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Sensory Nerve Action Potential Analysis in a Cohort of Patients With Spinal Muscular Atrophy Aged 12 Years and Older

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Keywords: electrophysiology | sensory nerve action potential | *SMN*-modulating therapy | spinal muscular atrophy

ABSTRACT

Introduction/Aims: *Survival Motor Neuron 1 (SMN1)*-related spinal muscular atrophy (SMA) is characterized by α-motor neuron degeneration, with sensory function assumed to be clinically preserved. However, recent studies in severely affected patients and animal models have challenged this view. Therefore, we assessed the maximum sensory nerve action potential (SNAP) amplitude of the median nerve in patients with SMA and examined its changes during treatment with *SMN*-splicing modifying therapies.

Methods: We longitudinally assessed median nerve maximum SNAPs in 103 genetically confirmed patients with SMA (types 1c-4, aged ≥ 12 years) before and approximately 1 year after treatment with nusinersen or risdiplam. For comparison, we included 53 age- and sex-matched healthy controls, using identical settings. We also compared data with reference values from a previously published cohort.

Results: Maximum SNAPs were abnormal in 6 patients with SMA (6%), which was comparable to controls (8%), even when corrected for age. In patients younger than 50 years, abnormal maximum SNAPs were more prevalent in patients with SMA types 1 and 2. Maximum SNAPs were higher in SMA compared with controls. Maximum SNAPs showed an age-related decline in most cohorts, but the decline was steeper in patients with SMA type 1c. There was no difference in SNAPs after 1 year of treatment.

Discussion: Our findings suggest the preserved sensory integrity of the median nerve in the majority of patients with SMA (94%), even in longstanding disease. The resilience of sensory neurons of the median nerve, and whether this extends to other peripheral nerves, warrants further investigation.

Trial Registration: The study was approved by the local medical ethics committee (no. 20-143) and registered in the Dutch registry for clinical studies and trials (www.toetsingonline.nl—NL72562.041.20, March 26, 2020)

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CI, confidence interval; CMAP, compound muscle action potential; eGFR, estimated glomerular filtration rate; HC, healthy controls; HSD, honestly significant difference; NA, not applicable; PC, published controls; SD, standard deviation; SMA, spinal muscular atrophy; SMN1, survival motor neuron 1 gene; SMN2, survival motor neuron 2 gene; SNAP, sensory nerve action potential; STROBE, strengthening the reporting of observational studies in epidemiology; UMCU, University Medical Center Utrecht.

W. Ludo van der Pol and Renske I. Wadman contributed equally to this work.

1 | Introduction

Survival Motor Neuron 1 (*SMN1*)-related spinal muscular atrophy (SMA) is a hereditary neuromuscular disease caused by loss of function of the *SMN1* gene on chromosome 5q, leading to a shortage of the ubiquitously expressed SMN protein [1]. Sufficient levels of SMN protein are necessary to sustain basic cell biological functions, such as mRNA splicing, ubiquitination, endocytosis, ribosomal assembly and function, and translation [2, 3]. α-Motor neurons in the anterior horn of the spinal cord are particularly vulnerable to SMN protein deficiency, which ultimately causes their degeneration and subsequent progressive muscle weakness [4]. Nevertheless, other tissues and cell types also require high levels of SMN protein for proper function. Depletion of SMN therefore also results in pathological changes in tissues other than motor neurons [5–9].

SMN protein concentrations are particularly high throughout the nervous system, including the brain and spinal cord, during normal prenatal development [10–13]. SMN depletion results in abnormal connectivity of neural networks in addition to α-motor neuron degeneration, as was shown in human post-mortem and imaging studies, as well as experimental animal models of SMA [14–21].

The lack of sensory symptoms is a striking feature of SMA, but recent clinical and pathological studies in animal models showed sensory circuit alterations including disconnection of afferent nerve fibers and changes in sensory synapses [16, 17], abnormal sensory conduction or complete absence of sensory nerve action potentials [22–29], and axonal degeneration and loss of myelinated fibers of sensory nerves [18, 30–32], have challenged the view of sensory normality. Most clinical studies, however, are limited due to relatively small sample sizes and predominant selection of young and more severely affected patients (Table S1). Our understanding of the role of (sub)clinical sensory changes in milder phenotypes, whether sensory alterations occur in older patients with longstanding disease, and the sensory (side) effects of any of the recently introduced SMN-modulating treatments remains limited.

