION OF SYMPATHETIC NERVOUS ACTIVITY

After 2 years in Uppsala, Vallbo left for the University of Umeå while a number of other scientists joined Hagbarth, e.g., the Finnish psychiatrist Anders Hongell and Gunnar Wallin. Wallin, who came from the department of physiology in Uppsala where he had finished his PhD thesis on axon membrane function, focused already from the beginning on sympathetic nerve traffic.

At the time, the sympathetic nervous system was a black box to clinical neurophysiologists, and, therefore, it was a great advantage to be able to collaborate with physiologists and clinicians interested in the cardiovascular system. Bertil Hood, who was the professor of internal medicine in Uppsala, assisted Hagbarth to recruit the German cardiologist Wolfram Delius to the sympathetic project. Delius provided valuable knowledge on cardiovascular regulation and, in addition, he applied the catheters that were necessary for recordings of arterial and central venous pressures; noninvasive methodology was still not available. A few years later, when Delius returned to Munich, another cardiologist, Göran Sundlöf, joined the group.

Sympathetic Differentiation

The collaboration with Delius and Sundlöf meant easy access to patients from the department of internal medicine, and it was in a hypertensive woman that the first multiunit recording of skin sympathetic activity was made. Her neurogram revealed a high level of resting activity with irregular bursts of long duration that sounded like ocean waves in the loudspeaker. Because no clear cardiac rhythmicity was discerned, this activity was strikingly different from the series of regular short-lasting and pulse-synchronous bursts encountered in muscle nerves (Fig. 3). Several pieces of evidence proved that the bursts in skin nerves were of sympathetic origin (11). First, individual bursts were followed by sympathetic effector responses within the innervation territory of the impaled nerve. Second, simultaneous recordings with two sets of electrodes from the same nerve revealed that the bursts were conducted in the efferent direction with a velocity of ~1 m/s. Finally,

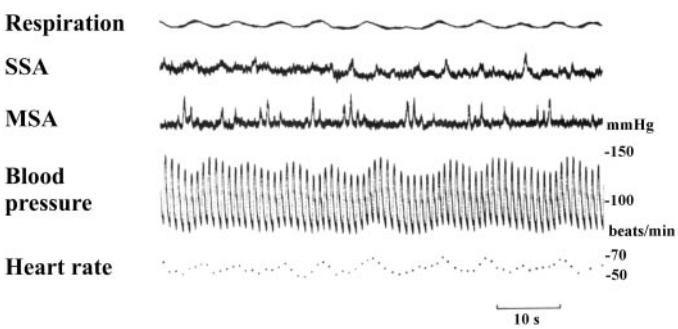

Fig. 3. First published simultaneous recording of resting skin (SSA) and muscle (MSA) sympathetic activity (mean voltage neurograms, time constant 0.1 s) in the two peroneal nerves. [Reprinted from Ref. 36, with permission of Lippincott William & Wilkins.] Note the cardiac rhythmicity and the inverse relationship to variations of blood pressure in MSA but not in SSA.

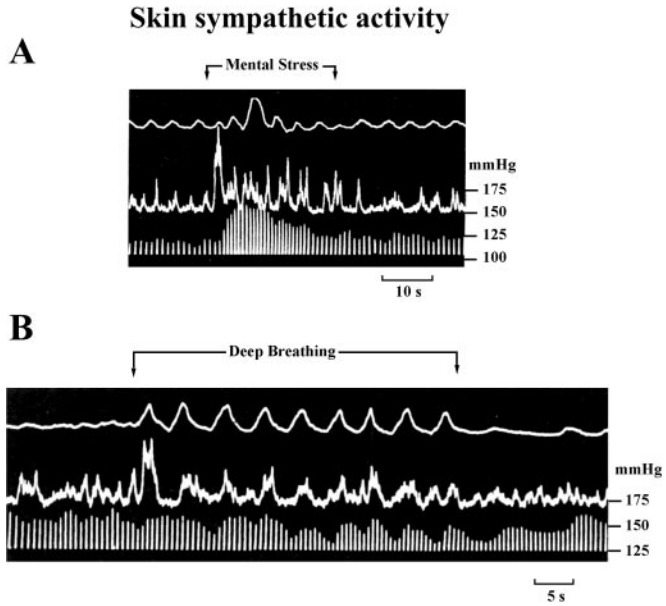

Fig. 4. Early records of skin sympathetic activity in association with mental stress (*A*) and deep breathing (*B*). Traces are, from *top*, respiration, mean voltage neurogram, intra-arterial blood pressure. [Modified from Ref. 5.]

intravenous infusion of the ganglion-blocking drug trimethaphan eliminated the activity.

