PREREGISTRATION

EEG gamma activity entrainment by periodic visual stimulation in sleep

Authors: Hainke, Laura [1,2,3]
Dowsett, James [2]
Priller, Josef [1,6,7]
Spitschan, Manuel [3,4,5]

Affiliations:
[1] Department of Psychiatry and Psychotherapy, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany
[2] Department of Psychology, Ludwig Maximilian University, Munich, Germany
[3] Department of Health and Sport Sciences, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany
[4] Translational Sensory & Circadian Neuroscience, Max Planck Institute for Biological Cybernetics, Tübingen, Germany
[5] TUM Institute of Advanced Study (TUM-IAS), Technical University of Munich, Garching, Germany
[6] Neuropsychiatry, Charité – Universitätsmedizin Berlin and DZNE, Berlin, Germany
[7] University of Edinburgh and UK DRI, Edinburgh, UK

Date: April 21, 2023

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1 Introduction

1.1 Background

Gamma-band EEG activity, especially at 40 Hz, is fundamental for cognitive functions such as memory and already impaired in early stages of Alzheimer's Disease (AD; Traikapi and Konstantinou, 2021). To counter this decline in gamma-band activity, periodic sensory stimulation has emerged as a new promising avenue: presenting auditory or visual stimuli periodically at 40 Hz can effectively entrain this form of neuronal activity in a non-invasive and non-pharmacological manner (McDermott et al., 2018). First studies reported multiple significant benefits of 40 Hz sensory stimulation in mouse models of AD (Iaccarino et al., 2016; Martorell et al., 2019) and human patients suffering from mild AD (Chan et al., 2022; He et al., 2021).

In these studies, patients were exposed to the intervention for one hour a day, for several weeks or months. It led to improvements in AD biomarkers, cognition, and even circadian rhythms, a domain also affected by AD. One of the proposed mechanisms is the clearance of neurotoxic molecules, which 40 Hz sensory stimulation seems to support, at least in mice (Iaccarino et al., 2016). This clearance process is usually thought to be most active during sleep (Wang and Holtzman, 2020). Moreover, it has been shown that pulses of light can be used to shift circadian phase, especially if applied during the night (Joyce et al., 2022). Considering the above, applying this intervention during sleep could add value in terms of convenience as well as effectiveness.

1.1.1 Prior evidence

The feasibility of 40 Hz visual stimulation during sleep, however, is unclear. So far, periodic visual stimulation during sleep has only been applied at frequencies lower than 10 Hz, to elicit circadian effects (Zeitzer et al., 2014) or to entrain brain activity in the corresponding frequencies, with effect magnitudes depending on sleep stage (Norton et al., 2017; Sharon and Nir, 2018). With subjects' eyes closed, visual stimulation at 40 Hz does seem to promote entrainment but so far, that has only been shown during wakefulness (Fitzgibbon et al., 2016; Jones et al., 2019; Murphy and Öngür, 2019). Therefore, determining whether 40 Hz visual stimulation can effectively

enhance gamma-band EEG activity without disrupting young, healthy subjects' sleep is a crucial first step toward a more convenient and effective intervention for AD patients.

1.2 Objectives

The goal of this study is to determine the feasibility of periodic visual stimulation during sleep to modulate EEG gamma activity. Specifically, we will:

1. Test whether EEG activity at 40 Hz can effectively be entrained through closed eyes in wakefulness (W) and different sleep stages (N2, N3, REM);
2. Evaluate how feasible visual stimulation during sleep is in a young healthy cohort;
3. Explore potential impacts of this form of stimulation on sleep quality and architecture.

These specific aims will be approached through a well-controlled within-subjects laboratory study.

2 Methods and Materials

2.1 Sample

2.1.1 Inclusion and Exclusion Criteria

In this study, we will recruit and enrol healthy young participants. Exclusion criteria as listed in (Table 1) and will be assessed by self-report through a survey before the first session.

2.1.2 Participant-level Characteristics

Participant-level data to be collected includes age, sex, handedness, education, average sleep quality, and chronotype.

2.1.3 Stopping guidelines

Participants will be recruited until a total number of 30 is reached, a number deemed reasonable given the available monetary and time resources.

Exceptionally, participation can be terminated early if on the baseline night, PSG outputs clearly suggest the participant suffers from a sleep disturbance. This would constitute an exclusion criterion (see Table 1).

