ORGANIZATION OF DETAILED PROTOCOL

Optimized tDCS for fibromyalgia: targeting the endogenous pain control system

Protocol #: 2017P002524

Date: 03/13/2023

I. BACKGROUND AND SIGNIFICANCE

This is a NIH R01 grant (1 R01 AT009491-01A1) funded by NCCIH.

Fibromyalgia (FM) pain affects upwards of 5 million people in US annually [1] and can have a considerable impact on the daily life routines and general well-being of patients, which results in generalized and debilitating conditions. To date, treatment options are limited and often associated with adverse effects. In addition, pain in FM is extremely difficult to assess and therefore difficult to treat. Common interventions to treat fibromyalgia involve the use of nonsteroidal anti-inflammatory drugs, antidepressants, and/or anticonvulsants, all of which carry relatively high risks and no guarantees of success. Recently, multi-modal interventions were developed, but their actual effectiveness has been challenged [2]. Therefore, the development of novel targeted non-pharmacological therapies that modulate cortical excitability and modify activity of the central nervous system (CNS) might provide a novel approach to analgesia. Recent evidence has suggested that FM pain can be related to deficits in pain endogenous regulatory control [3] and that novel non-pharmacological interventions, such as transcranial direct current stimulation (tDCS) can modulate this system and, consequently, reduce pain intensity [4]. Widespread pain in FM is thought to represent enhanced pain sensitivity that is maintained by central mechanisms [5]. Bosma et al. [5] acquired functional magnetic resonance imaging (fMRI) during Temporal Slow Pain Summation (TSPS) paradigm and showed that FM patients exhibited increased after-sensations and dorsal horn activity following TSPS compared to healthy participants. This suggests changes in the descending pain control mechanisms and a possible relationship with the central sensitization phenomenon [6]. Supporting this assumption, in one recent trial from our group, we showed that tDCS was effective in relieving pain only if patients with FM exhibited increased contact heat evoked potential (i.e., alteration of central pain sensitivity) at baseline [7]. Thus, the assessment of mechanisms underlying the response to treatment can help to predict treatment response in future trials and help to optimize treatments targeting specific pain pathways, such as the one highlighted in this proposal.

Chronic pain results from an imbalance between excitatory and inhibitory pain pathways. Recent evidence has suggested that pain inhibitory pathways are affected in FM; thus, further understanding these pathways' role in FM can significantly change how FM is treated. Two assessments can test pain inhibitory activity: Temporal Slow Pain Summation (TSPS) and

conditioned pain modulation (CPM). During TSPS, the progressive increase in pain intensity following repetition of identical nociceptive stimuli reflects C-fiber and other central neurons temporal summation and has been used as a marker for pain sensitivity. TSPS does not seem dependent on increased impulse C-fiber input, suggesting that TSPS is a CNS phenomenon rather than peripheral [8]. As a CNS phenomenon, several mechanisms contribute to TSPS, including ineffective central pain modulation [9] and rostral ventromedial medulla facilitation [10]. Moreover, TSPS is thought to rely on the same mechanisms that underline the initiation and maintenance of several chronic pain conditions, and thus TSPS has also been widely studied in FM [11, 12]. FM patients also demonstrate lower CPM inhibitory efficacy. Indeed, the rate of patients with FM reporting pain facilitation during CPM has been found to be significantly increased compared with that of the controls (41.7% vs 21.2%) [13]. Based on the current literature, there is strong evidence that for some FM patients the endogenous pain regulatory system is impaired. Moreover, CPM efficacy has already been related to pain development 6 months after surgery [14], making it another suitable marker for response prediction.

There is also evidence of CNS changes in FM that are associated with a deficit in the inhibitory control, namely abnormal cortical excitability as expressed by decreased short intracortical inhibition (SICI) and facilitation (SICF) and increased resting motor threshold [15]. This is thought to reflect an inhibitory deficit that is associated with altered thalamic anatomy and activity [16], which may result in abnormal thalamocortical circuits, as evidenced by the association between central pain and thalamic dysrhythmia [17-19]. Therefore, the unravelling of inhibitory deficits underlying pain maintenance mechanisms in patients with FM can also help the development of novel interventions for this pathology, as well as for other chronic pain conditions associated with a deficit in the regulatory pain system.

