

## **Supplementary Information**

### **Adult-onset Epilepsy in Klinefelter syndrome? Cognitive and neurophysiological evaluation with HD-EEG and high-frequency oscillations**

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Abbreviations:

KS= Klinefelter's syndrome, HFOs= high-frequency oscillations, EEG= electroencephalogram, HD-EEG= high-density electroencephalogram, EMU= epilepsy monitoring unit.

## Supplementary Section: Methods

### EEG examinations

Video EEG was recorded using Micromed System S.p.A (Mogliano, Italy) with SystemPlus Evolution and a SD LTM 64 Express Amplifier. Twenty-nine electrodes were applied, and the EEG was recorded with a bipolar montage. A sampling rate of 256 Hz was used.

HD-EEG was recorded with a 1000 Hz sampling rate using GES 400 Series Geodesic EEG Systems with NetStation 5.0 Geodesic EEG software and an EGI Net Amps 300 Amplifier. A Hydrocel Geodesic Sensor Net with 256 electrodes was applied as well as differential EMG on the chin using EGI Physio 16 MR.

During the HD-EEG in the morning and during the video EEG in the afternoon of the same day a cognitive testing was conducted.

### Psychological and cognitive tests

We used Beck Depression Inventory (1) and the Morningness-Eveningness Questionnaire (2).

The applied battery of tests included a daily questionnaire and five cognitive tasks: a motor task (Finger Tapping), a word-pair association (Word Pair) task, a spatial navigation virtual reality (Virtual Town) task, and two executive function tasks (Go/No-Go and Stroop).

The morning session recorded with HD-EEG started with a 1- minute resting phase. Cognitive testing included a learning part, followed by a 10-minute resting pause with eyes closed, and a recall and recognition part. The afternoon session recorded with video EEG took place in the EMU. We repeated the cognitive tasks using the same battery, but different versions. Each task included an initial training phase with written, step-wise instructions on the screen, to help the patient to familiarize with the task. The tasks were prepared with the 'Neurobehavioral Systems' Presentation (Version 18.1 Build 02.01.15) and they were presented in a standard 17'' screen. The participant was asked to respond via keyboard.

The two-part Finger Tapping task included 12 learning trials, and five recall trials. Each trial lasted 30 seconds with intertrial pauses of the same duration. The participants were instructed to repeatedly type as fast and accurately as possible a presented 5-digit sequence that included numbers associated with the four fingers index to pink using their non-dominant hand.

The two-part Word Pair Task included 60 pairs of German nouns presented in constant order. Of these word-pairs, 30 had an obvious semantic relationship (e.g. *apple juice - glass*), and 30 had no obvious relationship (e.g. *sky - book shelf*). In the first part, participants were

instructed to memorize each presented word-pair and report the presence or absence of a semantic relationship. In the second part, the first word of each word-pair was presented, and participants were supposed to recall the matching second word. Each recall trial was followed by a recognition trial providing with three options to choose the correct word.

The two-part Virtual Town task consisted of 10 town scenes presented before a road-turn. Each scene showed an eye-catching main object (e.g. a building), and at least one less accentuated side objects. In the first part, participants were asked to navigate through the town and memorize object details as well as allocentric and egocentric spatial information. The second part included a free recall of the memorized object information followed by a recognition trial including a 25-item true-or-false questionnaire on the presented scenes.

The Go/No-Go task included 70 trials. The participants were instructed to respond as rapidly as possible by pressing a key whenever a target stimulus (go) was presented (57,14% of trials), and not respond to an infrequently presented non-target stimulus (no-go) (42,86% of trials).

In the Stroop task, the color words “red”, “blue”, “violet” and “yellow” were displayed in a font color congruent or incongruent with the word meaning. The task comprised 16 congruent and 48 incongruent trials. Participants were instructed to respond to the font color by pressing an associated key.

During the Word Pair and Virtual Town tasks, the participants’ responses were logged in by the experimenter in order to reduce movement artefacts.

