Clinical Neurophysiology 130 (2019) 1781–1788

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Review

A review of acute responses, after-effects and chronic complications related to microneurography

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ARTICLE INFO

Article history:
Accepted 11 June 2019
Available online 15 July 2019

Keywords:
Microneurography
Recommendations
Reporting
Adverse outcomes

HIGHLIGHTS

- A review of adverse outcomes during and following microneurography.
- Provision of incidence rates for each outcome in a general population.
- Recommendations for risk assessment and future reporting of adverse outcomes in microneurography.

ABSTRACT

Microneurography, a technique used to detect postganglionic sympathetic nerve traffic in humans, is increasingly used to further the understanding of autonomic regulation in health and disease. The technique involves the transcutaneous insertion of a microelectrode into a peripheral nerve, following which, a variety of adverse acute responses; after-effect and chronic complications have been documented. Here, we comprehensively review the potential adverse outcomes of microneurography and provide updated quantifiable incidence rates of their occurrence within a general population. We also present recommendations for risk assessment and management of such outcomes, as well as recommendations to improve future reporting. This review aims to use objective evidence to improve the understanding of the rare, but present, adverse outcomes of microneurography.

Contents

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https://doi.org/10.1016/j.clinph.2019.06.228
1388-2457/

1. Introduction

Microneurography is a technique used in the study of human neurophysiology that allows the assessment of skin and muscle blood flow, neural control of movement and central mechanisms involved in pain (Hagbarth and Vallbo, 1968a, 1968b; Vallbo and Hagbarth, 1968a, 1968b; Vallbo et al., 2004). Of particular pathophysiological relevance, the measurement of efferent discharges in postganglionic sympathetic C fibers innervating vascular smooth muscle, known as muscle sympathetic nerve activity (MSNA), provides insight into the autonomic regulation of blood pressure (Joyner et al., 2008, 2010; Wallin, 1989; Sundlof and Wallin, 1978a, 1978b). Research using microneurography has shown that sympathetic hyperactivity of MSNA is evident in many clinical disorders, for example hypertension, obesity, hypertensive pregnancy (Reyes et al., 2018; Malpas, 2010; Grassi et al., 2018), may be observed prior to the development of overt disease (Fischer et al., 2004), and is associated with elevated cardiovascular morbidity and mortality (Malpas, 2010). As such, this technique is increasingly used to further the understanding of health and disease and this is reflected in the rising number of research studies using microneurography.

Consequently, assessment of sympathetic nerve activity using microneurography is an important and growing area of both physiological and pathophysiological research. However, the technique does have potential short- and long-term risks to the individual. With no formal training or certification, the onus is on practitioners to maintain best practice and reporting of adverse outcomes to inform ongoing and new assessments of risk. Adverse events following microneurography with various methodologies, nerves and populations have been previously reported (Anderson et al., 1989; Burg et al., 1973; Eckberg et al., 1989; Hagbarth, 1979b; Knutsson and Widen, 1967; Littell, 1981; Rice et al., 1994; Donadio et al., 2007; Dunham et al., 2018) and cited within review literature (Mano et al., 2006). These previous reports helped to guide best practice recommendations for practitioners, however, there has been a considerable lack of objectively reported data regarding adverse outcomes over the past 20–30 years, although much anecdotal evidence is shared between practitioners. The aims of this review were to: firstly, summarize the acute responses, after-effects and chronic complications related to microneurography reported within the literature; secondly, present quantified incidence rates of these adverse events; and thirdly, to provide recommendations for risk assessment and reporting of outcomes within future research.

