Comparison of Late Potentials for 24 Hours between Brugada Syndrome and Arrhythmogenic Right Ventricular Cardiomyopathy Using a Novel Signal-Averaging System Based on Holter Electrocardiogram

Running title: Abe et al.; Comparison of LPs for Brugada syndrome and ARVC

Atsuko Abe, MD1; Kenzaburo Kobayashi, MD1; Hitomi Yuzawa, MD1; Hideyuki Sato, MD1; Shunji Fukunaga, MD1; Tadashi Fujino, MD1; Yoshifumi Okano, MD1; Junichi Yamazaki, MD1; Yosuke Miwa, MD2; Hideaki Yoshino, MD2; Takanori Ikeda, MD1

1Dept of Cardiovascular Medicine, Toho University Faculty of Medicine; 2Second Dept of Internal Medicine, Kyorin University School of Medicine, Tokyo, Japan

Corresponding author:
Takanori Ikeda, MD, PhD
Department of Cardiovascular Medicine
Toho University Faculty of Medicine
6-11-1 Omorinishi, Ota-ku,
Tokyo 143-8541, Japan
Tel: +81-3-3762-4151
Fax: +81-3-3766-7810
E-mail: ikety3@gmail.com

Journal Subject Codes: [5] Arrhythmias, clinical electrophysiology, drugs; [171] Electrocardiology
Abstract:

**Background** - Late potentials (LP) detected with signal-averaged electrocardiograms (SAECGs) are known as being useful for identifying patients at risk of Brugada syndrome (BS) and arrhythmogenic right ventricular cardiomyopathy (ARVC). As the pathophysiology is clearly different between these disorders, we clarified the LP characteristics of these disorders.

**Methods and Results** - This study included 15 BS and 12 ARVC patients and 20 healthy controls. All BS patients had characteristic electrocardiogram changes and symptomatic episodes. All ARVC patients had findings that were consistent with recent criteria. Three LP parameters (fQRS, RMS40, and LAS40) were continuously measured for 24 hours using a novel Holter-based SAECG system. The incidences of LP determination in BS (80%) and ARVC patients (91%) were higher than in healthy controls (5%; $P<0.0001$ in both), but did not differ between BS and ARVC patients. In BS patients, dynamic changes of all LP parameters were observed and they were pronounced at nighttime. On the contrary, these findings were not observed in ARVC patients. When values of the standard deviation of three LP parameters (fQRS, RMS40, LAS40) over 24 hours were compared for the two patient groups, those values in BS patients were significantly greater than those in ARVC patients ($P<0.0001$ in all).

**Conclusions** - LP characteristics detected by the Holter-based SAECG system over 24 hours differ between BS and ARVC patients. Dynamic daily variations of LPs were only seen in BS patients. This may imply that mechanisms of lethal ventricular arrhythmia in BS may be more correlated with autonomic abnormality than that of ARVC.

**Keywords:** Brugada syndrome, arrhythmogenic right ventricular cardiomyopathy, late potentials, signal-averaged electrocardiogram, Holter monitoring
Introduction

The Brugada syndrome (BS)\(^1,2,3\) which is characterized by a pattern of right bundle branch blocks and ST-segment elevations in leads V\(_1\) and V\(_2\) on an electrocardiogram (ECG), is considered to be a primary electrical disease of the heart caused by a defect in the ion channel gene, resulting in abnormal electrophysiological activity in the right ventricle and a propensity to malignant ventricular arrhythmias. On the other hand, arrhythmogenic right ventricular cardiomyopathy (ARVC)\(^4,5\) is a primary myocardial disease, often familial, that is characterized histologically by structural abnormalities of the right ventricle due to replacement by fibrous and fatty tissue. The clinical manifestation of this disease is lethal ventricular arrhythmias which occasionally cause cardiac sudden death due to a degenerated myocardium. With both disorders, although their pathophysiology is clearly distinct, it has been reported that a conduction delay in the right ventricle plays an important role for arrhythmogenesis.\(^4-6\) Several studies\(^7-10\) have reported an overlap between the characteristics for BS and ARVC, such as ECGs similar to those with BS for ARVC patients with a sodium channel blocker\(^7\) and fibro-fatty replacement similar to that for ARVC found in right ventricular biopsies in BS patients.\(^8,9\)

