Original Article

Sural sensory nerve action potential: A study in healthy Indian subjects

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Abstract

Background: The sural sensory nerve action potential (SNAP) is an important electrodiagnostic study for suspected peripheral neuropathies. Incorrect technique and unavailability of reference data can lead to erroneous conclusions. **Objectives:** To establish reference data for sural SNAP in age-stratified healthy subjects at three sites of stimulation. **Materials and Methods:** A prospective study was conducted in 146 nerves from healthy subjects aged between 18 years and 90 years, stratified into six age groups (a = 18-30 years, b = 31–40 years, c = 41–50 years, d = 51–60 years, e = 61–70 years, and f >71 years). Sural SNAP was recorded antidromically, stimulating at three sites at distances of 14 cm, 12 cm, and 10 cm from the recording electrode. Mean – 2 standard deviation (SD) of the transformed data was used to generate reference values for amplitudes. Analysis of variance (ANOVA) test was used for inter-group and between three sites comparisons of amplitudes. **Results:** The lower limits of amplitude at 14 cm were 12.4 μV, 10.4 μV, 6.5 μV, 5.3 μV, 2.9 μV, and 1.9 μV; at 12 cm were 13.5 μV, 13.6 μV, 8.5 μV, 7.8 μV, 3.5 μV, and 2.8 μV; and at 10 cm were 16.3 μV, 16.3 μV, 11.1 μV, 10.0 μV, 4.8 μV, and 3.7 μV for groups a, b, c, d, e, and f, respectively. A statistically significant difference in amplitudes was noted from the three different sites of stimulation ($P < 0.001$). The amplitude differed significantly above the age of 60 years ($P < 0.01$) but not between groups e and f ($P > 0.05$). **Conclusion:** This study provides reference data for sural SNAP in Indian population at three different sites of stimulation along the calf in six age groups. It also shows significant variation in amplitude from the three different sites of stimulation.

Key Words

Age, amplitude, Indian, reference values, stimulation site, sural

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Introduction

The sural is a sensory nerve, distally and superficially placed in the foot making it most accessible for nerve conduction studies. It has a low risk for compressive injury[1] and being distal reflects the status of the peripheral nerve in length-dependent peripheral neuropathies. Nerve conduction testing helps in the objective evaluation of diabetic polyneuropathy and other length-dependent peripheral neuropathies.[2-4] Low amplitude or absent sural sensory nerve action potential (SNAP) bilaterally suggests involvement at the peripheral nerve level. Incorrect technique, excessive adipose tissue, edema in limbs, and absence of age stratified reference data can lead to abnormal reporting of a normal sural SNAP. This entails a host of unindicated investigations for the etiological diagnosis of a peripheral neuropathy that is not there to begin with. For example, in the elderly subjects, the normal sural SNAP would be much lower in amplitude than that in the younger subjects.[5-8] A review of

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published literature for reference data on sural SNAP reveals a dearth of studies in the Indian population as compared to the Western population.[9,10] Additionally, there are disparate reports regarding the presence or absence of sural SNAP in healthy elderly subjects who are aged above 60 years.[1,5] Also many Western studies antidromically record the SNAP at a distance of 14 cm from the lateral malleolus.[6,11-13] Our experience shows that a distance of 14 cm may not optimally apply to the Indian population, because the average height of an Indian is lesser than their Western counterparts, which implies a shorter limb length. Second, the large calf girth at a distance of 14 cm, especially in obese and short statured individuals, hinders the recording of an optimal sural SNAP.

This study was hence taken up to prospectively establish the reference data for the sural SNAP amplitude and latency at distances of 14 cm, 12 cm, and 10 cm from the active recording electrode in healthy Indian adults for different age groups and to assess for statistically significant difference in the amplitude recorded at these sites. Further, the effects of patient related parameters, such as age, body mass index (BMI), limb length, and limb girth at stimulating site, on the sural SNAP amplitude were assessed.

Materials and Methods

Subject selection

This was a prospective study approved by the Institutional Ethics Committee and conducted in the Clinical Neurophysiology department. The subjects selected for our study were:

1. Healthy volunteers,
2. Healthy relatives accompanying patients, or
3. Patients referred to the electrodiagnostic department for unrelated conditions affecting only the upper limbs.