## **Control group**

The control group for the Finger Tapping, Word Pair, and Virtual Town tasks consisted of 104 patients who were admitted to the EMU and who participated in another larger study on the effect of seizures on memory.

The control group was on average 34.5 years old (SD=15.32), 50 participants were male. For the purpose of matching the control group to the presented case, we selected only male patients who were older than 40 years, which resulted in a sample of 18 males who were on average 58.17 years old (median= 55; SD=8.81), all were right-handed, in accordance the patient's right-handedness. This matched sub-sample had an average score of 57.13 (SD=13.64) on the morningness-eveningness questionnaire and an average score of 11.47 (SD= 10.36) on the Beck Depression Inventory. Among these patients, two did not have epilepsy, six cases were uncertain, and 10 had an ascertained diagnosis of epilepsy. The epilepsy cases were temporal lobe epilepsy (N=2), focal epilepsy of unknown onset (N=6), juvenile myoclonic epilepsy (N=1), and frontal lobe epilepsy (N=1).

## **Behavioral analysis**

We counted correctly remembered words for the Word Pair task, correctly remembered details for the Virtual Town task, and speed as seconds per sequence as well as accuracy in percent of correctly typed sequences for the Finger Tapping task. We counted correct responses and calculated average and standard-deviation of reaction time to matching and interfering trials for the Stroop task. We counted correct responses (go) and false responses (no-go) as well as reaction time for these two categories for the Go/No-Go task.

## **Clinical evaluation**

For clinical evaluation of both standard video EEG and HD-EEG data were reviewed using a bipolar montage.

## **HFO analysis**

EEG analysis was done using the software MEEGIPS (Modular EEG Investigation and Processing System for visual and automated detection of HFOs [\(3\)](#)). The software package can be obtained via email request to [meegips@pmu.ac.at](mailto:meegips@pmu.ac.at). It is provided as binary application package for Mac OS under GNU Lesser General Public License v.3 (“LGPL”). The HD-EEG data were reviewed for HFO occurrence in a common reference montage, using the technique of split screens as described by Andrade-Valen  a et al. [\(4\)](#) and Zelmann et al. [\(5\)](#) with the following display settings: left screen, filtered EEG with an expanded view of 450 mm/sec, 10  $\mu$ V, 3s/view; right screen, raw EEG, 60 mm/sec, 50  $\mu$ V, 1 sec/view.

Yet, clear guidelines for HFO identification do not exist. Thus, we name the phenomena we identify “potential HFOs”, indicating the pilot nature of the method. Potential HFOs were visually identified according to the following criteria: HFOs must exhibit at least four consecutive oscillations in the filtered signal ( $>80$ Hz) of regular morphology distinguishable from the background signal and from artefactual components. Furthermore, events must exhibit an isolated blob in the Discrete Wavelet Power Density display and superimposed fast activity observable in the raw EEG.

As scalp HFO detection is dependent on low signal-to-noise ratio [\(5\)](#), EEG segments with best signal quality were selected. We extracted 1-minute segments of each Go/No-Go task, Stroop task, and resting with eyes closed, and a 2-minute segment of Finger Tapping task, including 1 minute of active tapping and 1 minute of inactive intertrial pause. HFOs were detected during Go/No-Go and Stroop tasks in fronto-central regions (Figure 1), during Finger Tapping task in central and adjacent regions (Figure 2), and during rest in a widespread area (Figure 3).

**Figure 1:** Colored channels indicate the regions where HFOs were searched during Go/No-Go and Stroop tasks (fronto-central regions).



**Figure 2:** Colored channels indicate the regions where HFOs were searched during fingertapping (central and adjacent regions).