Late potentials (LPs) detected by signal-averaged electrocardiograms (SAECGs), which reflect a conduction delay, have been widely utilized to detect high-risk individuals among patients with cardiac disorders such as myocardial infarctions, BS,\(^11,12\) and ARVC.\(^13-15\) At present, it is possible to monitor LPs continuously for 24 hours using a newly developed SAECG system that is applied to Holter ECG recordings.\(^16\)

In the present study, we assessed the value of monitoring LPs continuously for 24 hours using the developed signal-averaging system in order to compare daily varying LP characteristics and to investigate the clinical differentiation of arrhythmogenesis for BS and ARVC.
Methods

Patient Enrollment

**BS patients**

Fifteen patients (13 men and 2 women; mean age 42 ± 12 years) who were referred to our medical centers between 2005 and 2011 were enrolled. All the patients showed typical Brugada-type ECGs (i.e., a coved pattern of ST-segment elevations in the V₁ and V₂ leads) including transiently spontaneous changes during the sinus rhythm on 12-lead ECGs. In other words, patients who only had the saddle-back pattern were excluded from the study. Additionally, all BS patients had a history of syncope or life-threatening arrhythmic events. Before diagnosing BS, we excluded patients who had evidence of organic heart disease, including ARVC, myocardial ischemia of the right ventricle, and other infiltrative cardiomyopathies. These diagnoses were based on the Report of the Second Consensus Conference in 2005 using echocardiography, coronary angiography with acetylcholine, other diagnostic techniques such as scintigraphy, helical CT scanning, and magnetic resonance imaging (MRI), and endomyocardial biopsies in some cases. As a result, all BS patients studied were believed to have structurally normal hearts.

**ARVC patients**

During the same period, we enrolled 12 patients (9 men and 3 women; mean age 33 ± 12 years), who had right ventricular dilatation determined using imaging modalities and diagnosed with ARVC as their clinical findings fulfilled the Task Force Criteria of 2010. All patients underwent 12-lead ECGs, echocardiography, other diagnostic techniques such as scintigraphy, helical CT scanning, and MRIs, as well as those for BS. The dilatation and reduced wall motion in the right ventricle were observed in all ARVC patients. In addition, all ARVC patients underwent
endomyocardial biopsies for the diagnosis and to rule out ARVC phenocopies like cardiac sarcoidosis. We excluded patients who did not have either documented ventricular arrhythmias or syncopal episodes.

Before enrollment of patients with both cardiac disorders, informed consent was obtained from each patient. The study was approved by the Ethics Committees of our institutes.

**Control subjects**

Twenty healthy control subjects (16 men and 4 women; mean age 40 ± 11 years) who had routine health care examinations at health care department of our institutes between 2006 and 2008 were also included in the study. They are matched for age and gender to BS patients. All control subjects underwent a 24-hour Holter ECG to measure LPs for 24 hours as same as two patient populations.

**Measurements**

**Detection of Continuous LPs by Signal-Averaging System**

In this study, LPs were analyzed for 24 hours using a newly developed signal-averaging system (SCM-6600, Fukuda Denshi Co., Ltd. Tokyo), which is capable of analyzing LPs automatically every 30 minutes using data from a digital Holter ECG recorder (FM-180). This system was developed using the same algorithm as is applied in FDX-6500 from Fukuda Denshi Co., Ltd and now commercially available in Japan.

This analysis is based on the quantitative time-domain measurements of the filtered vector magnitude of the orthogonal X, Y, and Z leads. The QRS complexes (> 200 beats) were amplified, digitized, averaged, and filtered with a high pass filter (40 Hz). Three parameters were assessed using a computer algorithm: the filtered QRS duration (fQRS); the root mean square voltage of the terminal 40 msec of the filtered QRS complex (RMS₄₀); and the duration of...
low-amplitude signals (< 40µV) in the terminal, filtered QRS complex (LAS_{40}). These three parameters were assessed using a computer algorithm, every 30 minutes for 24 hours and the values for the parameters were presented on a trend graph covering the 24 hours.