Participants who have completed all sessions will be excluded from analysis if one of the following cases applies:

- Over the entire experimental night, >50 % of the PSG is scored as awake.
- For at least half of the experimental night, the participant chooses to remove the sleep mask and leave it off.

2.1.4 Participant Recruitment

We will recruit participants through a variety of means, including flyers, posters, and posts on social media. Additionally, participants will be recruited through mailing lists from General and Experimental Psychology, LMU, and Chronobiology and Health, TUM. On recruitment materials, a QR-code will be displayed that leads to study information and the screening survey. Participants will be remunerated for their time with 100 € upon completion of all three study sessions.

2.2 Protocol

2.2.1 Study Design

The planned study follows a 4 X 2 within-subjects design. It is a laboratory study and an interventional clinical trial. The within-subject factors are condition (control night, experimental night) and sleep stage [wakefulness (W), deep sleep stage 2 (N2), deep sleep stage 3 (N3), rapid-eye movement sleep (REM)]. All participants will undergo one stimulation session during wakefulness and one week later, one control night (con) followed by one experimental night (exp).

2.2.2 Environment and Context

The experiment will take place at the sleep laboratory of the Department of Psychiatry and Psychotherapy, TUM. Two similar rooms designed like simply built bedrooms, dominated by light brown, wooden tones, will be available. Room lights will be dimmed to a minimal level necessary for EEG setup and fully turned off during the night.

2.2.3 Timeline

The study will take place over the year 2023. Participants will be invited to the sleep laboratory on three occasions, over a study period of approx. one week. In an initial 1-hour session, the stimulation will be applied during wakefulness. Participants will be asked to maintain a regular sleep-wake schedule for one week before coming in for two consecutive nights on a following weekend (to avoid interference with clinical routine), for 20 hours in total. The first night will serve as a control and sleep baseline, with passive measurements only. Visual stimulation during sleep will be additionally applied in the second, experimental night.

2.2.4 Randomization

The order of conditions will be the same for all participants to help counter the "First Night Effect" (Agnew Jr et al., 1966). Moreover, any potential undiagnosed sleep disturbances can be spotted via PSG before subjects undergo the stimulation during sleep in the second night.

2.2.5 Screening

During screening prior to the first session, participants will complete a survey delivered via the online platform REDCap (Harris et al., 2009). The questionnaire will cover all exclusion criteria, including age, vision, shift work, long-distance travel, chronotype, and substance use, as well as neurological, psychiatric, and sleep disturbances. The Micro Munich Chronotype Questionnaire (μMCTQ; Ghotbi et al., 2020) will be administered to estimate chronotype. Additionally, the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993) and the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989) will be employed to assess alcohol abuse and average sleep quality, respectively. Subjects to whom none of the exclusion criteria apply based on their survey answers will be invited to the laboratory for the first session. At this session, colour blindness will be tested for with Ishihara plates (Clark, 1924). Additionally, at the first night, a urine drug test will be performed (nal von minden Drug-Screen Multi Test).

2.2.6 Procedure

The experimental procedure for each participant will be as follows. During the first session, the inclusion criteria will be verified and the Ishihara colour vision test will be administered before informed consent is obtained. Then room lights will be dimmed for the setup of EEG and stimulation mask, which participants will be asked to wear with eyes closed during measurements. At the target illuminance level, two stimulation blocks of 10 min will be recorded, while subjects remain awake with eyes closed. A short break between blocks should help maintain subjects' alertness.

Two nights in a row will then be scheduled at the laboratory on a following weekend. Participants will be asked to keep a constant sleep-wake schedule for one week before the sleep sessions; on the three days before the first night, participants should refrain from unusual amounts of caffeine and any alcohol or drug intake. On the two days of the sleep sessions, only if it is usual for them to nap during the day, subjects should not nap for longer than usual. Subjects will be asked to arrive at the lab 1,5 hours before their habitual bedtime on free days, as indicated in the μMCTQ.

Upon arrival at the lab on the first night, subjects will perform the urine drug test. If the test is negative, they will be given enough time to complete their usual sleep routine before PSG, EEG, and stimulation mask are set up. The mask will not be energized on the baseline night. Subjects will remain in a semi-recumbent position for 10 min in order to record a wakefulness baseline, then they will lay down to sleep. Participants will be given 8 hours of sleep opportunity before they are awakened. After the setup is removed, the Gröningen Sleep Quality Scale (SQS; Mulder-Hajonides van der Meulen et al., 1980) will be administered.