2. Methods

Many researchers using the microneurography technique refer to key publications (Anderson et al., 1989; Burg et al., 1973; Eckberg et al., 1989; Hagbarth, 1979a; Knutsson and Widen, 1967; Littell, 1981; Rice et al., 1994; Vallbo, 1976; Wall and McMahon, 1985) to identify the potential harms of microneurography; however, these data were never amalgamated. In the present review, we synthesized previously published data from nine papers reporting acute responses, after-effects and chronic complications of microneurography and combined the values to create incidence rates. Within these calculations, we also included data from our own laboratory where appropriate. To do so, data from all laboratory investigations including microneurography were identified and participant characteristics, ambient conditions, and details of microneurography (signal obtained, nerve site, number of sites, search time, signal quality if obtained) were extracted. Our dataset included 428 assessments of MSNA from the peroneal nerve in 303 individuals (125 repeat assessments). There were 93 males, 82 non-pregnant women (including women in the postpartum period) and 128 pregnant women (ranging from 16 to 40 weeks gestation) in our cohort. Files were screened for acute effects of microneurography, including (but not limited to) participant discomfort (dysesthesia, local pain), pre-syncopal symptoms and syncope. Within our dataset, we reported an 'event' of dysesthesia or local pain when the investigation was terminated (by the researcher and/or participant) specifically as a result of these sensations. We reported an 'event' of pre-syncope when the investigation was paused until relief of symptoms, or terminated as a result of symptoms. Data for after-effects or chronic complications following microneurography was primarily acquired through participant-initiated feedback, and as such, we did not systematically assess the incidence of such outcomes in our cohort. The combined incidence rate for each of the acute responses, after-effects and chronic complications of microneurography was then calculated using the sum of all investigations (including relevant previously published data and our own data if available) and the sum of events (as previous). Data (including source, number of investigations, number of events and incidence rate) are presented in Table 1.

3. Acute responses

Microneurography involves the insertion of a reference microelectrode, positioned subcutaneously, and a recording microelectrode positioned transcutaneously into a peripheral nerve. For a comprehensive explanation of the technical aspects of recording, refer to Hart et al. (2017). In brief, after insertion, the recording microelectrode is manipulated into position to obtain a sympathetic nerve signal using audio and physical feedback. During insertion and manipulation of the microelectrode, a number of acute responses may become apparent.

3.1. Dysesthesia

The search for an appropriate recording site can result in transient sensations of dysesthesia, muscle tension/cramp, paresthesia ("pins and needles") or apparent changes in local temperature that occur as the tip of the electrode reaches the nerve fascicle. Mild sensations are an anticipated response to intraneural investigation; however, these sensations lessen and are typically relieved when the microelectrode is no longer manipulated or is withdrawn. In our laboratory, we experienced a small number of investigations (n = 8/428, 1.9%) that were terminated by the researcher

Table 1
Incidence of acute responses, after-effects and chronic complications following microneurography.

	Source	Number of assessments	Number of events	Incidence rate
Acute responses				
Dysesthesia				
	Burg et al. (1973)	100	1	1.0%
	Knutsson and Widen (1967)†	6	"a few"	–
	Littell (1981)	22	10	45.4%
	Unpublished data	428	8	1.9%
	Combined totals	**550**	**21**	**3.8%**
Pain				
	Dunham et al., 2018	32	1	0.3%
	Rice et al., 1994	32	4	12.5%
	Unpublished data	428	2	0.5%
	Combined totals	**492**	**7**	**1.4%**
Pre-syncope				
	Dunham et al. (2018)	32	1	0.3%
	Donadio et al. (2007)	36	10	27.8%
	Unpublished data	428	33	7.7%
	**Males*	*93*	*3*	*3.2%*
	**Non-pregnant females*	*82*	*6*	*7.3%*
	**Pregnant females*	*128*	*24*	*18.8*
	Combined totals	**496**	**44**	**8.9%**
Syncope				
	Unpublished data	**428**	**1**	**0.2%**
After-effects				
Paresthesia				
	Anderson et al. (1989)	963	36	3.7%
	Burg et al. (1973)	100	1	1.0%
	Dunham et al. (2018)	32	0	0.0%
	Eckberg et al. (1989)	708	30	4.2%
	Knutsson and Widen (1967)†	6	0	0.0%
	Littell (1981)	22	16	72.7%
	Rice et al. (1994)	32	2	6.3%
	Combined totals	**1863**	**85**	**4.6%**
Pain				
	Anderson et al. (1989)	963	28	2.9%
	Dunham et al. (2018)	32	0	0.0%
	Eckberg et al. (1989)	708	17	2.4%
	Knutsson and Widen (1967)†	6	0	0.0%
	Rice et al. (1994)	32	4	12.5%
	Combined totals	**1741**	**49**	**2.8%**
Muscle weakness				
	Anderson et al. (1989)	963	13	1.3%
	Knutsson and Widen (1967)†	6	0	0.0%
	Littell (1981)	22	2	9.1%
	Combined totals	**991**	**15**	**1.5%**
Infection	**No available data**			
Chronic complications				
	Eckberg et al. (1989)	708	1	0.1%
	Hagbarth (1979b)	1000	3	0.3%
	Littell (1981)	22	1	4.5%
	Unpublished data	428	1	0.2%
	Combined totals	**2158**	**6**	**0.3%**