Detailed history regarding symptoms of polyneuropathy (such as tingling, numbness, gait abnormality, and weakness), diabetes mellitus, tuberculosis (past or present); family history of peripheral neuropathy; frequent alcohol consumption (more than two beverages per day for more than 4 weeks); and the use of medication known to cause neuropathy was taken and subjects with these symptoms were excluded from the study. The available serological reports of fasting glucose, vitamin B12 levels, and thyroid profile were evaluated and the subjects with abnormal readings were excluded. Only the subjects with preserved vibratory perception in the toes and bilaterally normal ankle jerks were included in the study. Subjects aged >60 years with reduced but not absent ankle jerks were included. Patients referred to the electrodiagnostic center for evaluation but then found not to harbor any disease were not included in this study.

One hundred and fifty healthy subjects aged between 18 years and 90 years were included in the study. Four subjects were excluded later as they had unilateral edema and/or ankle injury, thus making a total of 146 subjects. The study was done over a period of 5 years from 2008 to 2013, the main difficulty encountered being the recruitment of elderly subjects aged above 60 years without any preexisting risk factor.

For all the subjects data, such as age, height, weight, BMI, bilateral limb length from the tip of fibular head to the tip of the lateral malleolus, and the calf girth at each stimulus site, were documented. The calf girth was measured using a standard tape measure with the tape running below the site marked on the calf.

Nerve conduction technique

The procedure was explained to each subject to ensure maximum comfort and compliance. Both the sural nerves were sampled. The tests were done on a Synergy Oxford Medelec electromyograph (Natus Medical Inc.) with the following acquisition parameters: Filter settings at 3 Hz to 2 kHz, sweep speed of 20 ms, and gain of 10 microvolt (μV)/division. Lower limb temperature recorded at the lateral malleolus was maintained at 30°C. The temperature was measured before, during, and after the recording. All tests were done by neurophysiologists trained at the same center using the same protocol for each study.

The patient was placed in a comfortable lateral decubitus with the leg to be assessed on the top. The recording and stimulating sites were cleaned with spirit to ensure maximum electrical conductance. Surface electrodes of 10 mm square plates were used to record the potential. The active recording electrode was placed just behind the upper border of lateral malleolus and the reference electrode was placed 4 cm distal to it. The recording sites were marked at distances of 14 cm, 12 cm, and 10 cm proximal to the active electrode [Figure 1]. The ground electrode was then placed between the stimulating and recording sites. A supramaximal stimulus was used to obtain the maximum amplitude sural SNAP with the least stimulus artifact. The stimulating electrode was moved slowly from the midline of the calf laterally or medially till a maximum SNAP was obtained at each site of stimulation. Care was taken to reduce the stimulus artifact by relative rotation of the anode or reduction of stimulus intensity, without altering the amplitude of the response. Each optimal SNAP was then averaged for at least 8-10 responses to ensure a clear onset from the baseline [Figure 2]. The stimulus pulse duration was increased from the standard 0.1 ms to 0.2 ms in case the subjects had large calves, in the obese subjects or subjects with edema feet. For each averaged SNAP, the onset latency was measured in ms and the negative-to-positive peak amplitude was measured in μV.

Statistical analysis

All data were analyzed using the Stata Corp 12.2 (StataCorp LP, College Station, Texas) statistical program. Both limbs of

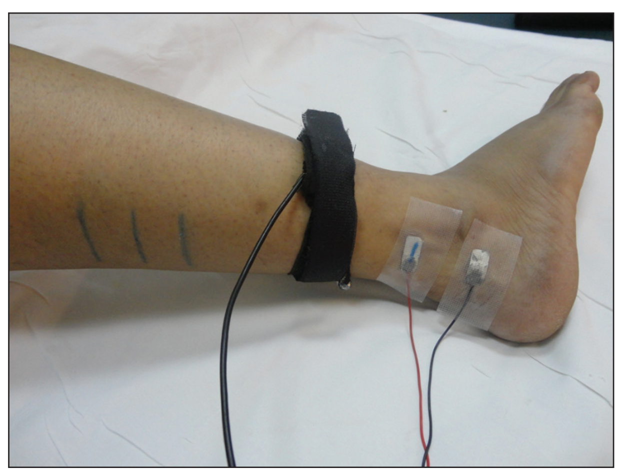

Figure 1: Recording sites for sural SNAP marked distances of 14 cm, 12 cm, and 10 cm proximal to the active electrode

each patient were assessed; the mean side-to-side amplitude difference was calculated as a ratio that was 0.99 at 14 cm, 0.97 at 12 cm, and 0.98 at 10 cm with standard deviation (SD) of 0.19. This was found to be statistically insignificant using the Student's paired "*t*"-test ($P < 0.01$). Also, the maximum difference in the side-to-side amplitude ratio at 14 cm, 12 cm, and 10 cm were 1.5, 1.5, and 1.6, respectively. The parameters of only the right lower limb were included for statistical analysis, making a total of 146 nerves. The subjects were stratified into six groups as per age: a = 18-30 years, b = 31-40 years, c = 41-50 years, d = 51-60 years, e = 61-70 years, and f > 71 years.