Non-invasive brain stimulation (NIBS) techniques such transcranial direct current stimulation (tDCS) are safe, cost-effective and powerful neuromodulatory tools, which have provided pain relief in several chronic pain conditions such as FM [20], chronic post-stroke pain [21] and chronic pain after spinal cord injury (SCI), [22] among others, with absent or minimal side effects. For over 10 years, we have been developing a neuromodulatory intervention for fibromyalgia using non-invasive brain stimulation (our first trial was published in 2006 [20]).

Physical exercise is another non-pharmacological intervention that has been related to analgesia in FM. For instance, following exercise, pain rating decreased in FM patients, with increased brain responses (as measured by fMRI) in the left dorsolateral pre-frontal cortex and anterior insula [23]. This suggests that exercise is able to modulate the activity of brain regions that are related to descending pain inhibition which results in decreased pain sensitivity. Thus, the development of new non-pharmacological interventions can help achieve two goals simultaneously: 1) improving patient well-being and 2) reducing the costs associated with symptom treatment [24]. Finally, these

two interventions –tDCS and exercise – are both relatively inexpensive with minimal side-effects, increasing the potential significance of these therapies for the treatment of FM.

In this study, we aim to understand the mechanisms of an optimized tDCS strategy for the treatment of chronic pain via a modulation of the endogenous pain control system by combining it with aerobic exercise and by increasing the number of tDCS sessions.

This study is built based on several studies showing that tDCS of primary motor cortex (M1) and AE have demonstrated significant analgesic effects in FM [2, 7, 23, 25-28], and both of them reduce the dysregulation of the inhibitory control system [4]. Furthermore, one of our recent studies has shown that combining M1 tDCS to exercise improves chronic allodynia and hyperalgesia associated with FM [29] and results in a greater degree of pain reduction as compared to each intervention individually.

The overall goal of this project is to investigate the mechanisms of optimized tDCS via two important markers of chronic pain, conditioning pain modulation (CPM) and slow temporal summation (STS). In addition, the results of this study will represent a significant step toward developing a novel low-cost therapy for FM and validating a therapeutic novel target.

Sponsors

This study is funded by NIH-NCCIH (1 R01 AT009491-01A1).

II. SPECIFIC AIMS

This trial aims at understanding the mechanisms of optimized tDCS (16 tDCS sessions combined with exercise) on pain control. Optimized tDCS can lead to stronger engagement of the endogenous pain regulatory system that will ultimately lead to increased pain relief in patients with FM. Therefore, we designed a 2x2 factorial mechanistic trial [tDCS (active and sham) and aerobic exercise (active and control)] to test the following aims:

Aim 1 (primary outcome): Evaluate the effects of 4 weeks of tDCS coupled with exercise on the endogenous pain regulatory system assessed by CPM and central sensitization as assessed by TSPS, and compared to either intervention alone and to no intervention. *We hypothesize that tDCS coupled with exercise will result in restoration of pain endogenous regulatory system as indexed by greater decrease in SPS and increase in CPM responses. We also hypothesize to observe differences between groups as exercises may have a larger effect on the insula and somatosensory cortex (which is indexed by SPS) while tDCS seems to have a greater effect on prefrontal and*

cingulate cortex (which is indexed by CPM). Thus, these markers will be important to understand to what extend the nodes of pain matrix may be more involved in pain relief and whether the effects of tDCS and exercises are mainly mediated by an increase in inhibition (increase of CPM) or a decrease of excitation (decrease of TSPS).

Aim 2: Determine the effect of these interventions on cortical markers of inhibitory control that are also affected in FM, such as intracortical inhibition as assessed by transcranial magnetic stimulation (TMS) and changes in thalamocortical dysrhythmia as assessed by EEG. *We hypothesize that active tDCS combined with exercise will result in greater increases in intracortical inhibition as measured by the short intracortical inhibition (SICI). These increases will be associated with greater reduction of pain when compared to either intervention alone. We also hypothesize that tDCS combined with exercise will induce a greater decrease in theta power than any intervention alone, as well as to the control group. Therefore, we hypothesize that decreases in theta EEG band power could be used as a marker of the normalization of the thalamic dysrhythmia normalization.*

Aim 3: Assess the number of sessions needed to induce significant changes in markers of endogenous pain inhibitory system and central sensitization (CPM and TSPS) and cortical changes (as indexed by paired pulse TMS and EEG). *We hypothesize that the group receiving the top-down and bottom-up strategies (tDCS and AE) will have a greater number of subjects with target engagement (change of at least 30% as compared to baseline) of pain and cortical marker.*

Sub-aim 3: Assess whether engagement of the two main targets tested in this study – TSPS and CPM – are associated with the secondary clinical outcomes (i.e., changes in pain outcomes: Brief Pain Inventory, Revised Fibromyalgia Impact Questionnaire). *We hypothesize that changes in TSPS and CPM will be correlated with these pain outcomes. We also hypothesize to find a correlation between baseline values of TSPS and CPM and pain relief immediately after the intervention.* This result will be important as to assess the suitability of such markers as potential treatment response predictors.