N.B. Number of assessments and events was extracted from specified studies. The incidence rate for each study was then calculated. The total number of assessments and events reported for each outcome were then calculated, and an incidence rate was calculated using the combined total. *Unpublished data* refers to the assessments and events observed in our laboratory.
No available data shows that reports of infection following microneurography were not identified within previous literature.
* Indicates that data presented is a breakdown of population data included within the *Unpublished data* of pre-syncopal symptoms.
† Indicates data were collected with glass-coated wire electrodes.

and/or participant as a result of a participant's inability to tolerate the sensation of dysesthesia. Additionally, previous researchers have reported moderate-to-significant dysesthesia during microneurography (Table 1), however it is unclear if this impacted upon data collection (Burg et al., 1973; Knutsson and Widen, 1967; Littell, 1981).

3.2. Pain

Microelectrode placement can result in sensations of pain in some participants (Knutsson and Widen, 1967) and the combined incidence of pain reported during microneurography was 1.4% (Table 1) (Dunham et al., 2018; Rice et al., 1994). Acute, superficial

pain is a typical response to the insertion of any needle across the skin and therefore, is a widely accepted consequence of microneurography (Dunham et al., 2018). The application of topical anaesthetic can decrease a participant's perception of pain during microelectrode insertion (Cooke, 2000), however, prolonged sensations of pain increase sympathetic activity and therefore, could affect data if participants are in considerable discomfort (Schobel et al., 1996). In particular, intraneural electrical stimulation can result in a radiating sharp or burning pain in the innervation territory of a cutaneous fascicle or a diffuse dull ache in the muscle innervated by a muscle fascicle if the current applied is too high. Additionally, deeper local pain as the tip penetrates the nerve sheath or fascicle can also cause considerable discomfort for some individuals. Most typically, such pain can be relieved through avoidance of further manipulation, very minor adjustments or complete withdrawal of the microelectrode from the nerve and/or site. Within our investigations, we have chosen to terminate a very small number of tests due to persistent participant discomfort or pain during the search for SNA (n = 2/428, 0.5%). It is unclear if the previous reports of pain during microneurography used this operational definition to record a pain 'event.' Future reports should state the nature and impact of the pain experienced during microneurography in order to improve our understanding of this outcome. Current evidence on participant's perception of pain during microneurography is lacking.

3.3. Nerve block

The insertion of a microelectrode into a peripheral nerve, as in microneurography, has been shown to a result in a transient pressure block. The pressure block may be induced by the space occupied by the microelectrode (Wall and McMahon, 1985). The pressure block causes a temporary impairment in function most likely reducing the number of afferents activated by electrical activity, but is not necessarily indicative of structural damage (Rice et al., 1993; Vallbo, 1976). A previous experiment demonstrated that after insertion of a microelectrode, fibers from a rat sciatic nerve either: were unable to transmit electrical activity, but upon removal of the microelectrode, regained the ability to relay impulses; or had reduced transmission across multiple fibers that were not fully restored following microelectrode removal (Wall and McMahon, 1985). The latter outcome may be reflective of acute nerve damage that could lead to transient paresthesia, pain or muscle weakness. It should be considered that these observations were made in animal models, and that application to humans may be flawed at least due to the differences in fascicle dimensions. In contrast to the rat model, data collected from the radial and peroneal nerve in humans by Inglis et al. (1998) showed that conduction of electrical activity across the site of impalement was secure, but conduction time was markedly prolonged. In some axons, increasing activity resulted in conduction failure but the ability to conduct across the site of impalement was restored after rest.