The coefficient of skewness was calculated for the sural latencies and amplitudes in each age group. While the latencies were found to show a Gaussian distribution, values for the sural amplitudes were positively skewed (0.00, 0.02, 0.02 for the sural SNAP amplitudes at 14 cm, 12 cm, and 10 cm, respectively) and required optimal transformation.

Statistical analysis for obtaining reference values was done using mean ± 2 SD as suggested by Robinson *et al*.[14] The percentile and the quantile regression methods could not be applied to our study as the sample size in each group was not adequate.

Mean + 2 SD was taken to define the upper limit of the sural latency. The sural amplitudes obtained at each stimulation site were square root transformed to bring the positively skewed data into a more Gaussian distribution. The mean – 2 SD of the transformed data was then computed and then reconverted into the original units for the lower limit of the sural amplitude.

The transformed sural amplitudes at each stimulating site at 14 cm, 12 cm, and 10 cm were compared using the analysis of variance (ANOVA) test for statistically significant difference at varying sites of stimulation. The effect of the variation was computed using the *R*-squared test and Cohen's *d*-test.

ANOVA was applied to compute the statistical difference in the sural amplitudes between each of the six age groups specified in order to assess the effect of age on the amplitude. Further linear regression analysis was done by model building to assess the effect of age, height, BMI, limb length, and calf girth on the amplitude of the sural SNAP.

Results

One forty six right sural nerves of healthy subjects (69 females and 77 males) between the ages of 18 years and 90 years (mean 51.24658, SD 18.92401) were included in the study. The anthropometric parameters of the subjects are shown in Table 1.

Paired *t*-test showed no significant differences in the sural SNAP amplitudes at each site of stimulation for males and females ($P > 0.1$). Hence, further analysis was carried out after pooling the data for both the genders.

The reference data for sural SNAP onset latency and peak-to-peak amplitude were calculated for each age group at distances of 14 cm, 12 am, and 10 cm from the recording electrode and were listed, respectively, in Tables 2 and 3.

Further, the difference in the amplitudes recorded at each stimulating site were found to be statistically different using ANOVA as shown in Table 4.

The most commonly used stimulating sites in recording the sural SNAP are at distances of 14 cm and 12 cm from the recording electrode. Paired Student's *t*-test detected a significant effect of the site of stimulation (14 cm and 12 cm) on the sural SNAP using both *R*-squared test for difference of means (0.84) and Cohen's *d*-test for difference of the SDs (1.64). Further, the sural SNAP amplitude at all three sites of stimulation showed a statistically significant difference only in the subjects aged above 60 years as compared to those aged below 60 years ($P \leq 0.01$).

Linear regression of the transformed sural amplitude data also showed age as the covariate with maximum effect ($r^2 = 0.39$), as depicted in Figure 3. Height, BMI, leg girth, and limb length had further minimal effect on the amplitude obtained ($r^2 = 0.41, 0.42, 0.43, 0.44$). The statistical power of our study was estimated to be >0.8.

Discussion

The SNAP is an important factor in the electrodiagnostic evaluation of a patient with suspected peripheral neuropathy.[15-18] Abnormality of this potential is decided by comparing primarily its amplitude to available reference data.

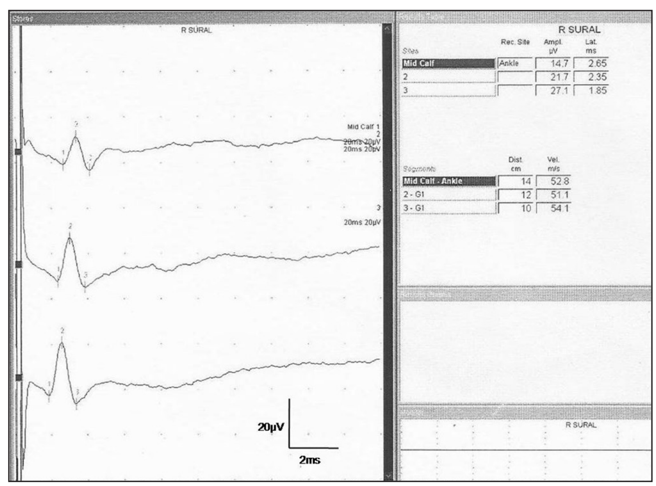

Figure 2: Averaged sural sensory nerve action potential recorded at three sites of stimulation