It was obvious that, in contrast to findings in muscle nerves (4), marked changes of skin sympathetic activity were evoked by arousal, emotions (Fig. 4*A*), deep breaths (Fig. 4*B*), and changes of environmental temperature (5). The initial thought that this unexpected new activity pattern had something to do with hypertension was quickly abandoned; the differences between skin and muscle sympathetic activities were similar in normotensive subjects.

The reason for being surprised by the finding of different patterns of sympathetic activity in skin and muscle nerves had to do with the prevailing view in the late 1960s: the sympathetic system was thought to be diffusely organized with similar traffic in nerves to different organs. Our new data showed that this was not the case and, thus, an important early achievement of microneurography was to provide strong evidence for today's concept of a highly differentiated sympathetic system, in which individual effectors are governed by their own control systems and specific reflexes (11).

The Strength of Activity

Much time was spent trying to quantify the amount of sympathetic activity in skin and muscle nerves. Because electrode movements, EMG, and afferent nerve traffic all could induce small shifts of the baseline in the mean voltage neurogram, the assessment of the area under the curve (measured with a planimeter!) proved to be an unreliable measure. For this reason another approach was tried; instead of using the mean voltage baseline of a long recording period as a zero level, each burst was measured from the baseline level immediately before and after the burst. The mean voltage amplitude of the burst was then taken as a measure of burst strength. For interindividual comparisons, however, only the number of bursts was used because the signal-to-noise ratio depends critically on the distance between the electrode tip and the

active fibers, a variable that is bound to differ between recording sites.

In muscle nerve branches, where the strict cardiac rhythmicity makes bursts easy to identify, this crude methodology turned out to be very useful. For skin sympathetic activity, on the other hand, the same procedure was less successful, primarily because the irregular shape and duration of the bursts as well as the lack of distinct cardiac rhythmicity made it difficult to define the onset and the end of a burst. In addition, bursts in skin nerves often contained a mixture of vasoconstrictor and sudomotor impulses, which made burst counting less useful from a functional point of view.

Simultaneous recordings in two nerves of muscle sympathetic activity at rest revealed a remarkable parallelism between the neurograms (28), much in the same way as found by Bronk et al. (2) for the left and right cardiac nerves in the cat (Fig. 5). On the other hand, between individuals the difference was large because some subjects had many and others had very few bursts. An even more surprising finding was that the large interindividual differences were reproducible from day to day over long time (28). Thus, even though different sympathetic effectors were controlled differentially, the vascular beds of skeletal muscles in all extremities seemed to receive a fairly uniform sympathetic drive at rest, the strength of which was characteristic for the individual.

The profound implications of these findings for future studies were obvious. First, a single recording in a randomly chosen muscle nerve fascicle provided representative and stable information on the strength of the subject's sympathetic traffic, thereby making it meaningful to compare resting activity between individuals in health and disease. Second, repeated recordings would provide longitudinal information on resting activity, allowing assessment of its changes with age, physical training, drug therapy, or other interventions.

Unfortunately, the interindividual differences in muscle sympathetic activity at rest were so wide that it was not possible to define a normal upper limit of burst incidence above which the strength of resting activity was pathological. Several obstacles prevented the definition of a normal range of the strength of resting activity also in skin nerves, and therefore the hope to use the strength of resting skin or muscle sympathetic activity for diagnostic purposes in individual patients was removed. Needless to say, this was a great disappointment.

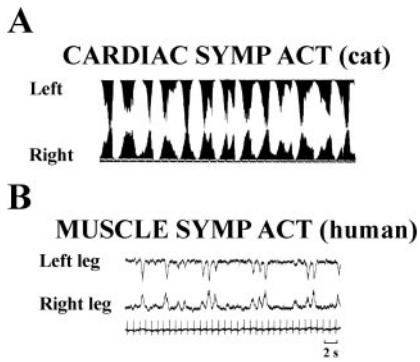

Fig. 5. Comparison of simultaneous bilateral recordings of (*A*) cardiac sympathetic activity in a cat (Ref. 2) and (*B*) muscle sympathetic activity in the peroneal nerves in a human subject. [Modified from Ref. 28.]