Subjects will return in the evening for the second study night. After nightly routines and experimental setup are complete, subjects will lay down to sleep right away. On this night, the intervention will be delivered as described in Section Section 2.3. In the morning, after 8 hours of sleep opportunity and setup removal, the SQS will be administered.

2.3 Intervention

2.3.1 Description and Delivery of Intervention

The intervention will consist of a visual stimulus temporally modulated at a frequency of 40 Hz, with the aim of entraining the brain's endogenous activity in that same frequency. The procedure for each participant will be as follows: On the experimental night, a trained researcher will actively monitor PSG starting at subjects' bedtime. As soon as four consecutive PSG epochs (2 min total) are are scored as a stage other than W or N1, the mask will be energized. To minimize arousals, the light will gradually fade in following a cosine pattern and then be kept constant overnight at the target illuminance level. If an awakening is registered by the experimenter, the stimulation will be interrupted and restarted following the same scoring criterion as above.

For EEG recording, sleep stage monitoring, and post-hoc sleep scoring, the Neurofax PSG system will be used, supported by Polaris.One and Polysmith software (Nihon Kohden Europe GmbH, Rosbach v.d.H., Germany). It is a wired system with traditional gold cup electrodes, which will be placed on mastoids, frontal / central EEG, chin EMG as well as diagonal EOG positions according to PSG standards. EEG data will be recorded at the occipito-parietal region.

The intervention will be delivered through a customized sleep mask with inbuilt LEDs, externally linked to a microcontroller. The design of the stimulation device leans on commercially available masks (e.g., https://noctura.com/) as well as previously published setups (Norton et al., 2017; Sharon and Nir, 2018); a custom build is preferable in order to guarantee the required level of experimental control. The device will feature LEDs of monochromatic red colour, with the spectral peak at 610 nm chosen to optimize both L-cone activation (Spitschan et al., 2015) and eyelid transmission (Bierman et al., 2011). Light will always be temporally modulated at 40 Hz with a square-wave flicker and 50 % duty cycle. The target illuminance will not exceed 50 lux at eye level, a value estimated based on pilot recordings.

2.3.2 Placebo or Control Condition

The first night at the sleep lab serves as the control condition, which all subjects will undergo. The planned setup is identical to the experimental night, except that the mask is not energized, so no manual control by the experimenter is required. Since sleep architecture analyses are only exploratory, to reduce participant load, the first night will combine environment adaptation and baseline measurement.

2.4 Outcomes

2.4.1 Primary Outcomes

The primary outcome will be spectral density power at 40 Hz (in dB), measured with EEG and estimated by means of Fast-Fourier-Transform (FFT). The number of measurements per condition will depend on the percentage of time per sleep stage; each 30-second epoch will be treated as one trial and subjected to FFT analysis. Within one condition, the FFT outputs of all trials will be averaged (induced FFT) and the value at 40 Hz selected for statistical analyses (FFT-40). EEG pre-processing steps are listed in Section 2.5.2, related confirmatory hypotheses are described in Section 2.5.3.

2.4.2 Secondary Outcomes

For information on sleep quality in the experimental night compared to the control night, total sleep duration (PSG) and subjective sleep quality (SQS) will be subjected to analysis. The distribution of time per sleep stage (PSG) will be reported descriptively.

To explore intervention effects on EEG activity under a different perspective, Steady-State Visually Evoked Potential (SSVEP) analyses will additionally be reported. Here, every 25 ms segment between two peaks of the light temporal modulation equals one trial; segments within one condition are averaged. From this time series approach, additional information can be gained on SSVEP peak-to-trough amplitude (in μV) as an indicator of entrainment magnitude, but also waveform and phase.

2.4.3 Covariates

We do not plan to include covariates in any of the analyses.

2.5 Statistical Analysis

2.5.1 Power Analysis

Simulation-based power analyses were run for planned pairwise comparisons. Assuming a sample of $n = 30$ and standard levels of $1-\beta = .8; \alpha = .05$, this study would be powered to detect effects of at least $d = 0.7$ with parametric and non-parametric tests (Figure A1, Figure A2). A one-way repeated measures ANOVA (see H5, Section 2.5.3) with equivalent parameters ($n = 30, 1 - \beta = .8, \alpha = .05$) would be powered to detect effects of at least $f = 0.22$ (Figure A3); this latter analysis was carried out with G*Power software (Faul et al., 2007).