III MEDICAL MONITOR

Responsibilities: The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator.

Medical Monitor: 

[redacted]

IV. SUBJECT SELECTION

We will recruit 116 fibromyalgia subjects (29 per group) to participate in this study. All subjects will need to meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion criteria:

1) age range 18-65 years,

2) diagnosis of FM pain according to the ACR 2010 criteria (existing pain for more than 6 months with an average of at least 4 on a 0-10 VAS scale) without other comorbid chronic pain diagnosis,

3) pain resistant to common analgesics and medications for chronic pain such as Tylenol, Aspirin, Ibuprofen, Soma, Parafon Forte DCS, Zanaflex, and Codeine,

4) must have the ability to feel sensation by Von-Frey fiber on the forearm,

5) Able to provide informed consent to participate in the study.

Exclusion criteria:

1) clinically significant or unstable medical or psychiatric disorder,

2) history of substance abuse within the past 6 months as self-reported (if subject reports a history of substance abuse, we will confirm using DSM V criteria),

3) previous significant neurological history (e.g., traumatic brain injury), resulting in neurological deficits, such as cognitive or motor deficits, as self-reported,

4) previous neurosurgical procedure with craniotomy,

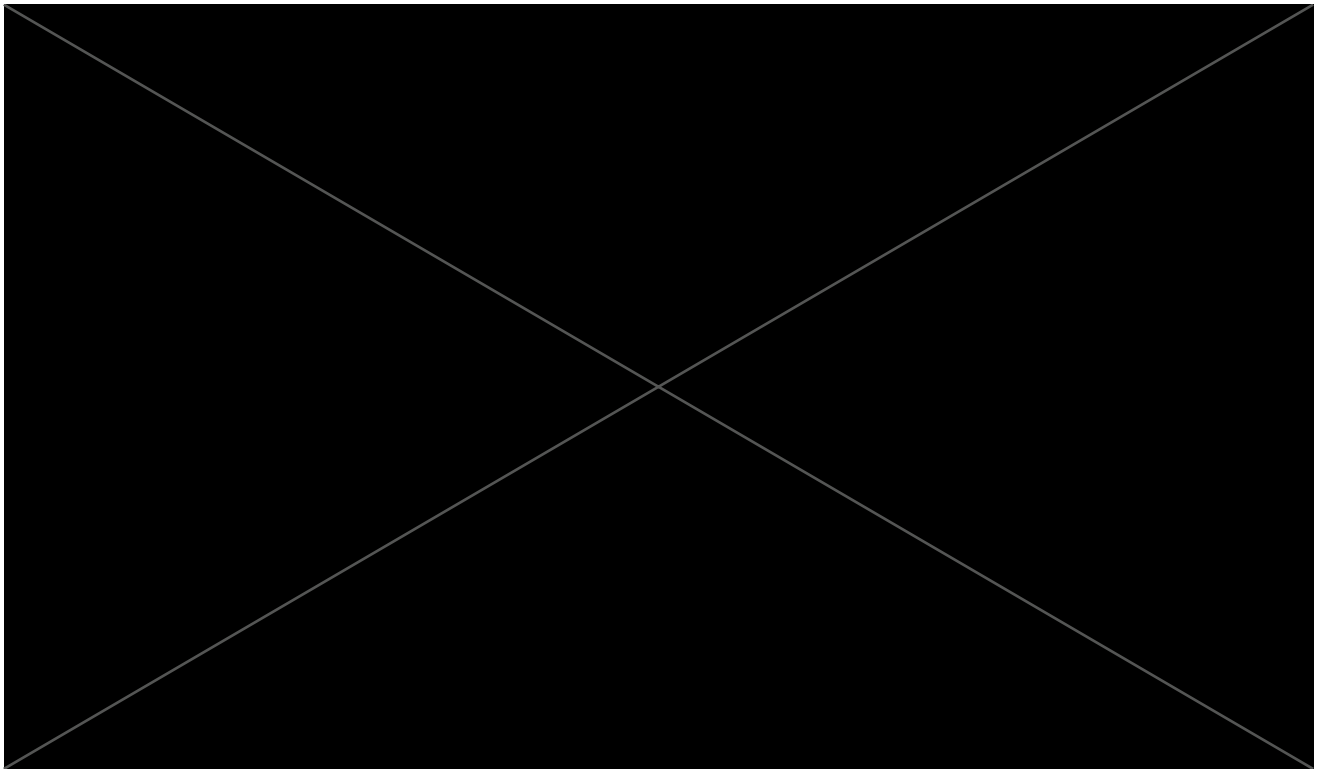
5) Severe depression (If a patient score >30 on the beck depression inventory, he/she will obtain clearance. If he/she does not pass the medical clearance, he/she will not be included in the study),