3.4. (Pre)syncope

Syncope refers to a transient loss of consciousness caused by systemic hypotension and or cerebral hypoperfusion (Moya et al., 2009). Prodromal symptoms, such as lightheadedness, nausea, cold/hot sweats or pallor, are warning signs that syncope is imminent, and are collectively termed "pre-syncope." Vasovagal syncope is a response that occurs because of a paradoxical reflex arc, known as the Bezold–Jarisch reflex, and is triggered in response to inadequate ventricular filling from reduced venous return. The syncopal reflex arc is stimulated by a drop in cardiac output and/or blood pressure, which is subsequently countered by an increase in cardiac inotropy. Vigorous contraction of volume-depleted ventricles enables a compensatory rise in heart rate to maintain cardiac output. However, this contraction activates mechanoreceptors and C-fibers in the heart that paradoxically stimulate the vagal nucleus in the medulla, signaling a drop in sympathetic activity and increase in vagal tone. Consequently, there is a rapid drop in both blood pressure and heart rate, during which, syncope may occur (Moya et al., 2009). Vasovagal syncope is neurally mediated and can be precipitated by various situational factors including emotional responses, for example to medical instruments such as needles, overheating, gastrointestinal stimulation, micturition, medication use, postural change and post-exercise or post-prandial redistribution of blood (Moya et al., 2009).

In our laboratory, we have documented symptoms of pre-syncope and syncope during microneurography, that have only recently been reported recently within the literature (Dunham et al., 2018; Donadio et al., 2007). Our dataset included 428 assessments of MSNA from the peroneal nerve in 303 individuals (125 repeat assessments). Out of 428 assessments conducted in our laboratory, 33 individuals (7.7%) experienced pre-syncopal symptoms during microneurography. The majority of individuals experiencing pre-syncope were pregnant females (ranging from 16 to 40 weeks gestation), with lower incidence rates in non-pregnant females and males (Table 1). In all individuals, symptoms were alleviated after withdrawal of the recording microelectrode, movement into a supine position and/or ingestion of water or juice. In combination with previous reports of pre-syncopal symptoms during microneurography (Dunham et al., 2018; Donadio et al., 2007), the combined incidence rate was 8.9%. We also observed a single case of syncope during microneurography in which the participant was a pregnant female (incidence rate of 0.2%, Table 1). After recovery from transient loss of consciousness the participant was medically assessed, and the incident was confirmed as vasovagal syncope with no further symptoms or negative health outcomes.

There are a number of controllable factors that increase the risk of (pre)syncope in the general population. Fear of blood or medical instruments, such as needles, is known to induce vasovagal syncope (Moya et al., 2009). Therefore, the technique of microneurography may result in (pre)syncope in phobic patients, as demonstrated previously by Donadio et al. (2007). In contrast to this, our standard practice is to screen for an aversion to needles prior to enrolment and additionally, we collect blood samples via an intravenous (IV) catheter inserted prior to instrumentation. Thus far, we observed no adverse symptoms related to IV placement but have observed symptoms during the microneurography search. As such, we do not believe the (pre)syncopal symptoms observed in our cohort were emotionally mediated. Anecdotally, we have observed the onset of pre-syncopal symptoms following the insertion of the electrode into a nerve fascicle, and this is also noted within Dunham et al. (2018). It is possible that dysesthesia experienced at this point may result in a centrally mediated vasovagal reflex; however, we did not identify a clear association between such sensations and symptom development. Finally, we complete the majority of our investigations in the semi-recumbent position in order to avoid potential inferior vena cava compression that may be experienced by pregnant women in the supine position. Sub-diaphragmatic blood pooling in the upright posture, combined with an absence of muscular movement, may reduce venous return and induce (pre)syncopal symptoms (Stewart et al., 2017). As such, both the population and position

should be considered when determining the risk of (pre)syncope during microneurography.

4. After-effects

4.1. Paresthesia and muscle weakness

Following microneurography, there is a generally accepted low incidence of ongoing neural symptoms, including paresthesia; pain or tenderness at, or distal to, the site of insertion; and muscle weakness (Anderson et al., 1989; Eckberg et al., 1989; Hagbarth, 1979a; Littell, 1981; Rice et al., 1994). With data from previous publications, we calculated the incidence of paresthesia, pain and muscle weakness following microneurography as 4.6, 2.8 and 1.5%, respectively (Table 1). These symptoms were reported to manifest a few hours to days following microneurography and to persist for a period of 1 day up to several weeks. Due to the latency time of symptom development, a delayed process following neural injury, such as inflammatory edema, has been suggested to be the cause (Gandevia and Hales, 1997).