2.5.2 Pre-processing

EEG data pre-processing for the main analysis will entail the following steps:

1. Bad channel rejection (visual inspection,for high levels of line noise)
2. Averaging of electrodes at the parieto-occipital region of interest
3. High-pass filtering (0.1 Hz)
4. Bad trial rejection (every 30 sec epoch divided into 5 sub-segments; if at least 3/5 show a signal range > 1 mV peak-to-peak, reject)
5. Applying FFT to every trial, then averaging FFT outputs within conditions

For supplementary SSVEP analyses, the criterion for bad trial rejection (4.) will be a signal range > 1 mV peak-to-peak for a 1 s segment around each 25 ms trial; no FFT will be applied (5.) before trial data are aggregated within conditions to form the average SSVEPs.

2.5.3 Confirmatory Analysis

Concerning the main outcome variable, FFT-40, five hypotheses are defined *a-priori*. We postulate that the intervention will lead to an increase in FFT-40 in the four stages compared to baseline (H1 - H4) and that the effect magnitude will differ between stages (H5). Concretely:

- H1: $W_{exp} > W_{con}$
- H2: $N2_{exp} > N2_{con}$
- H3: $N3_{exp} > N3_{con}$
- H4: $REM_{exp} > REM_{con}$
- H5: $W_{exp} \neq N2_{exp} \neq N3_{exp} \neq REM_{exp}$

For hypotheses H1 - H4, one-tailed pairwise t-tests will be performed; if assumptions are not met, we will resort to Wilcoxon signed rank tests. A significance threshold of $\alpha = .05$ will be chosen for null hypothesis rejection. For H5, a repeated-measures ANOVA will be performed unless assumptions are violated, in which case a Friedman ANOVA will be performed. Here, the significance threshold of $\alpha = .05$ also applies. Tukey tests will be employed to test for *post-hoc* contrasts, with a Bonferroni-Holm correction for multiple testing. Confidence levels, effect sizes and SNR levels will be reported.

2.5.4 Exploratory Analysis

SSVEP analyses will be based on hypotheses and statistical tests equivalent to those for FFT-40 and reported as supplementary. Where relevant, information on waveform, shape, and SNR will be reported.

To estimate differences in subjective sleep quality as measured by the Groningen Sleep Quality Scale score between experimental and control night, a two-tailed Wilcoxon signed rank test will be performed. Differences in total sleep time between the two nights will be tested for with a two-tailed paired t-test or a Wilcoxon signed rank test, in the case of assumption violations.

2.5.5 Data Storage and Privacy

Data collected in this project will be labelled using assigned participant IDs and stored directly in a pseudonymised form. I.e., data will be collected under a numerical ID without a reference to contact details and processed without any assignment to personal data of the participants. Demographic data, to be stored in a separate password-

protected sheet, will include age, gender, and chronotype. Experimental data, to be stored on-site, will encompass EEG recordings, percent time per sleep stage, total sleep duration, and subjective sleep quality.

In terms of open science practices, we plan to make analysis code available on GitHub (https://github.com) and fully anonymized data available on Open Science Framework (https://osf.io/).

Ethics Approval

A proposal will be submitted to the ethical committee of the Technical University of Munich.

Funding

The study relies on internal funding from the Faculty of Medicine and the Faculty of Sports and Health Sciences, both at the Technical University of Munich.

Conflicts of Interest

The authors report no conflicts of interest.