Baroreflex Influence

Already in the first experiment with intra-arterial blood pressure recording, it was obvious that there was an inverse relationship between transient variations of blood pressure and muscle sympathetic activity: when blood pressure decreased sympathetic activity increased, and when pressure increased the sympathetic bursts disappeared (Fig. 3). This, together with the cardiac rhythmicity, suggested that muscle sympathetic activity was modulated from the arterial baroreceptors, and using a new electronic device, i.e., a computer of average transients, it was possible to define the baroreflex latency (3).

Even with this information, however, it was not until the first recording from a patient with cardiac arrhythmia (33) that the true strength of the baroreceptor influence became obvious. It was found that an "arrhythmic" beat with low diastolic blood pressure and long cardiac interval usually was associated with a much stronger burst of longer duration than bursts of regular heartbeats. In other words, a given sympathetic discharge was always related to a specific heartbeat, an insight that paved the way for quantitative analyses of arterial baroreflex effects (29).

Relationship to Transmitter Release

Around 1980, a positive correlation was found between the number of bursts in muscle nerves and the concentration of the sympathetic transmitter norepinephrine in forearm venous plasma (37). The first blood samples in this study were sent to Grobecker's lab in Germany, which, at the time, was one of the few laboratories where norepinephrine in plasma could be analyzed.

Because sympathetic outflow had been found to be differentiated between target tissues, it was not clear why the venous plasma concentration correlated with muscle sympathetic activity. In retrospect, the explanation is probably multifactorial. First, norepinephrine from muscle is overrepresented in forearm venous blood, and, second, the relationship is strengthened by a parallelism between sympathetic drives to muscle, heart (34), and kidney (38), as later demonstrated together with Murray Esler in Melbourne.

Cardiovascular Diseases

In early recordings of muscle sympathetic activity in patients with essential hypertension (36), we found slightly more bursts at rest in patients than in controls. The patients were older, however, and because the activity was known to increase with age, a hypertension-related augmentation of sympathetic activity seemed unlikely. In later studies from other laboratories, with control and patient groups matched for age and other confounding factors, results have varied, but most studies have shown a higher resting activity in the hypertensive group. On the other hand, individual patients with clear hypertension may have quite low levels of activity, illustrating that the relationship between mean levels of sympathetic traffic and blood pressure is multifactorial and still incompletely understood. A collaborative study with Allyn Mark on patients with cardiac failure revealed a marked increase of muscle sympathetic activity in the patients compared with the control group (21). On the other hand, the pulse-synchronous pattern of activity was similar in patients and normal subjects, indicating that arterial baroreflex modulation of sympathetic outflow was still

present. This was of interest because literature data from sympathetic effector recordings had suggested a weakening of the baroreflex in cardiac failure.

Neurological Diseases

There were frequent contacts between the laboratory of clinical neurophysiology and the neurological department at the Academic Hospital in Uppsala, and in the late 1970s Jan Fagius, who is a neurologist, became engaged in a study of autonomic symptoms in polyneuropathy. Part of the project involved an attempt at finding a method of diagnosing slowed conduction in sympathetic fibers. The studies were successful in all aspects but one: no slowing of conduction was ever detected, and microneurography found no place as a diagnostic method in polyneuropathy.

After his thesis, Fagius remained a member of the research group and made several elegant studies. He initiated what later became known as "the heroic experiment," which involved bilateral anesthesia of the vagus and glossopharyngeal nerves, the idea being to study effects of baroreceptor deafferentation on sympathetic nerve traffic (9). The procedure was performed only on members of the research group and, needless to say, Jan was the first subject. As expected, the deafferentation increased muscle sympathetic activity and eliminated its cardiac rhythmicity, whereas skin sympathetic activity remained largely unaffected. Other expected effects were marked increases of heart rate and blood pressure. The big surprise was that during the nerve block, brisk sensory stimuli delivered unexpectedly to the subject evoked distinct arousal bursts, not only in skin nerves as in unanesthetized subjects, but in muscle nerves as well. This finding suggested that such responses normally are suppressed by the afferent activity from arterial or cardiopulmonary baroreceptors. Apparently, the deafferentation uncovered the excitatory response to several kinds of sensory stimuli, much in the same way as the Babinski reflex is uncovered after an upper motor neuron lesion.