Previous work in animal models has demonstrated that the insertion of a microelectrode into an exposed nerve results in anatomical lesions that have a transient impact on function. Penetration with a microneurography electrode results in damage to the endo-/perineurium, myelinated and unmyelinated axons that can be detected with electron microscopy. However, undamaged fibers within the 'zone of injury' are also present (Fried et al., 1989). In the post-operative period following microelectrode penetration in rodent models there were no overt behavioural indications of pain or discomfort (Fried et al., 1989). However, there was a transient period of hypoalgesia, assessed by hind limb withdrawal latency, that fully resolved within 2-weeks following the procedure. As such, investigations in a rat model indicate that microneurography results in a limited and isolated degeneration of nerves that is followed by regenerative processes. The injury caused by insertion of a tungsten microelectrode, now used as standard in microneurography, may be result of pressure caused by the electrode itself, mechanical disruption of neural tissue during insertion and the resultant inflammatory responses. Such disruptions to the nerve structure may be the cause of paresthesia and/or muscle weakness following microneurography in humans.

The fascicular lesions following microneurography observed in animal models provide valuable insight into the acute neural impact of the technique; yet, the direct application to human investigations may be limited. Fried et al. (1989) identified that microelectrode impalement in a rat typically requires short single or double insertions into a dissected nerve, whereas in humans, the search for an appropriate signal may require repeated insertions through the skin over longer periods of time. Previous work has demonstrated that advancement of a microelectrode repeatedly placed into a cell tissue culture results in distortion and fragmenting of cells (Wall and McMahon, 1985). It may therefore be suggested that neural disruption as a result of microneurography in humans could be greater than that observed in animal models. In support of this, Eckberg et al. (1989) demonstrated that microneurography investigations lasting over 45 min were associated with a greater incidence of after-effects, and therefore, could be indicative of greater negative impact to the nerve. Based on these data, the authors suggested limiting searches to 60 min, using slow, controlled movement during microelectrode manipulation and keeping intraneural insertion to a minimum where possible (Eckberg et al., 1989). In light of this, the use of ultrasound guidance or electrical stimulation may speed up the placement of the microelectrode within a fascicle and reduce search time. However, neither technique will provide any advantage to finding a robust signal. Additionally, electrical stimulation has deleterious effects on the impedance of commercially available electrodes and can affect the signal-to-noise quality of a recording. As such, there are different advantages and challenges with varying search methods and as such, the technique used is usually the preference of the practitioner.

Participants should also be encouraged to restrict physical activity for 24 h following the procedure, as symptoms may be worsened by exercise (Eckberg et al., 1989). It has also been suggested that the after-effects of microneurography may be more significant in patients with diagnosed peripheral nerve damage such as neuropathies (Rice et al., 1994), however, no difference in the incidence of paresthesia or muscle weakness symptoms between patient groups and healthy participants has been observed (Anderson et al., 1989; Eckberg et al., 1989; Gandevia and Hales, 1997).

4.2. Infection

It is somewhat surprising that there are no reports of infection following microneurography reported within the literature, as this is recognized as an adverse outcome following any invasive procedure. As a relative comparison, acupuncture is a common technique that includes the transcutaneous insertion of a microneedle. A recent analysis by the World Health Organization identified a low but present risk of bacterial and viral infection that was rated as *probable* to *certainly* related the to the acupuncture technique (Zhang et al., 2010). In all cases, individuals recovered fully. Other authors (Ernst et al., 2011; Park et al., 2016) supported these findings and the incidence of infection following acupuncture was attributed to poor hygiene and sterilization techniques (Zhang et al., 2010; Park et al., 2016). Considering the popularity of this technique, the incidence of infection-related complications following acupuncture is very low and is further reduced by appropriate training and use of universal aseptic precautionary measures (Walsh, 2001). These control measures are also recommended for researchers completing microneurography. Microelectrodes may be sterilized and re-used, similar to other surgical equipment, however practitioners should consider that considerable degradation of the recording electrode occurs during a standard search, and this may render the impedance of the electrode ineffective. Additionally, as with any other invasive technique (e.g. IV placement), microneurography may pose a greater risk of infection in immunosuppressed individuals. As such, researchers should consider the population and/or individual risk of infection and detail this clearly within their informed consent. It should also be noted that investigations have previously been conducted in theoretically (not proven) immunosuppressed populations without adverse reports (Haarmann et al., 2015; Jordan et al., 2017; Strom et al., 2011).