Table 1

Exclusion criteria

Aspect	Screening modality	Exclusion criteria
Age	Self-report	< 18 years or > 35 years
Vision	Self-report, Ishihara test	Red-green colour blindness, any history of eye disease
Neurological disturbances	Self-report	Any history of neurological symptoms, especially epilepsy, migraine, stroke, brain tumour, concussion
Family history of epilepsy	Self-report	Any first-degree relative diagnosed with seizures
Sleep disturbances	Self-report, Pittsburgh Sleep Quality Index (PSQI)	Any symptoms in the past 6 months, especially insomnia, sleepwalking, bruxism, narcolepsy, restless legs syndrome, sleep apnoea; bad average sleep quality (PSQI > 5)
Psychiatric disturbances	Self-report	Any symptoms in the past 6 months, especially depressed mood, (hypo)mania, excessive worries, hallucinations or delusions, substance abuse, suicidal thoughts
Shift work	Self-report	Any shift work in the past month
Long-distance travel	Self-report	Any travel across 2 or more time zones in the past month
Substance use	Self-report, Alcohol Use Disorders Identification Test (AUDIT)	Any use of illicit drugs, cannabis, nicotine, or psychopharmacological medication in the past month; current alcohol abuse (AUDIT > 15)
Chronotype	Self-report, Munich Chronotype Questionnaire Micro (uMCTQ)	Chronotype measure MSFsc < 01:30 (“extremely early”) or > 06:00 (“extremely late”)

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Appendix

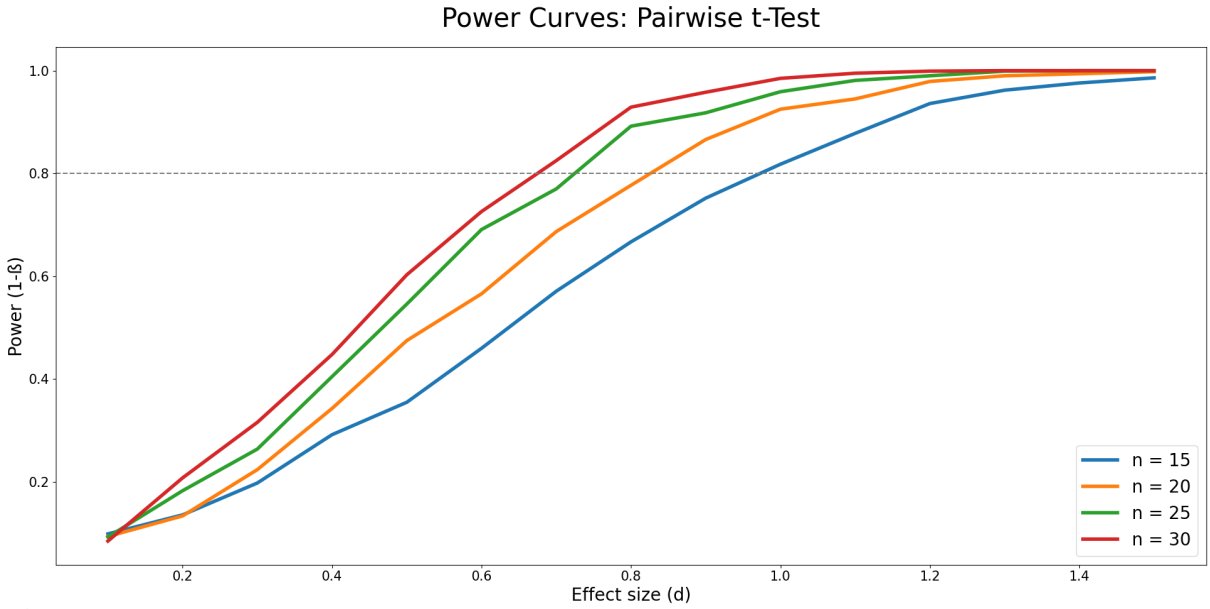

Figure A1

Power levels by effect size and sample size, simulated for one-tailed pairwise t-tests.