Spinal Cord Injury

At about the same time, extensive studies were made on patients with traumatic spinal cord lesions (27, 35). The key person in these experiments was Leif Stjernberg, who was responsible for the clinical care of the patients at the Department of Rehabilitation. The clinical impetus was to try to understand the mechanisms underlying attacks of "sympathetic hyperreflexia," i.e., acute episodes of peripheral vasoconstriction, sweating, and hypertension that sometimes are evoked in such patients by peripheral stimuli below the level of the lesion. A more theoretical incentive was that the patients also offered a possibility to clarify to what extent spontaneous sympathetic activity originated at spinal levels.

Already the first recording in a tetraplegic patient revealed an important finding that turned out to be representative: little or no spontaneous activity was found, but nevertheless distinct neural discharges were evoked by phasic stimuli such as short-lasting pressure over the bladder or pinching the skin in the legs. Responses were similar in skin and muscle nerves. Because of the weak or absent resting activity below the level of the lesion, a main conclusion was that the strength and temporal pattern of spontaneous sympathetic activity in normal subjects depends to a large extent on supraspinal influence; below a spinal cord lesion, afferent baroreceptor activity cannot influence sympathetic neurons. Moreover, as sympathetic differentiation was reduced, the finding agreed with the idea mentioned above that baroreceptor afferent activity is essential for sympathetic differentiation between skin and muscle nerves.

Sympathetic recordings after spinal cord injury were technically difficult and for several reasons failures were more common than in intact subjects. First, many patients had peripheral nerve lesions distal to the spinal cord injury, which occasionally made it impossible to locate the nerve. Second, the lack of spontaneous activity made it more difficult to find sympathetic recording sites, and third, involuntary muscle cramps due to spasticity often dislodged the electrode.

Intraneural Electrical Stimulation

The most common application of microneurography for sympathetic studies has involved recordings of spontaneously occurring nerve traffic. Another approach is to induce nerve activity via the microelectrode by intraneural electrical stimulation with the purpose to study effector mechanisms.

The main drawback with this method is that the interpretation of the results is complicated by the fact that electrical stimuli may activate several types of nerve fibers. This is most noticeable when studying vascular effector responses, which may result from stimulation of sympathetic efferents as well as afferent unmyelinated fibers. However, by carefully grading the stimulation strength and using local anesthetic blocks of the nerve proximal and distal to the stimulation site, it has been possible to obtain evidence for several vasodilating mechanisms in human skin (1).

Sweating is evoked by cholinergic sudomotor nerve fibers exclusively, and therefore results about sudomotor mechanisms obtained with intraneural electrical stimulation are easier to interpret. By stimulating the median nerve at the wrist (after application of local anesthetic block of the axillary plexus), the relationship between stimulation frequency and sweat production in the hand was found to depend on previous activity (20). The findings led to the conclusion that the amplitude of a skin resistance change is useful as a measure of sympathetic activity only if background sympathetic activity remains constant, i.e., that thermal conditions are constant and that all forms of stress are minimized.

Recordings From Single Sympathetic Fibers

As mentioned above, Torebjörk and Hallin (30) had demonstrated that recordings could be made from single unmyelinated fibers, afferents as well as sympathetic efferents. On and off over the years Wallin also noted deflections above the noise, suggesting single sympathetic action potentials. However, the low signal-to-noise ratio and the lack of adequate methods to inspect the spike wave form made it difficult to prove their single-unit nature.

The opportunity to make a serious attempt at single-unit recordings came when Vaughan Macefield from Sydney spent a postdoctoral year with Wallin in Göteborg. Macefield was familiar with a sophisticated computer program developed in Umeå (Zoom), and the analyses allowed by this program became a key element for the study, which resulted in the first published material on single-fiber sympathetic activity in mus-

cle nerves (23). A main difficulty in such experiments is the low signal-to-noise ratio, which makes the analysis very time consuming: it is necessary to inspect every spike of all candidate units to make sure that they derive from a single fiber.

Subsequently, Macefield, Elam, and Wallin published a series of single-unit studies, which described firing characteristics in different types of sympathetic fibers in healthy subjects and patients with cardiovascular diseases. A common finding in these studies was an unexpectedly low firing frequency both at rest and during provocations (22). In fact, impulse rates above 1 Hz in individual sympathetic fibers are rare even in diseases associated with marked hyperactivity as seen in multiunit recordings (such as cardiac failure or obstructive sleep apnea). Another interesting finding is that high levels of multiunit activity may be brought about in different ways, i.e., recruitment and frequency modulation. As an example, the normal intersubject variation with regard to burst frequency at rest in muscle nerves is due to a larger number of active fibers in subjects with many bursts; the firing frequency of the individual fiber is not increased. In contrast, increased firing frequency contributes to the increase of multiunit activity during an inspiratory apnea in healthy subjects and to the increased multiunit activity at rest in patients with cardiac failure or obstructive sleep apnea.