We therefore conducted an exploratory longitudinal study of sensory integrity by means of electrophysiological assessment of the median nerve in a cohort of adolescents and adults with SMA types 1c-4. We used the maximum sensory nerve action potential (SNAP) amplitude of the median nerve to explore: (1) whether patients with SMA types 1c-4 show (subclinical) alterations compared with healthy controls and to investigate the influence of patient and disease characteristics, and (2) the effects of one-year treatment with the *SMN2*-splicing modifiers nusinersen and risdiplam.

2 | Methods

2.1 | Study Design and Participants

We conducted a longitudinal cohort study between May 2020 and December 2022 at the Netherlands SMA Center at the University Medical Center Utrecht (UMCU). We performed baseline electrophysiological assessments in patients with SMA before starting treatment with *SMN2*-splicing modifying therapy (nusinersen or risdiplam). Follow-up electrophysiological assessments were performed in patients with SMA, approximately 10–14 months after the start of risdiplam and nusinersen treatment, respectively.

All participants took part in a longitudinal cohort study (the "SMA Motor Map" protocol) that we previously described [33]. All participants were aged ≥ 12 years and all patients had a clinical diagnosis of SMA types 1c-4 [4, 34–36], a confirmed loss of function of the *SMN1* gene, and had not been previously treated with *SMN2*-splicing modifiers (nusinersen or risdiplam). Longitudinal assessments were performed only for patients who started and continued therapy during the study.

We included two reference cohorts: (1) A group of 53 healthy controls assessed at our center to allow direct comparisons under identical conditions and settings by protocol (hereafter referred to as HC). Participants were age- and sex-matched to the patient cohort, with no relevant medical history (specifically no history of neurological disorders or sensory symptoms in the hands). We recruited these participants through our website (www.smacentrum.nl), the newsletter for patients with SMA, and the newsletter of the patient organization Spierziekten Nederland. (2) A reference cohort derived from a previously published study with 258 healthy participants assessed with peak-to-peak analyses from antidromic wrist stimulation and third digit recordings (hereafter referred to as "published controls" [PC]) [37]. We based reference values for abnormal maximum SNAP values on this published cohort. The cut-off value was determined based on the third percentile of the overall cohort (17 μV). In addition, subcategories were defined based on age (< 50 years vs. ≥ 50 years) and body mass index (BMI) (< 24 kg/m^2 vs. ≥ 24 kg/m^2) based on calculated −2 standard deviation (SD) thresholds, resulting in cut-offs for maximum SNAP values in participants < 50 years: 27 and 19 μV for BMI under or above 24 kg/m^2, respectively; and in participants ≥ 50 years of age: 18 and 8 μV for BMI under or above 24 kg/m^2, respectively. As BMI data were not available for our patients with SMA and HC, we compared data to both BMI-based reference values to ensure comprehensive analysis.

We compared data from patients with SMA to both reference cohorts (HC and PC), and in addition, compared the HC to the PC.

We obtained written and oral consent from all participants and, when appropriate, from their parents or legal representatives. We used Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria [38].

2.2 | Clinical Assessments

We collected clinical characteristics, including age and sex at the time of inclusion, SMA types (as defined previously; i.e., types 1c, 2a, 2b, 3a, 3b, and 4) [4, 34–36], age at symptom onset, *SMN2* copy number, disease duration, contractures in the forearm, and ambulatory status. We also documented medical history (including risk factors known for developing polyneuropathy (i.e., diabetes mellitus, neurotoxic chemotherapy, and decreased renal function)) and use of medication. We determined renal

function using cystatin-C estimated glomerular filtration rate (eGFR) [39] and defined decreased renal function as an eGFR $<60\,mL/min/1.73\,m^2$. We specifically asked whether participants experienced sensory symptoms in their hands.

2.3 | Electrophysiological Assessments

We described the protocol, including assessment, tolerability, and feasibility of sensory nerve action potential (SNAP) of the median nerve, previously [33, 40]. The SNAP measurement was a part of a larger, primarily motor-focused protocol [33]. Before and during assessments, we warmed the forearm with a water blanket with a constant flow of warm water (37°C), using a previously described procedure [41]. Patients were either in a supine or seated position depending on their ability to lie down.