Table 1: Anthropometric parameters of the subjects in the study

Variable	Mean	SD	Min	Max
Age (years)	51.24	18.92	18	89
Weight (kg)	60.92	11.28	38	100
Height (cm)	158.85	8.74	139	180
BMI–weight in kg/height in m^2	24.13	4.01	15.81	41.62
Limb length (cm)	35.83	3.11	27	44
Leg girth (cm) at 14 cm	24.64	3.08	16	35
Leg girth (cm) at 12 cm	23.24	2.85	15	32.5
Leg girth (cm) at 10 cm	21.89	2.60	14	30

Table 2: Age stratified upper limit of normal for sural SNAP onset latency in milliseconds (ms) at stimulating distances of 14 cm, 12 cm, and 10 cm from the recording electrode

Age groups (years)	*N*	Latency at 14 cm (ms)			Latency at 12 cm (ms)			Latency at 10 cm (ms)		
		Mean	SD	Upper limit of normal (Mean + 2 SD)	Mean	SD	Upper limit of normal (Mean + 2 SD)	Mean	SD	Upper limit of normal (Mean + 2 SD)
18-30	26	2.86	0.30	3.47	2.47	0.30	3.09	2.11	0.29	2.70
31-40	28	2.72	0.27	3.28	2.39	0.28	2.95	2.01	0.24	2.50
41-50	19	2.77	0.39	3.56	2.42	0.34	3.11	2.03	0.29	2.63
51-60	16	2.90	0.28	3.48	2.54	0.25	3.04	2.14	0.20	2.54
61-70	28	2.95	0.31	3.59	2.56	0.27	3.12	2.17	0.26	2.703
>71	29	3.08	0.36	3.81	2.64	0.34	3.32	2.20	0.29	2.785

Table 3: Age stratified lower limit of normal for sural SNAP peak-to-peak amplitude in microvolts (μV) at stimulating distances of 14 cm, 12 cm, and 10 cm from the recording electrode

Age groups (years)	*N*	Amplitude at 14 cm (μV)			Amplitude at 12 cm (μV)			Amplitude at 10 cm (μV)		
		Mean*	SD*	Lower limit of normal†	Mean*	SD*	Lower limit of normal†	Mean*	SD*	Lower limit of normal†
18-30	26	4.82	0.65	12.35	5.23	0.78	13.50	5.67	0.81	16.36
31-40	28	4.82	0.80	10.35	5.27	0.79	13.62	5.68	0.82	16.34
41-50	19	4.48	0.97	6.48	4.87	0.97	8.54	5.19	0.92	11.15
51-60	16	4.31	1.00	5.31	4.86	1.03	7.83	5.33	1.08	10.01
61-70	28	3.47	0.88	2.94	3.85	0.98	3.51	4.27	1.04	4.81
>71	29	2.84	0.71	1.97	3.16	0.73	2.83	3.51	0.79	3.73

*Computed from transformed sural SNAP amplitude, †Computed as (Mean – 2 SD) and converted back to original units

Table 4: Comparison of the amplitude obtained at stimulating distances of 14 cm, 12 cm, and 10 cm from the recording electrode using ANOVA

Summary of sural amplitude (transformed data)		
	N	Mean (SD)
14 cm	146	4.07 (1.13)
12 cm	146	4.48 (1.19)
10 cm	146	4.88 (1.23)
Comparison of amplitude by site (Bonferroni)		
14 cm and 12 cm	14 cm and 10 cm	12 cm and 10 cm
P<0.05	*P*<0.001	*P*<0.05

Low amplitude or absent SNAP is the basic abnormality in a length-dependent peripheral neuropathy. Incorrect recording techniques and unavailability of age matched and correctly generated reference data could give erroneous conclusions regarding the presence of a peripheral neuropathy that could then lead to multiple unwarranted investigations.