International Collaboration

For over 10 years, sympathetic recordings were made only in Sweden. After that, however, colleagues from various countries came to learn the technique and bring it back home. In the mid-1970s David Burke from Sydney spent 2 years with Hagbarth to work on motor physiology, but he also participated in sympathetic recordings. Another early visitor was Giorgio Bini from Rome, who spent a year at the lab in 1978–79. Bini was a charming Italian fellow who sadly died in a traffic accident shortly after returning to Italy. As a consequence, microneurography was not introduced in Italy until many years later.

In 1979 Dwain Eckberg was the first American to participate in microneurography projects, and in 1983, a year before Wallin moved to Göteborg, Allyn Mark came to Uppsala for a 4-month period. Soon after returning home, Mark had a microneurography laboratory running in Iowa City, and within a few years he had trained a considerable number of microneurographers.

During the last two decades, many other scientists have been trained both in Sweden and elsewhere to use microneurography for sympathetic recordings. This, and the widespread interest in autonomic function, has had the effect that, today, the largest field of microneurography is sympathetic recordings. The technique is practiced in many countries in Europe, North and South America, and Australia. In Japan, Tadaaki Mano initiated the method and he has been teacher for many Japanese microneurographers.

CONCLUDING REMARKS: POTENTIALS AND LIMITATIONS OF MICRONEUROGRAPHY

Over the years sympathetic nerve recordings have been used to explore many areas. Various reflexes have been studied, and links between the sympathetic system, metabolism, and hormones have been explored as well as sympathetic effects of drugs or anesthetic or operative procedures. Sympathetic recordings have also been used in high-altitude research and even in space.

Although no diagnostic applications have been developed, microneurographic data have been obtained in several cardiovascular and neurological diseases, thereby providing a pathophysiological basis for evaluation of mechanisms and clinical symptoms. In addition, microneurographic results have improved the understanding of mechanisms underlying sympathetic effector tests used as diagnostic tools.

The number of physiological and pathophysiological problems explored with microneurography has expanded substantially from the initial period, partly thanks to important innovations in a number of laboratories.

The present paper offers only a short account of the progress within a single branch of the microneurography research, i.e., the analysis of sympathetic efferent activity. Examples of important achievements in other areas are single-unit recordings from unmyelinated afferents, microstimulation of identified single afferent and efferent nerve fibers, recordings from intraoral nerves to study sense organs in the structures that anchor the teeth to the bone, and the combination of microneurography with brain imaging and magnetoencephalography to demonstrate cerebral targets of myelinated or unmyelinated tactile afferents. Moreover, microneurography has inspired to other types of studies of the human nervous system, for instance, analyses of the control of grip forces involved in delicate manual work and analyses of muscular thixotropy.

Although microneurography is a powerful technique, it has limitations. First, the experiments are time consuming and demanding for both the experimenter and the subject, particularly when the aim is to study single-unit activity. Short recording sessions may be preceded by long search periods requiring maximal attention to delicate visual and auditory cues while the experimenter has to make minute manual adjustments of the electrode position. Second, the technique does not allow large movements of the subject, which is a limitation particularly in studies of the motor systems. Finally, it should not be forgotten that a single-unit recording implies an extreme zooming in, limiting the analysis to the one nerve fiber one has happened to come by, whereas the features and the amount of activity in parallel neural elements remain unknown. In the design of clinical studies, it may be particularly relevant to keep in mind that microneurography can seldom reveal to what extent activity is lacking in parts of the system.

The unique potentials of microneurography are based essentially on three features. First, the absolute resolution of single-unit recordings allows the sampling of all impulses passing along an identified nerve fiber. Second, the possibility to record from awake, intact, and cooperative individuals invites truly physiological analyses of causal relationships between peripheral neural activity and various higher functions, e.g., perception, emotions, voluntary movements, and electrical activity of the brain. Finally, the fact that the subjects are human beings implies that the pathophysiology of a number of diseases may be explored.

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