In short, we used QTrac-S software (Institute of Neurology, Queen Square, London, United Kingdom) and stimuli were applied with the cathode positioned at the wrist, 7 cm from the active recording surface electrode on the thenar muscles for compound muscle action potential (CMAP) responses and the anode placed 10 cm proximal to the cathode on the radial side of the arm, using disposable Red Dot electrodes (3 M Health Care, Neuss, Germany). We obtained baseline-to-peak maximum CMAP (in mV) per protocol [42]. We used ring electrodes placed on the proximal and distal interphalangeal joints of the third finger of the dominant hand for SNAP responses. The stimulation sides were the same for sensory and motor testing for the convenience of the participant and minimizing time to perform the complete protocol.

For the median nerve sensory assessment, we amplified signals by a factor of 10,000 with filter settings of 10 Hz to 3 kHz. We applied stimuli of 0.5 ms duration, and stimulation intensity was incrementally increased in 2% steps until the maximum SNAP was recorded. We recorded the peak-to-peak maximum SNAP amplitude (in μV) and the stimulus intensity (in mA). To reduce the potential influence of noise, we calculated the maximum SNAP using three consecutive SNAP amplitudes.

We standardized all procedures across participants, and all tests were performed by the same investigator (LR). Due to COVID-19 regulations in the Netherlands during the study period, we could not perform repeated assessments to evaluate the reproducibility of the complete protocol (including motor assessments).

2.4 | Statistical Analysis

We used descriptive statistics for baseline characteristics of participants. The normality of the data was verified through visual inspection using histograms and QQ-plots.

To assess differences between patients and controls, we applied Fisher's exact test or Pearson's Chi-squared test based on expected frequencies. Differences in electrophysiological parameters between patients and controls were assessed using a Student's *t*-test reported together with a 95% confidence interval (CI) for the difference in case of normally distributed data or the Mann–Whitney *U*-test in case of non-normally distributed data. For analyses of more than two groups we used an analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) adjustments to investigate subsequent pairwise comparisons or the Kruskal–Wallis test with Dunn post hoc analyses for non-normally distributed data.

As an exploratory objective, we used linear regression models to evaluate the impact of patient and disease characteristics on maximum SNAPs. Firstly, age and participant group (comprising patients with SMA and controls) were included as covariates to assess the influence of age on SNAP across the entire cohort. Subsequently, age, participant group (comprising controls and SMA types 1c, 2a, 2b, 3a, and 3b/4), and the interaction of age by group were included as covariates to assess whether age had varying effects on the maximum SNAP across disease severities. Additionally, we investigated the relationship between motor nerve degeneration and sensory integrity, using maximum CMAP, SMA type (comprising SMA types 1c, 2a, 2b, 3a, and 3b/4), and age as covariates.

For patients with SMA with complete follow-up data, we compared the change in mean maximum SNAP values between baseline and follow-up using a paired Student's *t*-test and reported the mean difference together with a 95% CI. We also explored the change in maximum SNAP between baseline and follow-up in subgroups based on therapy (nusinersen vs. risdiplam) and SMA types (SMA types 1c, 2a, 2b, 3a, and 3b/4).

We considered (corrected) *p*-values <0.05 as statistically significant. We used R (version 4.2.1 for macOS with RStudio version 2023.09.1 + 494, 2009–2023 Posit Software, PBC, Boston, MA, USA) for all analyses.

3 | Results

We included 103 patients with SMA. Clinical characteristics and electrophysiological parameters at baseline from patients with SMA and HC are presented in Table 1.

Sex was evenly distributed ($\chi^2=0$, $p=1$) and there was no difference in age (39 vs. 37 years, $p=0.421$) between patients with SMA and HC. Patients with SMA type 2 were younger compared with patients with SMA types 3/4 (median age 29 vs. 50 years, $p<0.001$) and compared with HC (median age 29 vs. 37 years, $p=0.012$). One patient (SMA type 3) had confirmed carpal tunnel syndrome in the dominant hand and was therefore tested on the other (asymptomatic) side; none of the other patients or HC experienced sensory symptoms in their hands. Eight patients with SMA (8%) (SMA type 2: $n=1$ and type 3: $n=7$) had diabetes mellitus, and one patient with SMA (1%) (SMA type 3) had a history of neurotoxic chemotherapy (paclitaxel). Seven patients (7%) (all SMA type 3) had a decreased renal function based on glomerular filtration rate.