Prior to the present study, we did a preliminary study to assess the correlation between the Holter-based SAECG (SCM-6600) and standard SAECG (FDX-6500) that was used the same method. Twenty subjects including both 10 patients (3 with dilated cardiomyopathy, 5 with coronary artery disease, and 2 with BS) and 10 healthy controls were measured using the two SAECG manufactures simultaneously. No subjects demonstrated ventricular conduction disturbance such as bundle branch block and persistent atrial tachyarrhythmias on the 12-lead ECGs. First, all 20 subjects had the Holter-based SAECG examination for 24 hours and then underwent the standard SAECG examination in the supine position in the same day. Three parameters (fQRS, RMS_{40}, and LAS_{40}) for LP determination are measured and compared between two manufactures in the same time period by Pearson product-moment correlation coefficient as statistical analysis. The correlation coefficient for fQRS, RMS_{40}, and LAS_{40} between two measurements in all subjects were 0.98 (P < 0.0001), 0.97 (P < 0.0001), and 0.95 (P < 0.0001), respectively. Consequently, we concluded that there is the correlation of three LP parameters between the standard SAECG and Holter-based SAECG. Kelen et al.17 supports results of our preliminary study.

Through the use of this system, we assessed daily variations in LP parameters in 15 BS patients and 12 ARVC patients. All LP acquisitions were done without the use of antiarrhythmic drugs. The LPs were considered positive when any one of three criteria (fQRS > 135 msec, RMS_{40} < 15 µV, and LAS_{40} > 39 msec)\textsuperscript{16,18,19} were met, with the maximum values over 24 hours.
Statistical analysis

Data of age in each group are expressed as mean ± standard deviation (SD). Data of LP parameters are presented as the median with first and third quartiles (25% - 75%). Differences between the BS patients, ARVC patients, and control subjects were examined using an extension of a Fisher’s exact probability test for a $3 \times 2$ table for categorical data (i.e., the incidence of LPs) and a Kruskal-Wallis test as the nonparametric analysis for continuous data (i.e., numerical values of fQRS, RMS$_{40}$, and LAS$_{40}$). If the global test is significant among the 3 groups, we conduct pair-wise comparisons using the Bonferroni procedure that adjusts for multiple comparisons. A value of $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using a statistical software, SPSS version 15.0 for Windows.

Results

Characteristics of BS and ARVC patients

Characteristics of the 15 BS and 12 ARVC patients are summarized in Table 1. With regard to the features of the 12-lead ECGs, a coved pattern of ST-segment elevations was demonstrated in all BS patients. An epsilon wave was documented in 4 of the ARVC patients.

All patients in both groups had a history of at least one event which was defined as either a ventricular arrhythmia (8 in BS patients and in all ARVC patients) or syncopal episode (11 in BS patients and 10 in ARVC patients). In 8 BS patients with ventricular arrhythmias, ventricular fibrillation (VF) or polymorphic ventricular tachycardia (VT) was documented, but sustained monomorphic VT was not. On the contrary, in 10 of 12 ARVC patients with ventricular arrhythmias, sustained monomorphic VT was documented by ambulatory monitoring ECGs and/or 12-lead ECGs, and the remaining 2 patients had either VF or polymorphic VT.
In consideration of the time of events, 11 (73%) of the BS patients had events at night or in the early morning (22:00 to 08:00). No life-threatening events occurred during exercise for the BS patients. Conversely, all 12 ARVC patients had events during the day (08:00 to 22:00). Events occurred during physical exercise for 6 (50%) of the ARVC patients.