6) pregnancy (as the safety of tDCS in pregnant population (and children) has not been assessed (though risk is non-significant), we will exclude pregnant women (and children). Women of child-bearing potential will be required to take a urine pregnancy test during the screening process and at every week of stimulation),

7) current opiate use in large doses (more than 30mg of oxycodone/hydrocodone or 7.5mg of hydromorphone (Dilaudid) or equivalent),

8) Patients will be excluded when they have increased risk for exercise as defined as (i) not fulfilling the american college of sports medicine (acsm) criteria (i.e., risk of cardiovascular complication [30]) and in this case not cleared by a licensed physician.

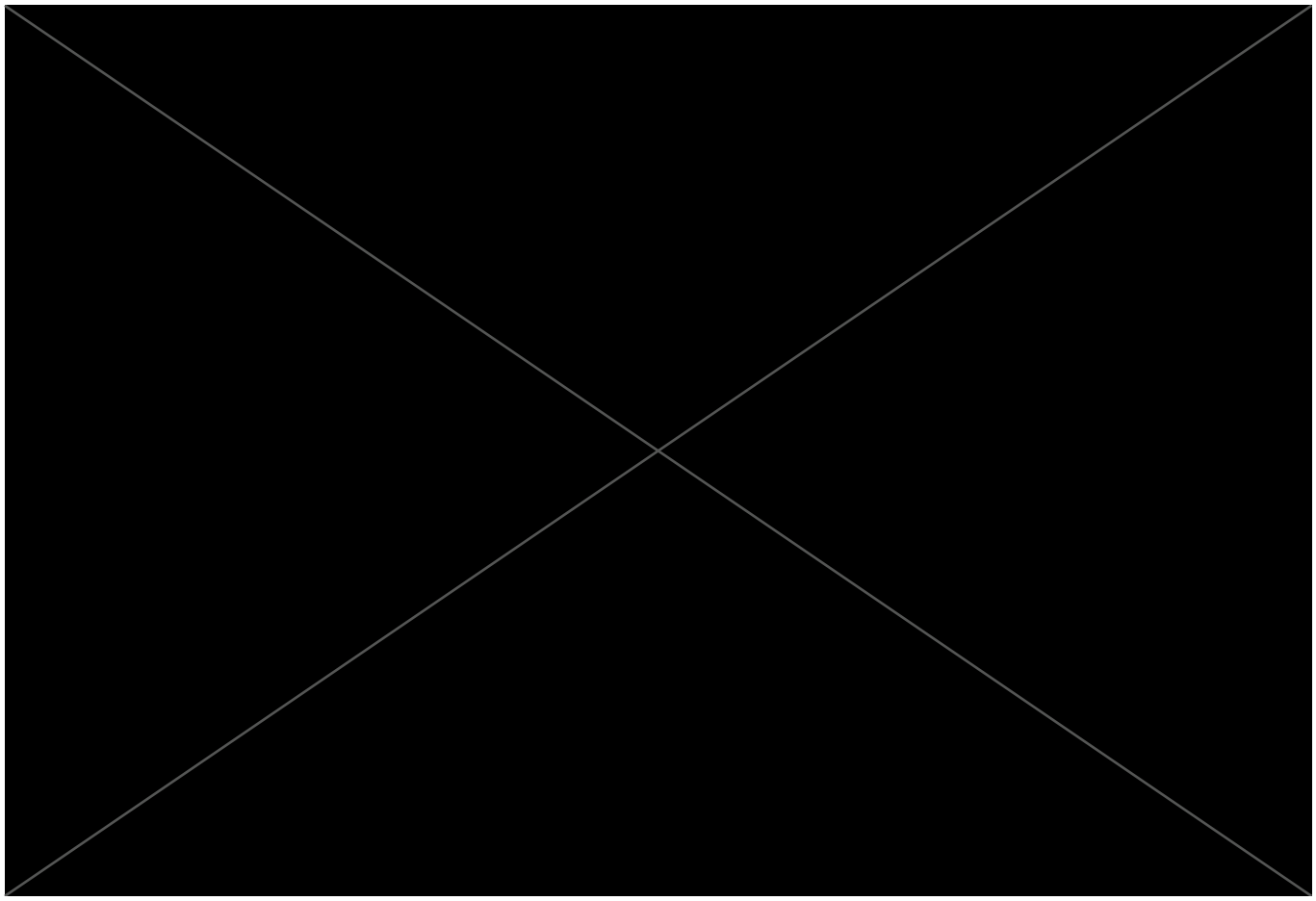
(If a patient meets the inclusion criteria but is found to have a risk factor (according to the ACSM guidelines), he/she will undergo a medical visit in order to obtain clearance. If he/she does not pass the medical clearance, he/she will not be included in the study.