We could elicit median nerve maximum SNAPs in all patients with SMA and HC. Six patients with SMA (6%) (SMA type 1: $n=2$, type 2: $n=1$, and type 3: $n=3$) had maximum SNAPs $<17\,\mu V$ (range 8–16 μV) (Table 2). Two out of three SMA type 3 patients with abnormal values had diabetes mellitus and decreased

TABLE 1 | Clinical characteristics and electrophysiology of HC and patients with SMA based on type.

	HC	Total SMA	Type 1[a]	Type 2	Type 3/4
Clinical characteristics					
N	53 (100)	103 (100)	8 (8)	45 (44)	50 (48)
Age at time of inclusion, Y	41 (13, 71)	38 (13, 67)	38 (18, 51)	31 (13, 49)	45 (13, 67)
Females	30 (56)	57 (55)	4 (50)	29 (64)	24 (48)
Age at onset, Y	NA	3.1 (0.3, 42.5)	0.4 (0.3, 0.5)	0.8 (0.3, 2.0)	5.5 (0.7, 42.5)
SMN2 copy number					
2	NA	2 (2)	0 (0)	1[b] (2)	1[c] (2)
3		65 (63)	8 (100)	39 (87)	18 (36)
4		34 (33)	0 (0)	5 (11)	29 (58)
5		2 (2)	0 (0)	0 (0)	2 (4)
Disease duration at time of inclusion, Y	NA	35 (5, 63)	38 (18, 51)	31 (13, 49)	39 (5, 63)
Ambulatory	53 (100)	19 (18)	0 (0)	0 (0%)	19 (38)
Contractures[d]	0 (0)	56 (54)	7 (88)	34 (76)	15 (30)
Electrophysiology					
Maximum SNAP (μV)	46 (11, 116)	62 (8, 217)	53 (8, 106)	77 (14, 217)	51 (14, 128)
Stimulus intensity (mA)	9.5 (5.5, 19.4)	10.6 (4.6, 20.3)	13.5 (7.4, 18.5)	10.7 (4.6, 19.4)	10.1 (4.6, 20.3)
Maximum CMAP (mV)	9.5[e] (5.1, 14.1)	5.1 (0.3, 14.0)	2.0 (0.4, 4.5)	3.5 (0.3, 7.8)	7.1 (1.0, 14.0)

Note: Data are presented as mean (range) or count (%).
Abbreviations: CMAP, compound muscle action potential; HC, healthy controls; NA, not applicable; SMA, spinal muscular atrophy; *SMN2*, survival motor neuron 2 gene; SNAP, sensory nerve action potential; Y, years.
[a]All patients with SMA type 1c, a subcategory of type 1 patients with three *SMN2* copies and relatively long survival into adulthood [34].
[b]Patient with point mutation in *SMN2* exon 7 (c.859G>C) [43].
[c]Patient with heterozygous *SMN1* deletion and point mutation in exon 4 (c.542A>G) on the other allele [43].
[d]Contractures in the measured arm.
[e]Only measured in the newly recruited 25 HC in this study.

TABLE 2 | Maximum SNAP of the median nerve in patients with SMA and HC.

	HC (*n* = 53)	Total SMA (*n* = 103)	Type 1 (*n* = 8)	Type 2 (*n* = 45)	Type 3/4 (*n* = 50)
Abnormal maximum SNAP (< 17 μV)[a]	4 (8)	6 (6)[b]	2 (25)	1 (2)	3 (6)[b]
Age < 50 years[c], *n*	36 (68)	77 (75)	7 (88)	45 (100)	25 (50)
Cut-off < 27 μV	5 (14)	4 (5)	2 (29)	2 (4)	0 (0)
Cut-off < 19 μV	2 (6)	3 (4)	2 (29)	1 (2)	0 (0)
Age > 50 years[c], *n*	17 (32)	26 (25)	1 (12)	0 (0)	25 (50)
Cut-off < 18 μV	3 (18)	4 (15)	0 (0)	0 (0)	4 (16)[b]
Cut-off < 8 μV	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: Data are presented as count (%).
Abbreviations: BMI, body mass index; HC, healthy controls; n, number; PC Published controls; SMA Spinal muscular atrophy.
[a]Based on third percentile data of the total group of the PC by Buschbacher 1999 [37].
[b]Including two patients with SMA type 3 with diabetes mellitus and decreased renal function (eGFR < 60 mL/min/1.73 m^2).
[c]Based on calculated mean − 2 SD data of the PC by Buschbacher 1999, divided over patients with BMI more or less than 24 (kg/m^2) (with higher BMI accepting lower maximum SNAP values) [37].