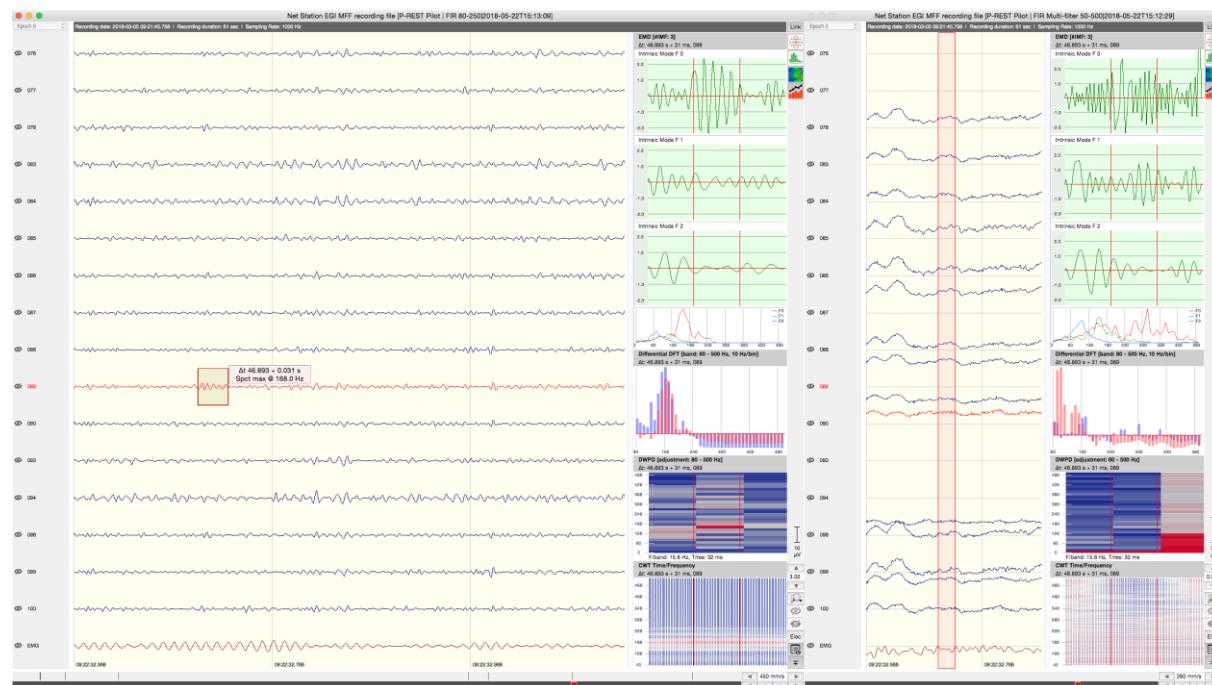


**Figure 3:** Colored channels indicate the regions where HFOs were searched during rest.



## Supplementary Section: Results

**Figure 4:** Channel over which a potential HFO was detected during rest is colored green.



**Figure 5:** Example of a potential HFO detected in the HD-EEG during rest.

## Potential HFO occurrence during Finger Tapping task recall

Duration: 136.617 sec

ROI electrodes: central and adjacent (124 channels)

Channels excluded: due to insufficient signal quality (41 channels)  
003, 004, 005, 012, 013, 020, 021, 027, 028, 034, 035, 040, 041, 043, 048, 049, 056, 057, 063, 064, 070, 071, 161, 163, 164, 171, 180, 181, 193, 194, 203, 204, 205, 206, 212, 213, 214, 215, 222, 223, 224