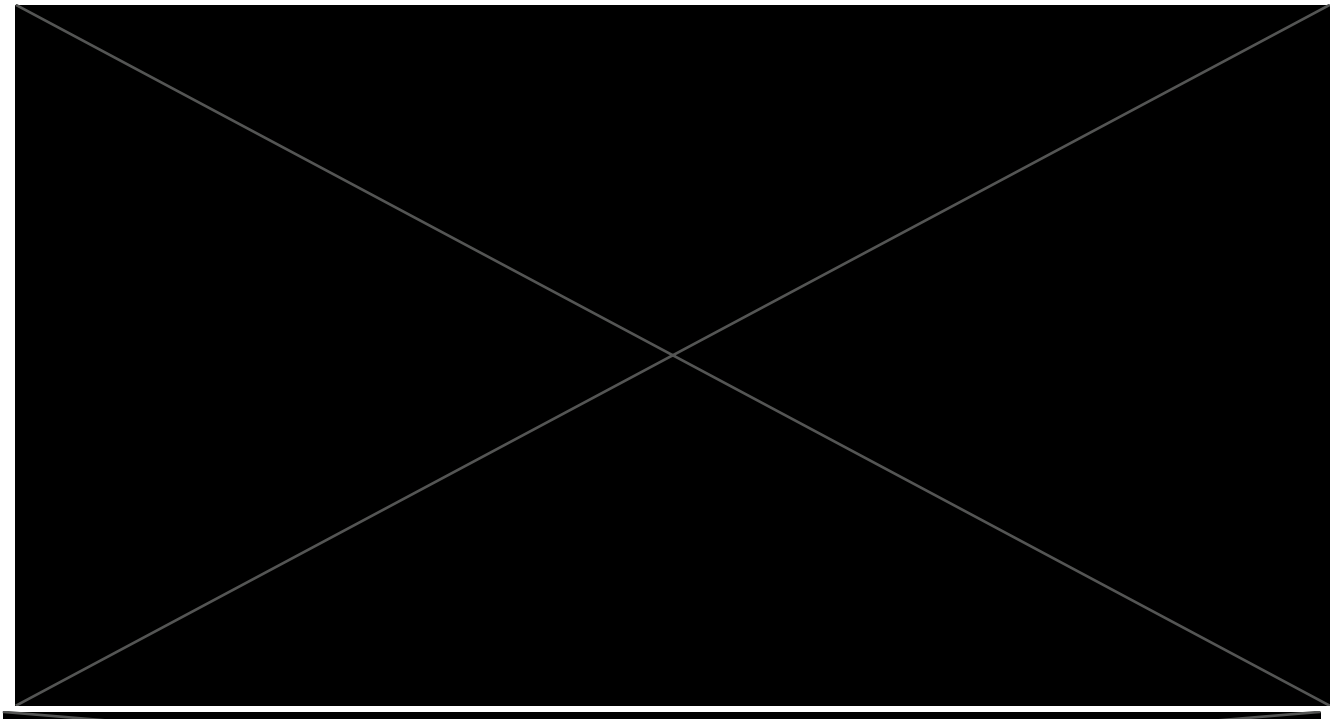


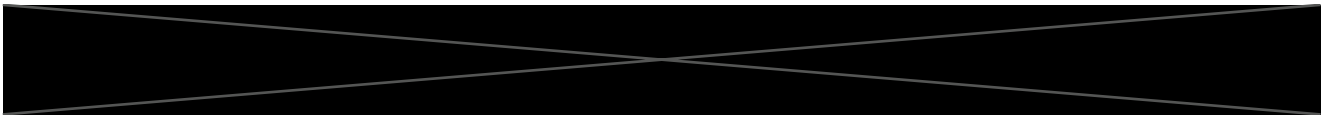
V. SUBJECT ENROLLMENT