renal function. An abnormal maximum SNAP was most prevalent in patients with SMA types 1 and 2 younger than 50 years (8%); none of them had diabetes mellitus, renal impairment, or signs of carpal tunnel syndrome. In patients with SMA above the age of 50 years, there was no difference in abnormal maximum SNAP values between SMA types ($p > 0.05$).

Four HC (8%) had an abnormal maximum SNAP (range 11–15 μV). Proportions of abnormal maximum SNAP values did not differ between patients with SMA and HC, even when adjusted for age or BMI category (all $p > 0.05$).

The maximum SNAP was higher in patients with SMA compared with HC, as shown in Figure 1A. Within patients with SMA, there was a difference in the maximum SNAP between SMA types ($p < 0.001$), as shown in Figure 1B. The maximum SNAP was higher in patients with SMA type 2 compared with types 3/4 and controls. There was no difference in the stimulus intensity to elicit maximum SNAPs (mean difference = 0.10, 95% CI [−0.01, 0.21], $p = 0.07$) or temperature (mean difference 0.09, 95% CI [−0.29, 0.49], $p = 0.61$) between patients with SMA and HC. There was no difference in the stimulus intensity to elicit maximum SNAPs between SMA types ($p = 0.06$), but the stimulus intensity in patients with SMA type 1 was slightly higher compared with HC (mean difference = 0.34, 95% CI [0.02, 0.66], $p = 0.03$). Temperature was higher in patients with SMA type 2 compared with patients with SMA types 3/4 (mean difference = −0.62, 95% CI [−1.19, −0.05], $p = 0.03$).

Maximum SNAPs correlated with age in patients with SMA and HC, with a comparable decline over time (Figure 2). In SMA types 2a–3b/4, the decline of maximum SNAPs with age did not differ from HC, while in SMA type 1c, the decline was steeper.

Patients with SMA had a lower maximum CMAP compared with HC (mean difference = 4.4, 95% CI [2.9, 5.7], $p < 0.001$). Maximum CMAPs differed between SMA types ($p < 0.001$), with lower values for patients with SMA type 1 and 2 compared with patients with SMA types 3/4 (both $p < 0.001$). There was no correlation between maximum SNAPs and CMAPs in patients with SMA when adjusted for SMA type and age ($p > 0.05$).

We assessed maximum SNAPs in 90 out of the 103 (87%) patients after 1 year of treatment. Forty-seven patients had started treatment with nusinersen (mean follow-up interval of 14.5 months [range 13.5–19.2]) and 43 patients with risdiplam (mean follow-up interval of 10.1 months [range 8.9–14.9]). Six patients did not start any treatment (all SMA type 3), four patients stopped treatment before follow-up (one patient with SMA type 2 and three with type 3), one patient (SMA type 3) died before follow-up, and two patients (both SMA type 1) were unable to complete the follow-up. Maximum SNAPs did not differ between baseline and follow-up measurements, as shown in Figure 3. We also did not observe any differences between baseline and follow-up measurements within subgroup analyses for therapy or SMA types (all $p > 0.05$).

4 | Discussion

In this study, we show that the majority (94%) of adolescents and adults with SMA types 1c–4 have normal maximum SNAP values. Interestingly, patients with SMA exhibited higher maximum SNAPs compared with HC, particularly driven by higher values observed in patients with SMA type 2. The maximum SNAP showed an age-related decline in both patients with SMA and HC, with patients with SMA type 1c demonstrating a steeper decline compared with all others. Maximum SNAP values remained stable over approximately 1 year of treatment. Our data support the sensory integrity of the median nerve in adolescents and adults with SMA types 1c–4, even in those with longstanding disease.