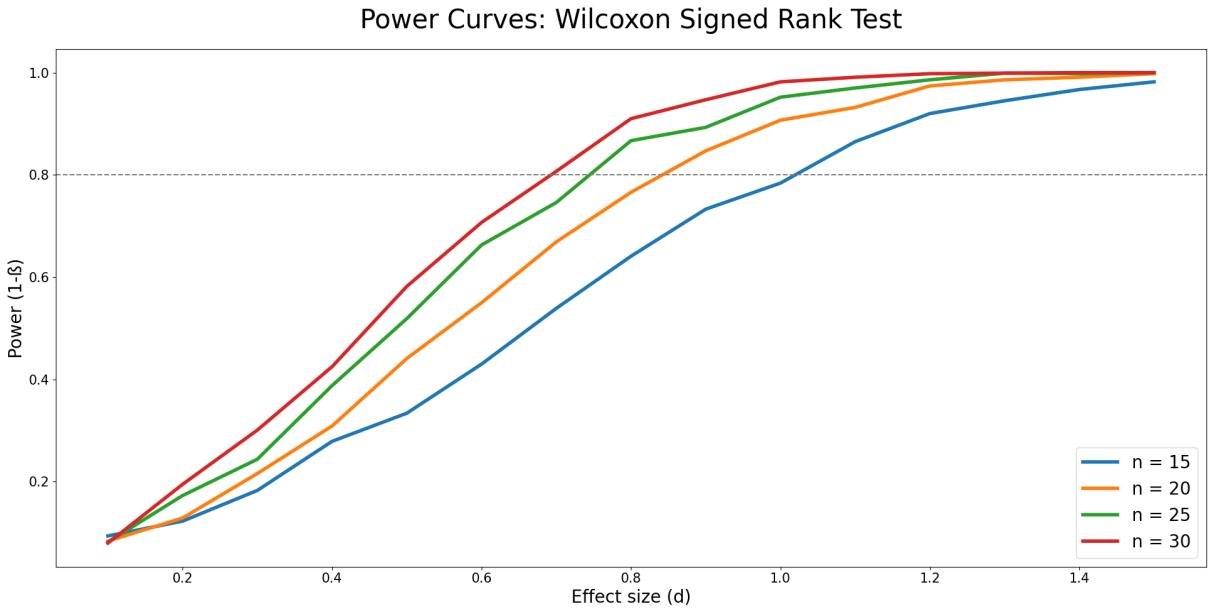

Figure A2

Power levels by effect size and sample size, simulated for one-tailed Wilcoxon signed rank tests.

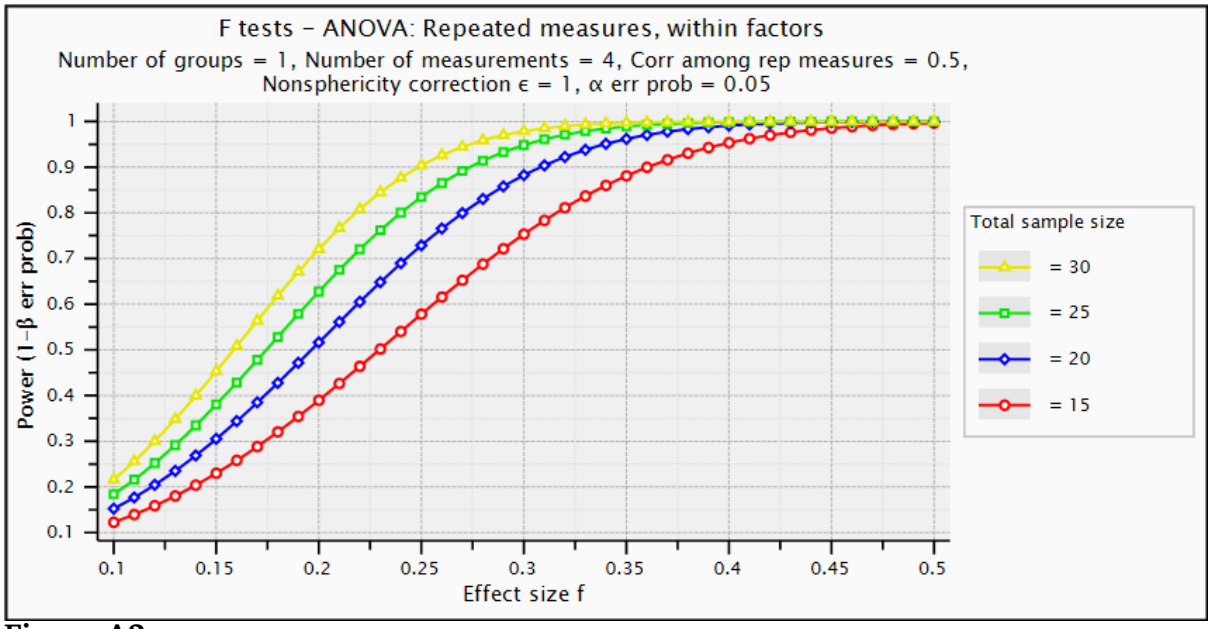

Figure A3

Power levels by effect size and sample size, simulated for one-factor repeated measures ANOVA.