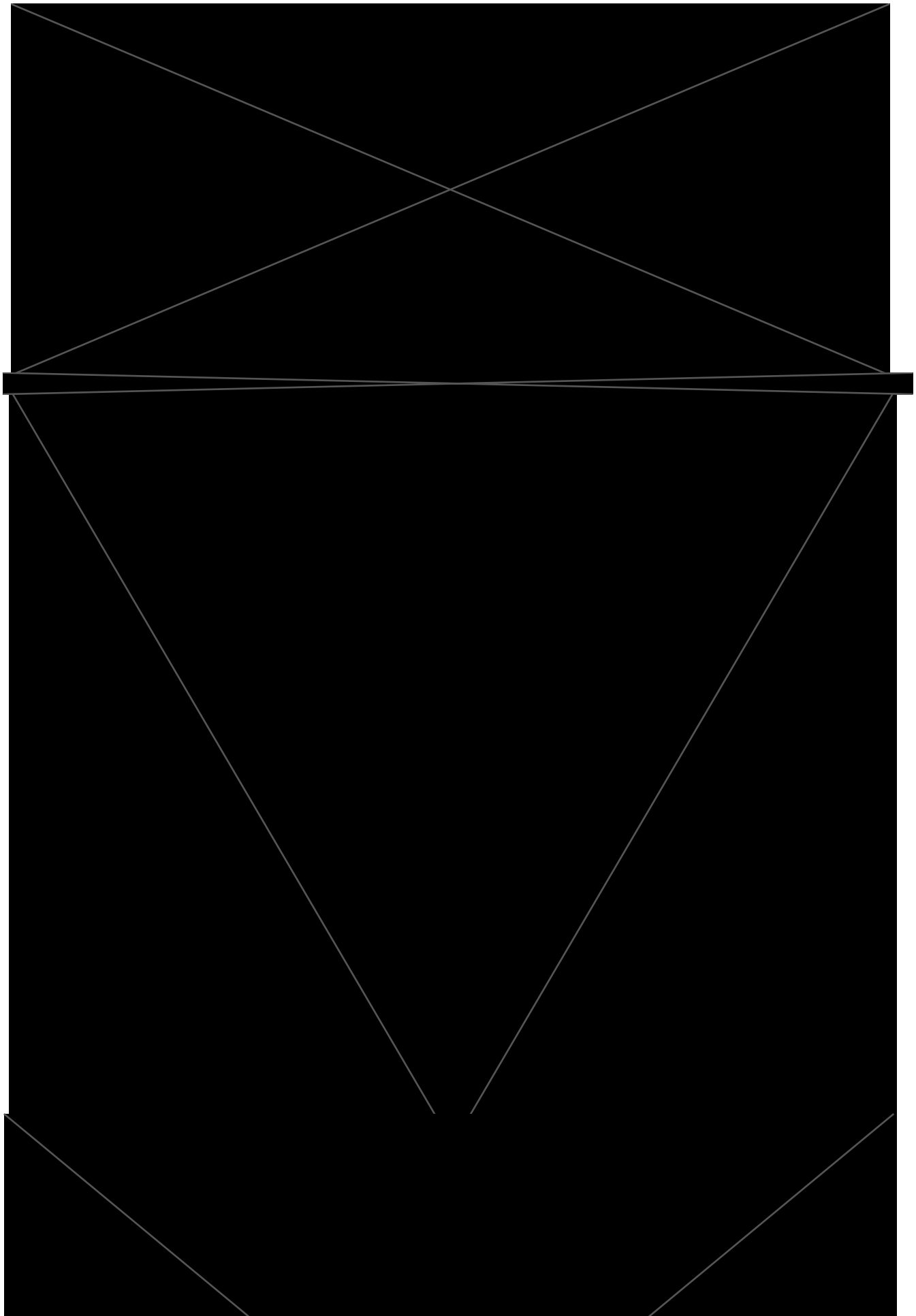
Subjects will be sought through the following sources:

- Attending physicians, therapists, clinicians and other healthcare providers may refer out subjects to the study.
- We will provide them with study information sheets and flyers. Prospective subjects will be encouraged to contact the study co-investigators.
- Flyers posted in the outpatient specialist clinics and public posting boards (i.e., digital signage).
- Internet (i.e., Google Ads) and newspaper advertisements.
- Advertisings posted in public transportation (The T).
- Via the Partners Healthcare Research Patient Data Registry (RPDR), Patient Gateway, and Research Match (Vanderbilt Recruitment Tool).
- Potential subjects might also be identified though their medical records (epic LMR, etc.) and their physicians might be asked to inform the subjects about the study.
- Support groups presentations.









The method of randomization is a 1:1:1:1 permuted block randomization, generated by a web-based randomization tool. Randomization order will be kept in individual sealed and opaque envelopes with order of entrance and the randomization master list will be kept in a locked drawer; therefore, subjects will get their assignment according to the order of entrance in the study.

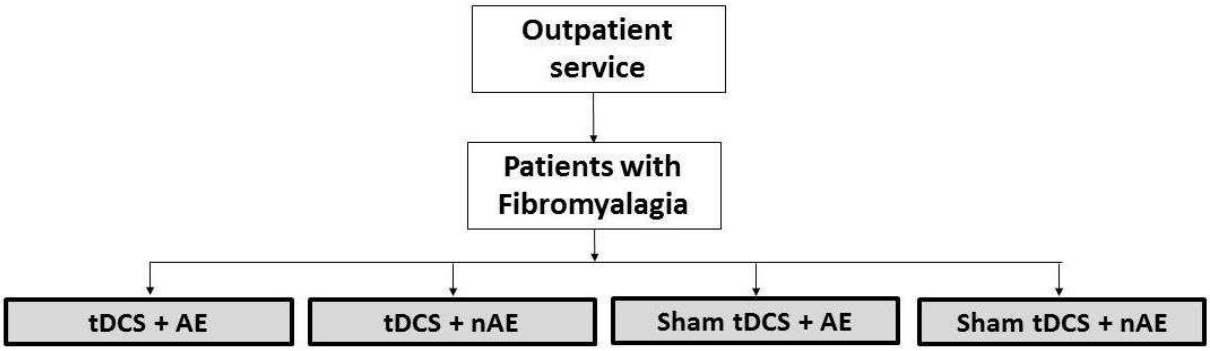


Figure 1 - Intervention groups with 1:1:1:1 allocation ratio.

Blinding Procedure:

Participants, family members, treating staff/ physicians, data collectors, site investigators and the statistician will be blinded to group stimulation assignment.

If the subject receives sham stimulation, he/she may re-enroll into an open label portion of the study, where he/she will receive 10 days of active tDCS. Un-blinded co-investigators (e.g., who provided the exercise intervention) will coordinate with subjects electing to enter into the open label phase, which will be conducted by the un-blinded study staff. In the open label phase, we will complete the VAS assessments at baseline, Day 5 and Day 10 of stimulation. This collected data will not be analyzed with the main data collected in the study. This data will be used for exploratory analyses only. If the subject refuses to enroll to the open-label portion of the study, this will be documented and stored in the unblinded binder.

Intervention

Transcranial Direct Current Stimulation (tDCS)

Active tDCS: A 1x1 Low-intensity DC Stimulator that uses coding such as the Soterix Medical Clinical Trial will be used to deliver direct current through rubber electrodes in saline soaked sponges. The anode will be placed over the left primary motor cortex (M1) while the cathode will be placed over the contralateral supra-orbital area. Primary motor cortex will be localized using the 10/20 EEG system (C3 or C4), a reliable method for the technique of tDCS [22]. During active tDCS, a 2.0mA constant current will be delivered across 35 cm^2 electrodes for 20 minutes while the subject performs exercise. The primary motor cortex is a reliable "entry port" to modulate dysfunctional activity in pain-related neural networks. Up-regulation of motor cortex excitability

modulates pain perception through indirect effects on pain-modulating areas, such as thalamic nuclei and cingulate gyrus. Garcia-Larrea, et al performed neuroimaging studies in patients with chronic pain showing that motor cortex stimulation results in activity changes in the motor thalamus (lateral thalamus) that is then transmitted to sensory thalamus (medial nuclei) and other pain-related structures such as subthalamic areas and cingulate gyrus [31, 32]. Subjects will also perform physical exercise while receiving stimulation.