The classic pathological hallmark of SMA is motor neuron degeneration resulting in muscle weakness, without sensory symptoms [44]. This is surprising, given the ubiquitous expression of SMN protein throughout various regions of the nervous system, albeit to a lesser extent in the dorsal root ganglia compared with the brain and spinal cord [45, 46]. In addition, a clinical phenotype of motor neuron disease combined with sensory neuropathy may initially suggest alternative diagnoses such as non-5q SMA or spinal and bulbar muscular dystrophy [47–49]. However, more recent (pre)clinical studies have suggested that

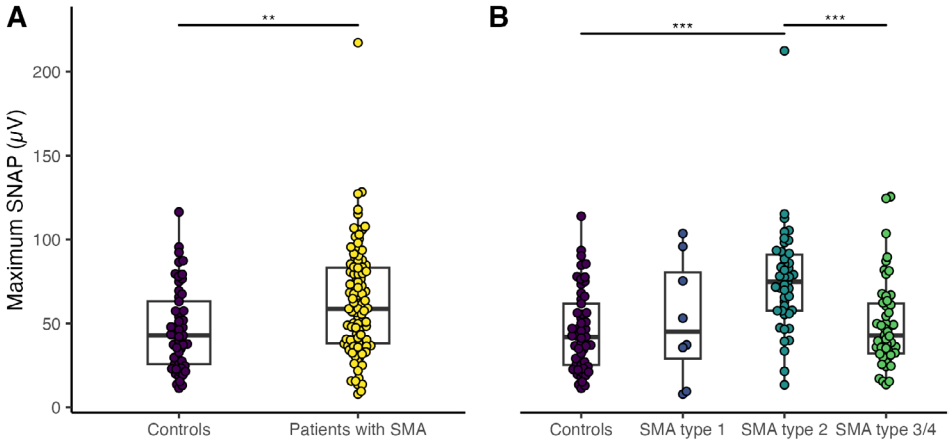

FIGURE 1 | Maximum SNAP values of patients with SMA and HC. (A) Boxplots of maximum SNAP values in patients with SMA at baseline and HC. Colored dots represent individual data points. Patients with SMA had higher maximum SNAPs compared with HC (mean difference = 16, 95% CI [6, 26], $p = 0.002$). (B) Boxplots of maximum SNAP values within patients with SMA based on types and HC. Colored dots represent individual data points. Patients with SMA type 2 had higher maximum SNAPs compared with types 3/4 (mean difference = 26, 95% CI [12, 41], $p < 0.001$) and HC (mean difference = 31, 95% CI [16, 46], $p < 0.001$). HC, healthy controls; SMA, spinal muscular atrophy; SNAP, sensory nerve action potential. $^{**}p < 0.01$, $^{***}p < 0.001$.