#HFO identified: 6 potential HFOs discussed

<b>Channel</b>	<b>Description</b>
006	117 Hz, regular morphology, standing out from background, blob, superimposed fast activity in raw data. Potential artefact: similar pattern on adjacent channels 005, 014, 015, 2015 and particularly on 001-003 artefactual activity visible in raw data.
008	218 Hz (very small amplitudes), regular morphology, blob, superimposed fast activity in raw data. Potential artefact: similar pattern on adjacent channels 001-007.
022	162 Hz, regular morphology, standing out from background, blob, superimposed fast activity in raw data, visible time-event 022+023. Potential artefact: relatively high amplitudes but could be also explained by source synchrony, blob and superimposed activity debatable. Similar pattern on adjacent channels 014-015, 030, noisy activity 028-030, 036 in raw data involved.
023	166 Hz, see 022.
089.1	180 Hz (relatively small amplitudes compared to background), regular morphology, blob, superimposed fast activity in raw data. Potential artefact: similar pattern on adjacent channels 079-080, 088, 100, also in distant channels 056 and 074.
089.2	113 Hz, regular morphology, standing out from background, blob, superimposed fast activity in raw data. Potential artefact: Event before artefact but outside our defined +/- 200 ms artefact-window, superimposed fast activity in raw data not clearly identifiable, similar pattern on adjacent channels 078-080, 088.

Additional markings: 9 HFO-like artefacts

Channels: 017 (1), 060 (1), 066 (1), 075 (1), 126 (1), 139 (1), 140 (1), 183 (1), 197 (1)

## Potential HFO occurrence during Go/no-go task performance

Duration: 61.000 sec

ROI electrodes: fronto-central (96 channels)

Channels excluded: due to insufficient signal quality (44 channels)  
001, 002, 003, 004, 005, 006, 010, 011, 012, 018, 032, 033, 037, 043,  
046, 047, 048, 049, 050, 054, 055, 056, 057, 058, 059, 060, 061, 062,  
063, 064, 065, 066, 164, 182, 185, 186, 194, 195, 196, 197, 211, 212,  
213, 214

#HFO identified: 0

Channels: –

Additional markings: 2 HFO-like artefacts

Channels: 025 (1), 132 (1)

## Potential HFO occurrence during Stroop task performance

Duration: 61.000 sec

ROI electrodes: fronto-central (96 channels)

Channels excluded: due to insufficient signal quality (62 channels)  
001, 002, 003, 004, 005, 006, 010, 011, 012, 013, 014, 018, 025, 026,  
027, 028, 029, 030, 032, 033, 034, 035, 036, 037, 043, 048, 049, 050,  
051, 052, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065,  
066, 155, 164, 184, 194, 203, 204, 205, 206, 207, 211, 212, 213, 214,  
215, 220, 221, 222, 223, 224

#HFO identified: 0

Channels: —

Additional markings: 3 HFO-like artefacts

Channels: 007 (1), 019 (1), 039 (1)

## Potential HFO occurrence during resting

Duration: 61.000 sec  
ROI electrodes: all regions (154 channels)  
Channels excluded: due to insufficient quality (14 channels)  
011, 019, 026, 033, 043, 170, 178, 190, 191, 192, 193, 201, 202, 211  
  
# HFO identified: 4 potential HFOs discussed

<b>Channel</b>	<b>Description</b>
087	213 Hz (small amplitudes), regular morphology, blob, superimposed fast activity in raw data. Potential artefact: similar pattern on adjacent channels 083-088.
089	168 Hz (small amplitudes but standing out from background), regular morphology, blob, superimposed fast activity in raw data. Potential artefact: very small but similar pattern (1 <sup>st</sup> + 2 <sup>nd</sup> peak) on adjacent channels 083-089.
094	116 Hz, regular morphology, standing out from background, blob. Potential artefact: superimposed fast activity in raw data debatable, critical location (behind the ear), similar pattern on all adjacent channels 083-085, 093-095, 103-105.
139	188 Hz (small amplitudes) regular morphology, blob, superimposed fast activity in raw data. Potential artefact: similar pattern (1 <sup>st</sup> + 3 <sup>rd</sup> peak) on adjacent channels 137-141, 149-151. Raw data view 1: noisy activity. Raw data view 2: superimposed fast activity and positive peak debatable.

Additional markings: 4 HFO-like artefacts

Channels: 047 (1), 149 (1), 150 (2)

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