5. Chronic complications

Persistent effects of microneurography have previously been reported within the literature. Specifically, three different authors have identified complications following microneurography including ongoing paresthesia (Littell, 1981; Hagbarth, 1979b) and suspected thin-fiber neuropathy with symptoms of persistent right lower leg pain and numbness, without weakness (Eckberg et al., 1989). Such complications of microneurography may have significant impacts on the individual that are prolonged (lasting up to 6 months) (Hagbarth, 1979b) or ever-lasting (Eckberg et al., 1989). We have identified one case of prolonged (>6 months) symptoms including lower leg pain and weakness. These complications are likely the result of significant fascicular injury during

intraneural recording. In cases where symptoms were resolved by 6 months, it is possible that the lesions caused by microelectrode insertion (see above) required a prolonged regenerative process in order to regain normal nerve function. However, in the case of neuropathy, the fascicular injury may have been too great for repair. While these chronic complications following microneurography have significant impact to an individual, it must be reinforced that the reported incidence remains extremely low (0.2%), as shown in Table 1 (Anderson et al., 1989; Knutsson and Widen, 1967; Littell, 1981).

In rare circumstances, chronic complications following microneurography can occur, however, control measures have been suggested to manage this risk. Eckberg et al. (1989) provided recommendations for human microneurography that have been adopted as best practice in the majority of laboratories. These measures aim to reduce the risk of both after-effects and chronic complications, and include precautions such as: limiting the search to 60 min, technique completed by a suitably trained investigator, and investigations should not be repeated at the same site within 1 month (Eckberg et al., 1989). It should be noted that no reports of chronic complications following microneurography were identified after the publication of these recommendations by Eckberg et al. 1989. The reduction in the number of subsequent cases could be attributed to both the adoption of best practice techniques in microneurography, but also a considerable lack of reporting of outcomes.

6. Recommendations for the assessment, management and reporting of adverse outcomes to microneurography

6.1. Risk assessment

Risks associated with microneurography are well known by researchers in the field, yet these risks are only addressed incidentally within a limited number of publications. Consequently, improved transparency and reporting of adverse outcomes is needed, so that researchers are able to complete informed and adequate risk assessment prior to conducting microneurographic investigations.

The nerve selected for microneurography is determined by the experimental question, as well as practical considerations such as easy identification of the nerve, proximity (depth) in relation to skin, proximity to other anatomical structure (e.g. blood vessels), the ability to stabilize the limb during recording and finally, the size, and therefore potential, of the nerve to support the needle when a signal is obtained. As such, the peripheral nerves of the lower extremities, including the deep and superficial peroneal, sural and tibial nerves, as well as the upper extremities, including the radial, median and ulnar nerves, are commonly used in microneurography. The choice of nerve determines the location of acute afferent responses to microneurography, but also the site of potential after-effects and chronic complications. As such, researchers using the microneurography technique should consider the nerve in terms of feasibility of their experiment, their experience of obtaining a signal from the nerve, but also the distal innervation of the nerve in question. The latter is important for identifying anticipated zones of dysesthesia and the functional consequences of any after effects. Of the nine papers included in this review, investigations were completed in the peroneal (n = 3), median (n = 1), ulnar (n = 1) and 'peripheral' nerves (n = 4). Additionally, our data were collected exclusively in the peroneal nerve. The incidence of adverse events during and following microneurography may vary according to nerve site, but with the current extent of reporting, it is impossible to determine these differences.

As detailed previously, the risk of after-effects following microneurography may be increased with longer search times and repeated intraneural insertion of the microelectrode. In nerves that are less easily palpated or stimulated, researchers should consider additional methods, such as ultrasound guidance, to improve accuracy and decrease the duration of the microneurography search (Curry and Charkoudian, 2011; Dunham et al., 2018). Supporting techniques that guide microelectrode placement may reduce the requirement for searching within the nerve, and therefore could reduce the extent of nerve lesions, resulting in less symptoms following microneurography. However, it should be noted that this practice does not guarantee an appropriate signal will be obtained (Curry and Charkoudian, 2011).