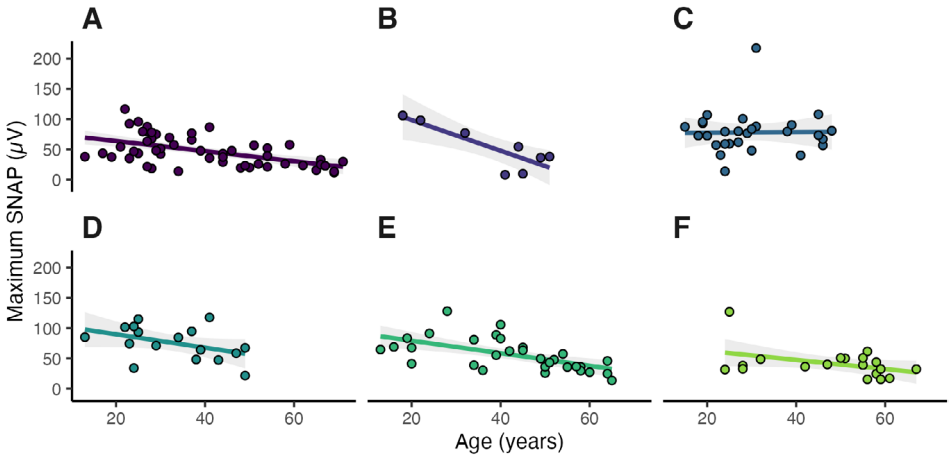

FIGURE 2 | Relationship between age and maximum SNAP values in patients with SMA based on SMA subtypes and HC. Scatterplots illustrating the relationship between age and maximum SNAPs in patients with SMA categorized by SMA subtypes and HC. Colored dots represent individual data points. Solid lines represent the linear regression line fitted to the individual data points within each panel. The shaded light gray area represents the 95% CI of the regression line. (A) HC: $\beta = -0.83$, 95% CI [−1.19, −0.48], $p < 0.001$; (B) SMA type 1c: $\beta = -1.71$, 95% CI [−3.27, −0.14], $p = 0.033$; (C) SMA type 2a: $\beta = 0.88$, 95% CI [−0.17, 1.93], $p = 0.101$; (D) SMA type 2b: $\beta = -0.27$, 95% CI [−1.49, 0.94], $p = 0.659$; (E) SMA type 3a: $\beta = -0.20$, 95% CI [−0.93, 0.52], $p = 0.582$; (F) SMA type 3b/4: $\beta = 0.08$, 95% CI [−0.86, 1.02], $p = 0.860$. In the total group of patients with SMA, maximum SNAPs had a decline with age comparable to controls ($\beta = -1.15$, 95% CI [−1.54, −0.77], $p < 0.001$). This decline was also observed in patients with SMA types 2b–3b/4, whereas in SMA type 2a, maximum SNAPs did not decline with age. In contrast, patients with SMA type 1c showed a steeper decline of maximum SNAPs with age compared with HC. CI, confidence interval; HC, healthy controls; SMA, spinal muscular atrophy; SNAP, sensory nerve action potential.

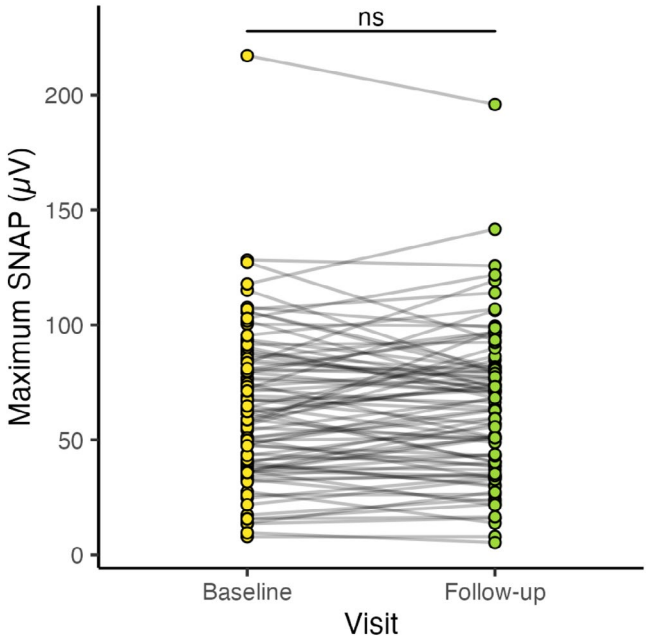

FIGURE 3 | Maximum SNAP values at baseline and follow-up in patients with SMA. String graph of maximum SNAP values of patients with SMA at baseline and follow-up. Colored dots represent individual data points and strings represent longitudinal changes within each patient. There was no difference in the mean maximum SNAP between baseline (63 μV) and follow-up (63 μV) measurements (mean difference = −1, 95% CI [−4, 3], $p = 0.711$) in patients with SMA after 1 year of treatment with *SMN2*-splicing modifying therapy. ns, not significant ($p > 0.05$); SMA, spinal muscular atrophy; SMN2, survival motor neuron 2 gene; SNAP, sensory nerve action potential.

motor neuron degeneration is not an isolated feature but is preceded by widespread dysfunction of the motor-sensory system including changes in neuromuscular junction function, axonal hyperexcitability, and decreased synaptic efficacy between efferent and afferent fibers. Nevertheless, our data show a clear difference between motor and sensory functions of the median nerve. There are few other electrophysiological studies that assessed the integrity of sensory function across the severity spectrum of SMA (Table S1). One study of sensory function of the arms also confirmed sensory integrity of median and ulnar nerves in 10 patients with SMA type 2 [26]. However, studies of sural nerve SNAPs in the legs yielded conflicting findings, ranging from normal SNAPs and conduction properties in patients with SMA type 2 [26] to absent SNAPs in patients with SMA types 2 and 3 [27]. Consistent with our findings in patients aged 50 years and younger, abnormal sural nerve SNAPs and conduction properties were most often found in patients with SMA type 1 [26, 27]. Nonetheless, it is important to consider that both study designs are retrospective and encompass either a limited sample size and/or include only young patients. Additionally, the study reporting absent sural SNAPs in patients with SMA types 2 and 3 does not provide further information on whether the remaining measured SNAPs fall within normal ranges [27].