**Comparisons of LP parameters**

Test results of LP parameters in 15 BS and 12 ARVC patients and control subjects are shown in Table 2. For BS patients, LPs were determined in 12 patients (80%). For ARVC patients, LPs were identified in 11 patients (91%). In healthy controls, LPs were identified in only one subject (5%). The incidences of LP determination were significantly different among three groups. Subsequently, pair-wise comparisons between BS patients and ARVC patients and control subjects were performed. The incidences of LP determination in BS and ARVC patients were higher than that in healthy controls ($P < 0.0001$ in both), but did not significantly differ for both BS and ARVC patients.

The numerical value of fQRS, RMS$_{40}$ and LAS$_{40}$ for LP determination were significantly different among three groups. Subsequently, pair-wise comparisons between BS patients and ARVC patients and control subjects were performed. The numerical value of fQRS in BS patients was significantly lower than that in ARVC patients ($P = 0.0004$), but the values of RMS$_{40}$ and LAS$_{40}$ did not differ between both patients. When compared between BS patients and control subjects, the values of fQRS, RMS$_{40}$ and LAS$_{40}$ in BS patients were significantly greater than those in healthy controls ($P < 0.0001$ in all).

**Daily Variation of LP parameters**

In all BS patients, dynamic daily variations of LP parameters were observed. Figure 1 shows a representative example of the trend graphs of each LP parameter (fQRS, RMS$_{40}$, LAS$_{40}$) in a BS
patient. All parameters fluctuated over 24 hours, and measured values of LP parameters increased at night and decreased during the day. In this patient, the maximum LP values are recorded at 03:00-05:00 (middle of the night) and minimum values at 12:00-14:00 (afternoon). In 11 (73%) of the 15 BS patients, similar trend graphs were recorded. LPs were identified and events occurred at night in these 11 patients. In contrast, the dynamic daily changes of LP parameters were not observed in any ARVC patients (Fig. 2), whereas LPs were identified in most (91%) of the ARVC patients.

Since LP parameters showed dynamic change in BS patients, we calculated the SD of continuous values of QRS, RMS40, and LAS40 from each patient over 24 hours (i.e., daily variation of the parameters) and compared these values for the three groups (Table 2). The SD of a patient’s observations over 24 hours of each LP parameters was significantly different among three groups. We did pair-wise comparisons between BS patients and ARVC patients and control subjects. All SD values for BS patients were significantly higher than those for ARVC patients ($P < 0.0001$ in all). We also compared each value between BS patients and control subject. Similarly, all SD values of BS patients are significantly higher than control subjects ($P < 0.0001$ in all).

**Discussion**

The major finding of the present study was to document the different dynamics of LPs detected by SAECG over 24 hours, for both BS and ARVC. Although the incidences of LPs that reflect depolarization abnormalities or conduction delays were high for both disorders, BS patients had daily variations of LPs with nighttime ascendancy, but ARVC patients did not. This finding may reveal that the pathophysiology of lethal ventricular arrhythmias differ for both BS and ARVC.
The pathophysiological mechanism of LPs in BS patients with structurally normal heart may be closely associated with both depolarization abnormality and autonomic modulation. The characteristic 12-lead ECG features of BS are dynamic and often concealed. Modulation abnormalities of the autonomic system may directly affect the ECG properties and increase vulnerability to fatal arrhythmias. Several reports showed, for BS, ST-segment elevations on 12-lead ECGs have diurnal and/or daily variations; therefore it is considered that the occurrence of events may also have circadian and/or daily variations. Matsuo et al. analyzed the circadian pattern of 30 VF episodes in 12 BS patients who underwent an implantable cardioverter defibrillator treatment, and reported that VF occurred more frequently at night (93%) than during the day (7%). In this study, 73% of episodes of BS patients occurred at night or in the early morning. The remaining 27% of episodes occurred during the daytime. Of note, no patients had life-threatening events during exercise. The circadian pattern of the events in our population was similar to those of previous studies.