Pre-syncope and syncope in response to microneurography is not well established within previous microneurography literature, however, the combined evidence from recent work (Dunham et al., 2018; Donadio et al., 2007) and our dataset highlight that this is an adverse response to the technique. While the mechanisms for (pre) syncope during microneurography remain to be elucidated, the risk of such events should be carefully considered on an individual and population basis. The increased incidence of (pre)syncopal events in pregnant women within our dataset was unsurprising, as this group are at greater risk compared to the general population, with over a quarter of women experiencing at least one pre-syncopal episode during gestation (Gibson et al., 2001). Pregnancy is just one example of a population with a higher risk of syncopal events however; patients with cardiovascular disease, the elderly and individuals experiencing orthostatic hypertension also have an increased risk (Moya et al., 2009). Additionally, syncope is common side effect of many common medications. As such, these predisposing factors could result in an increased incidence of (pre) syncopal events in clinical populations during microneurography. Researchers should identify the risk of syncope associated with their population of interest (e.g. incidence rates of syncopal events in habitual activity) and in the individual (e.g. prior history of syncope and precipitating factors, disease status, medication use) prior to completing microneurography.

6.2. Informed consent

All individuals undergoing microneurography should be informed of the potential adverse outcomes of microneurography. Indeed, the incidence rates of acute responses, after-effects and chronic complications following the technique are relatively low (Table 1), yet this information, especially for less reported outcomes like (pre)syncope, is poorly documented within research. As such, the incidence of adverse responses to microneurography may also be poorly represented on participant information sheets and within ethical approval applications. In addition to clear information regarding potential outcomes, researchers are encouraged to detail control measures used to reduce the risks of such outcomes (i.e. limited search time, avoidance of exercise following technique, etc.) and recovery procedures that would be employed should an adverse event occur as a result of microneurography. The following are examples of evidence-based statements we have incorporated within ethics and information documents:

- *As we search for a suitable location to record your sympathetic nervous system activity, you may feel odd or new sensations. This may include change in temperature, tingling, mild cramping, or pressure in your lower leg. These are all normal and may help us to know if we are recording from the right location within the nerve. These sensations occur only briefly and go away quickly if searching is paused or if the needle is pulled back from the spot that caused the sensation. You will be asked to indicate the presence of any*

of these feelings to the person looking for the signal. Between one and four people out of 100 may find these sensations intolerable, if that is the case, we will stop searching for your nervous system activity.

- *Approximately 9 out of 100 people may start to feel faint, dizzy or nauseous during the search for your nervous system activity. Fainting can occur in extremely rare circumstances (less than 1 out of 100), however if you should start to feel any symptoms, we will stop the search, will recline you and/or elevate your legs, and give you some water. This should stop you feeling faint and prevent fainting.*
- *Following the experiment, there is a minimal risk that you may experience lasting effects of the procedure (up to two weeks). Between 1 and 5 out of 100 people report mild tenderness, numbness, increased sensitivity, and redness around the location of the needles. In extremely rare circumstances (less than 1 out of 100), there is also the risk of persistent muscle weakness (lasting up to 6 months). This can affect how you move and can result in numbness or tingling of the area associated with the experiment. To minimize the risk of any persistent effects, we limit the amount of time for which we search for a suitable signal (10 min in one spot and no more than 45 min in total). We also recommend that you do not take part in any physical activity greater than walking in the 24 h after the protocol is complete to help reduce any inflammation that may result following the procedure.*

6.3. Reporting

The majority of data concerning acute responses, after-effects and chronic complications of microneurography arises from data published over 20 years ago. Much anecdotal evidence is shared between researchers, yet there is a considerable lack of empirical published data regarding the safety of microneurography, however rare adverse outcomes may be. This aim of this review is to provide an update to the safety of microneurography, but also to encourage researchers using the technique to improve the practice of reporting outcomes. Such information is invaluable in the planning of efficient and effective microneurographic investigations. With the current lack of reporting within microneurography studies, it is not possible to determine if there are any differences in the incidence of adverse outcomes when using different methodologies (i.e. palpation, electrical stimulation, and ultrasound guidance), different nerves or between different populations. The latter is of particular relevance, as microneurography assessments are increasingly utilized and important in individuals with various disease states and it is imperative to understand if safety is compromised in certain clinical populations, especially those with pre-existing neuropathy. There have also been considerable changes in the methodology of microneurography (as with the findings from Knutsson and Widen (1967) in which glass-coated wire electrodes were used for recording) which may challenge the relevance of our calculated incidence rates.