Two patients with SMA type 1 (29%) and two patients with SMA type 2 (4%) had an abnormal maximum SNAP value of the median nerve. These findings are consistent with previously reported case series (Table S1). The other patients with SMA type 1 (all type 1c) and type 2 had normal electrophysiological sensory function of the median nerve. Both SMA type 1 and 2 imply low levels of SMN protein expression, specifically SMA type 1c, which might predispose them to innate structural abnormalities

of the afferent fibers of the median nerve. Absent SNAPs, slowed sensory conduction velocities, and marked axonal loss in sural nerve biopsies have been reported in babies with SMA type 1 [18, 22–26, 28, 29]. Absent SNAPs have been reported only for the sural, but not the median nerve, in SMA type 2 [27]. However, a broad range of abnormalities of the sensory system such as reduced size of sensory neurons and dorsal root ganglia [50], a decrease in myelinated dorsal root axons and sensory fibers going into the ventral horn [16], and a reduced number of synapses onto motor neurons originating from proprioceptive neurons [16, 17, 50], have been reported in mouse models of severe SMA, suggesting that involvement of the sensory compartments in SMA is confined to the more severe forms of SMA. Furthermore, patients with SMA type 1c showed a steeper decline of SNAP with age, which suggests vulnerability of sensory function with increasing age. However, this was not observed in the other SMA types. The only patients with abnormal maximum SNAP values in patients aged above 50 years were four patients with SMA type 3 (16%). Comorbidities, including diabetes mellitus, high BMI ($\geq 24\,kg/m^2$), and impaired renal function may have contributed to the lower SNAPs in at least two of these patients, and when corrected for BMI their maximum SNAPs could be defined as normal.

We found higher maximum SNAPs in patients with SMA compared with HC. This difference was predominantly driven by patients with SMA type 2. Notably, these patients were younger than both patients with SMA types 3/4 and HC, which may also explain the less apparent age-related decline in the SMA type 2a subgroup. In addition, the skin temperature during assessments was slightly higher in patients with SMA type 2, which is known to potentially increase SNAP amplitudes [51].

We did not detect a reduction in maximum SNAPs as a sign of toxicity that was reported in animals treated with *SMN1*-gene therapy [52, 53]. Notably, such complications have not been reported in patients receiving *SMN2*-targeting treatments.

There are several limitations of our study. This study was part of an extensive electrophysiological protocol primarily focused on motor assessments of the median nerve. Consequently, we limited our analysis to the maximum SNAP due to considerations of time, tolerability, and feasibility within the context of the broader protocol. A more comprehensive evaluation of potential sensory neuropathy in this cohort of patients with longstanding disease would have benefited from the inclusion of additional nerves. Previous studies have reported abnormalities in other nerves, such as the sural nerve, particularly in patients with milder SMA types [27]. Moreover, additional sensory electrophysiological variables such as latency and conduction velocity were not included in this analysis. Even when maximum SNAP amplitudes appear normal, subtle changes in these parameters could reveal subclinical sensory alterations as reported previously [22, 25, 26, 29].

Our findings indicate preserved sensory integrity of the median nerve in the large majority of a representative cohort of adolescents and adults with SMA, including those with longstanding disease, with no significant changes observed after 1 year of *SMN2*-splicing modifying treatment. The reasons for this apparent resilience of sensory neurons in the median nerve, and whether this extends to other peripheral nerves, particularly in the lower limbs, require further investigation.

Author Contributions

Leandra A. A. Ros: data curation, investigation, formal analysis, visualization, project administration, writing – original draft, writing – review and editing, methodology. **Boudewijn T. H. M. Sleutjes:** conceptualization, investigation, methodology, project administration, resources, software, supervision, validation, writing – original draft, writing – review and editing. **H. Stephan Goedee:** writing – review and editing. **Fay-Lynn Asselman:** writing – review and editing. **Inge Cuppen:** writing – review and editing. **Ruben P. A. van Eijk:** methodology, validation, writing – review and editing. **W. Ludo van der Pol:** conceptualization, methodology, project administration, supervision, validation, writing – review and editing. **Renske I. Wadman:** conceptualization, funding acquisition, methodology, project administration, supervision, validation, writing – original draft, writing – review and editing.

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Ethics Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

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