In contrast, islands of fibro-fatty tissues generate the arrhythmogenic substrate and form an electrical reentrant circuit for malignant ventricular arrhythmias responsible for serial events in ARVC patients. These arrhythmias are typically induced by adrenergic stimulation such as a catecholamine infusion or physical exercise. It has been shown that physical exercise is a precipitating factor for lethal ventricular arrhythmias in patients with ARVC. Corrado et al. reported sudden cardiac death happened among young ARVC patients and Leclercq et al. documented VT on a 24-hour Holter ECG after an autonomic imbalance of increasing sympathetic tone in ARVC patients. Furthermore, it has been reported that adrenergic stimulation, an isoproterenol infusion mimicking the catecholamine effect, induced VT with ARVC. Succinctly, VT with ARVC is due to sympathetic stimulation, but not vagal tone. In this
study, the events during the day, when physical activity is high, occurred in all the ARVC patients. Conversely, none of the patients had an event at night. The events occurred during physical exercise for 50% of the ARVC patients.

The presence of LPs is an established marker that is useful for risk stratification in patients with structurally abnormal hearts, for example, patients having had a myocardial infarction. Several clinical studies support that LPs are a strong predictor for risk stratification of malignant arrhythmias with BS as well. However, with ARVC, the clinical values are still controversial as to whether they are markers for the pathophysiological substrate, namely fibro-fatty substrate, or the index of vulnerability to ventricular arrhythmias. The relationship between the features of ECGs (epsilon wave and inverted T wave in leads V1 and V2) and LPs is still unknown.

Assessing LPs by SAECGs over 24 hours for BS patients who have been identified as having a high risk of lethal ventricular arrhythmias, a dynamic manner of changes for LP parameters were found during this study. These dynamic changes are accentuated at night, and all the LP parameters at night are greater than during the day. In contrast, all the values for LP parameters for ARVC over 24 hours did not fluctuate largely as they did in BS patients. The SD values of the LP parameters over 24 hours for the ARVC patients are significantly less than those for BS patients. ARVC patients who were identified as being at high risk for ventricular arrhythmias were considered as being less likely to be influenced by autonomic balance in comparison with BS patients, as no clear peak for either night or day was observed. Therefore, the role of LPs in ARVC may be as a pathophysiological substrate rather than electrical instability. The analysis of LPs over 24 hours may play an important role as a noninvasive technique for diagnosing both disorders in their early stages.
Clinical implications

In identifying patients with BS and ARVC at high-risk for serious arrhythmic events, noninvasive methods are more desirable than invasive or genetic methods. Our study shows that, for asymptomatic BS patients, the detection of LPs using the SAECG system with a 24-hour Holter ECG could be a useful technique for identifying the subgroup at high risk for arrhythmic events. If such patients have dynamic changes of LPs over 24 hours, with night ascendancy, they could be considered candidates for an implantable defibrillator.

One application of the information from this study would be to consider doing SAECG using commercial non-Holter-based SAECG at two times; one at middle of the night and the other at afternoon. These are the times reported at maximum daily changes of LPs in this study. However, the Holter-based SAECG would be necessary when we assess daily variations of LPs in BS patients because BS patients are awake (not sleeping) to undergo the standard SAECG and have the same situation as daytime.

Study limitations

In this study, we only analyzed LPs as the tool to determine electrophysiological abnormalities and did not realize the value of other markers such as electrophysiological testing, drug infusion tests, and genetic analysis, which were assessed in previous studies.

Since we have not investigated daily variations of LPs in the terms of arrhythmic event, an additional or follow-up study will be necessary to determine the prognostic significance of LP variability for stratifying patients at risk in BS patients.

Conclusions

The LP characteristics detected using SAECG over 24 hours differ between BS and ARVC
patients. LPs in BS patients dynamically change during the day, whereas this is not the case for ARVC patients. It may imply that the mechanisms of lethal ventricular arrhythmias in BS patients are more correlated to autonomic abnormalities than for ARVC patients.

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Conflict of Interest Disclosures: None.