Regardless of positive or negative outcomes, we believe additional reporting of acute responses, after-effects and chronic complications are invaluable in determining incidence rates for such events. As shown in Table 1, the incidence for these various outcomes is relatively low (between 0.2 and 8.9%); however, this may be substantially lower if all authors documented the presence, or lack, of adverse events during and following microneurography. In addition to recording events during the search, practitioners should follow up with participants after microneurographic investigations. This may in the form of a questionnaire or telephone call completed within 7–14 days of the study. Any participant that identifies ongoing symptoms at this point should be followed up routinely until the cessation or treatment of complications.

At present, there is no standardized method or registry for practitioners to report adverse outcomes, which creates some difficulty in determining incidence rates of acute responses, after-effects and chronic complications following current practices of microneurography. We suggest that practitioners publishing their research should consider including 1–2 sentences within the results section of their manuscript detailing the incidence of outcomes during or following microneurography in their study cohort. Operational definitions of an adverse event as a result of the technique should be established. We suggest the following:

Acute responses

- Participant experienced significant sensations of pain and/or dysesthesia that prevented or impaired data collection. It should be identified if these sensations were on going or evoked by the practitioner (e.g., when the nerve was tapped or stretched, during microelectrode placement).
- Participant experienced pre-syncopal symptoms during data collection.
- Participant experienced syncope.

After effects

- Participant experienced significant pain, muscle weakness and/or paresthesia that affected daily activity for up to one month following microneurography. Specifically, it should be identified if these sensations were on going or evoked by the participant or practitioner (e.g., when the nerve was tapped or stretched).
- Participant developed an infection at the site of microneurography investigation following microneurography.

Chronic complications

- Participant experienced persistent muscle weakness, paresthesia or neuropathy lasting for 1 to 6 months following microneurography.
- Participant experienced chronic muscle weakness, paresthesia or neuropathy following microneurography (>6 months).
- Where possible, information from clinical assessment should also be collected for any chronic complications.

7. Conclusions and specific recommendations

In summary, in the current paper we provide updated quantifiable incidence rates for acute and chronic outcomes associated with the technique of microneurography. We also include new data that specifically identify the risk of (pre)syncope during microneurography. Using this evidence we provide recommendations regarding the dissemination of risks associated with microneurography (e.g. to ethics boards, patients, etc.) and recommendations regarding documentation of adverse outcomes. We believe this information is relevant and important for conducting risk assessments or expressing known risks to others; this includes: individuals learning and initiating the technique, current practitioners/researchers, ethics boards, and patient/participant groups.

- Participants should be *adequately informed about the acute responses, after-effects and chronic complications* previously documented following microneurography.
- Researchers should *identify the risk associated with the population* (e.g. incidence rates of syncopal events, infection risk) *and the individual* (e.g. prior history of syncope, diagnosis of neuropathy, medication use) prior to completing microneurography.
- *In individuals with higher risk of syncopal events, we recommend continuous heart rate and blood pressure monitoring during the search for sympathetic activity.* Monitoring of hemodynamic

changes prior to symptom development may provide additional physiological indications of syncope and may elucidate the mechanisms underlying these responses to microneurography.
- We suggest that authors should always *document the presence of, or lack of, adverse events during and following microneurography within manuscripts*. Standard reporting would help to address gaps in the literature regarding symptoms, but also continue to objectively support the safety of the technique if no adverse outcomes are identified.
- Specifically to improve the understanding of the incidence of after-effects of microneurography, researchers should ask participants to complete a *follow-up questionnaire* approximately 1 week after the study visit. If participants are still experiencing symptoms after 1 week, contact should be maintained until resolution.

Sources of funding

This research has been funded by the Natural Sciences and Engineering Research Council of Canada (NSERC, RGPIN 06637; CDS) and the Heart and Stroke Foundation of Canada (HSFC G-16-00014033; MHD, CDS). VLM is funded by a Women and Children's Health Research Institute Postdoctoral Fellowship. CDS is funded by a HSFC Joint National and Alberta New Investigator Award (HSFC NNIA Steinback) and MHD is funded by an HSFC Joint National and Alberta Improving Hearth Health for Women New Investigator Award (HSFC NNIA Davenport).

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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