Literature search reveals a few studies for reference data on the sural SNAP in healthy Indian population.[9,10] Multiple international studies have used a distance of 14 cm or 12 cm from the recording electrodes for stimulation.[1,6,11-13,19] In case of our subjects, we stimulated the nerve at three sites—14 cm, 12 cm, and 10 cm above the active recording electrode in the calf. ANOVA showed a significant difference in the leg girth at all three sites ($P < 0.001$). Using a distance of 14 cm from the recording electrode placed the stimulating electrode over a very fleshy part of calf. The large calf girth at 14 cm especially in short and obese individuals posed technical difficulties in recording an optimal SNAP. Excess pressure on the stimulating electrode and an increase in the stimulation duration were required to get supramaximal stimulation, increasing the subject discomfort. On the other hand, stimulation at a distance of 10 cm from the recording electrode produced a large stimulus artifact and needed anodal rotation to optimize the SNAP. However, this is useful for short subjects with excessive adipose tissue in the legs, where the calf girth is large even at 12 cm above the recording electrode. Stimulation at 12 cm from the recording electrode was found to be technically most convenient and suitable for our subjects.

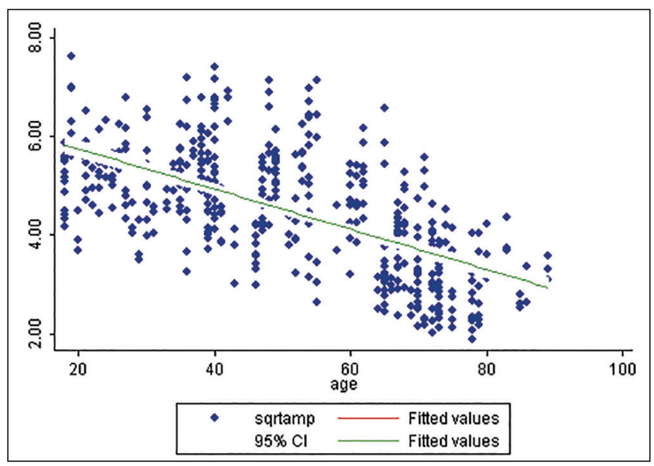

Figure 3: Linear regression of square root of sural SNAP amplitude with age of the subjects

Ascertaining the reference data to help diagnose a peripheral neuropathy is of utmost importance in the elderly subjects aged above 60 years, and misdiagnosis could lead to multiple unwarranted investigations. Previous studies have shown disparate results about obtaining a sural SNAP above the age of 60 years with incomprehensible results of the lower limit of amplitude, sometimes being less than zero.[5,19] We found no normal subject with an unrecordable surface sural SNAP in our study even in groups e and f. Our finding is supported by a study using near nerve techniques which show that sural responses, though decreasing in amplitude with age, were obtained in healthy subjects of all ages (5–90 years).[20]

It was also observed that the optimum site of stimulation for the sural nerve did not always lie in the midline of the calf but often 2-4 cm lateral to it, which was detected as we slid the stimulating electrode both medially and laterally at each site to get the maximum evoked amplitude of the SNAP.[21]

As expected, the amplitude of the SNAP negatively regressed with age and was significantly different in subjects aged above 60 years (groups e and f) as compared to the younger groups (groups a, b, c, d). Similar correlation with age has been established in previous studies.[1,6,22,23] Covariates, such as height, BMI, leg girth, and limb length did not add significant variation to the model.

The reference sural SNAP amplitude obtained in our study is comparable to the results obtained by Esper *et al*.[1] In this study, we have utilized the Mean ± 2 SD method after transforming the skewed data as suggested by Robinson *et al*.[14] Many studies have used the percentile method to establish the lower limit of the normal sural SNAP amplitude.[1,19] However, the number of subjects in each group in our study was inadequate to reliably apply the same methodology.[14] Besides, most of the subjects included in this study were from north-western Maharashtra, hence a larger multicentric study is being planned to include a larger number of subjects from a wider geographic distribution to reassess the reference data of sural SNAP. Similarly, a comparison of various prescribed statistical methods for establishing reference data is also being postulated. A shortcoming of this study is that we have not accounted for inter-examiner variability of data that could be about 10 percent.[24,25]

Conclusion

This is the first study to provide age stratified reference data for SNAP in Indians, using three sites of stimulation at distances of 14 cm, 12 cm, and 10 cm from the active recording electrode. Our study shows that the sural SNAP amplitude varies significantly with both the site of stimulation and age of the subject. Hence, the use of a single cutoff value for establishing a normal sural SNAP without the consideration of age and site of stimulation is not advised as it may lead to erroneous conclusions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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