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### Table 1. Characteristics of BS and ARVC patients enrolled in this study

<table>
<thead>
<tr>
<th>Variables</th>
<th>BS patients (n=15)</th>
<th>ARVC patients (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD) (yr)</td>
<td>42 ±12</td>
<td>33 ± 12</td>
</tr>
<tr>
<td>Gender, male</td>
<td>13 (87%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Family history of SCD</td>
<td>3 (20%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>ECG features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coven pattern ST elevation</td>
<td>15 (100%)</td>
<td>---</td>
</tr>
<tr>
<td>Epsilon wave</td>
<td>---</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>History of events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syncope</td>
<td>11 (74%)</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>Aborted sudden death</td>
<td>4 (27%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Document of ventricular arrhythmias</td>
<td>8 (53%)</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>VF and/or polymorphic VT</td>
<td>8 (53%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Sustained VT</td>
<td>0 (0%)</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>Time of events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>4 (27%)</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Nighttime</td>
<td>11 (73%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

VF indicates ventricular fibrillation; VT, ventricular tachycardia
Table 2. Comparisons of LP parameters for BS and ARVC patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>BS patients (n=15)</th>
<th>ARVC patients (n=12)</th>
<th>Control subjects (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determinate LP</td>
<td>12/15 (80%)</td>
<td>11/12 (91%)</td>
<td>1/20 (5%)</td>
<td>0.003</td>
</tr>
<tr>
<td>fQRS (msec)</td>
<td>147 (139 - 150)†‡</td>
<td>160 (156 - 163) †</td>
<td>122 (118 - 125)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RMS₄₀ (µV)</td>
<td>10.8 (8.2 - 16.8)†</td>
<td>11.5 (10.4 - 12.9)†</td>
<td>26.5 (18.2 - 32.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LAS₄₀ (msec)</td>
<td>47.0 (42.5 - 50.5)†</td>
<td>47.3 (44.0 - 54.0) †</td>
<td>35.3 (30.0 - 36.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD over 24 h of fQRS (msec)</td>
<td>4.52 (3.41 - 5.72)‡§</td>
<td>1.26 (0.89 - 1.68)</td>
<td>1.73 (1.50 - 2.44)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD over 24 h of RMS₄₀ (µV)</td>
<td>2.81 (2.44 - 4.05)‡§</td>
<td>0.89 (0.83 - 1.02)</td>
<td>1.22 (0.98 - 1.41)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD over 24 h of LAS₄₀ (msec)</td>
<td>4.50 (4.14 - 4.96)‡§</td>
<td>1.37 (1.37 - 1.97)</td>
<td>1.39 (1.20 - 1.59)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

LP parameters are shown as medians with 25% - 75% values. SD indicated standard deviation. P values attained by a single test that compares three groups simultaneously. These were done using an extension of Fisher’s exact test for a 3×2 table for categorical data and a Kruskal-Wallis test for continuous data.

* P < 0.0001 by a Fisher’s exact test comparing control subjects.
† P < 0.0001 by the Bonferroni procedure as pair-wise comparisons comparing control subjects.
‡ P = 0.0004 and § P < 0.0001 by the Bonferroni procedure as pair-wise comparisons comparing ARVC patients.

Figure Legends:

Figure 1. Dynamic change of LP parameters used for LP determination of a BS patient. A: fQRS, B: RMS₄₀, C: LAS₄₀, D: filtered QRS complex at time of minimum value of fQRS, E: filtered QRS complex at time of maximum value of fQRS. The arrows on panels A, B, and C indicate maximum and minimum values of each parameter. The three parameters dynamically change and the maximum values are recorded at night.

Figure 2. No dynamic change of LP parameters used for LP determination of an ARVC patient. The three parameters are almost fixed throughout the 24 hours.
A. $f_{QRS}$

B. RMS

C. $LAS_{40}$

D. $f_{QRS}$: 136 ms
RMS$_{40}$: 14 $\mu$V
LAS$_{40}$: 48 ms

E. $f_{QRS}$: 155 ms
RMS$_{40}$: 8 $\mu$V
LAS$_{40}$: 60 ms
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