For Visit 3, 5, 8 and 10 (only tDCS stimulation visit), we will have an option for Home-Based tDCS. If a subject is interested on this option, we will have a training onsite during Visit 2 (only patients who fulfill the checklist training will be able to proceed with the Home-Based tDCS visits). If applicable, during these four visits, the study procedures will be done at the subject´s house under strict remote supervision by the Neuromodulation Center researchers (according to the standard procedures that we use in other studies. As part of our Standard Operation Procedure for the Virtual Visits, appropriate business attire, neutral or virtual background, following Mass General Brigham Zoom Video Conference Etiquette, as well as no screenshots or recordings will be allowed. The use of a headset/earbuds will be mandatory for study staff to ensure confidentiality.

During the training visit, we will explain to the subject how to use the portable and home-based tDCS device (Soterix Medical 1X1 tDCS mini-CT stimulator), including how to place the electrodes and get an adequate impedance. It is important to mention that the stimulation parameters will be controlled remotely by the researcher, therefore, the participant cannot modify them.

Sham tDCS: We will apply sham tDCS on the primary motor cortex. We will use the same montage and parameters of active tDCS. However, the current will be applied for 30 seconds in the beginning of the procedure after which the current is turned off. This parameter for sham stimulation was chosen based on previous studies that have shown that perceived sensations on the scalp such as tingling usually fade out in the first 30 seconds of tDCS. It should be noted that less than 3 minutes of tDCS induces no effects on cortical excitability [33] and also using 30 seconds of sham is a reliable method of blinding as shown by a randomized controlled study [34]. We have also shown that this method of sham is reliable for longitudinal studies [35]. Subjects will also perform physical exercise while receiving stimulation.

Training and support for participants

Even though the device is practical to use and provides a safety guarantee, it is convenient to have a training session to address any question and check the compliant and proper usage of the device. Therefore, a checklist-based training will be conducted by using the device for practice without stimulation. Subjects who have completed all items of the checklist will be allowed to use the device for the study after the training session and a final test. This session will include practicing the placement and positioning of the device and preparation materials, starting the stimulator, and

troubleshooting common problems. Remote support will be provided via a remote-control program as well as video calling will be used to facilitate the study. After the training session, a certified investigator will evaluate the subject's performance before starting the stimulation sessions. If the subject does not meet the criteria for doing home-based tDCS, the subject would not be eligible for the optional home-based tDCS for visit 3, visit 5, visit 8 and visit 10.

Exercise

Subjects will undergo moderate physical exercise (corresponding, for instance, to brisk walking (about 3-4mph or light cycling (about 12mph)). All the sessions will be monitored by a co-investigator (who will be MD, PT or RN) and also certified in Heart Rate Monitoring Assessment Course (by ACMS), with CME credit from European Respiratory Society for Interpreting Cardio-Pulmonary exercise test and also with BLS/AED certification) and will have in-house coverage from a senior trained clinician.

The exercise level is what is recommended by the American Heart Association for adults (i.e. American Heart Association recommends: 30 minutes of moderate-intensity aerobic activity daily (http://www.heart.org/en/healthy-living/fitness/fitness-basics/aha-recs-for-physical-activity-in-adults). In addition, as detailed in the inclusion and exclusion criteria, subjects will be screened for risk factors to perform exercise (see safety plan below).

In addition, the exercise level we are using here is exactly the same we used in our previous study *(Mendonca ME, Simis M, Grecco LC, Battistella LR, Baptista AF, Fregni F.Transcranial Direct Current Stimulation Combined with Aerobic Exercise to Optimize Analgesic Responses in Fibromyalgia: A Randomized Placebo-Controlled Clinical Trial. Front Hum Neurosci. 2016 Mar 10;10:68)*. We have found this to be an effective and also reasonable level of exercise for FM patients.

Finally all subjects will participate in a 1-week exercise conditioning program according to their randomized group as explained below.

Aerobic Exercise: Each session of moderate intensity AE will last 30 minutes (brisk walking). FM patients will be walking on a treadmill with a workload intensity set to a maximum of 60-70% of age predicted maximal heart rate, following the formula HRmax = 208 − (0.7 * age) [36]. Before and after each AE session, there will be warm-up and cool down periods (explained below). During the AE session, the intensity will be modulated based on patient's HRmax.

Non-aerobic Exercise: The non-aerobic (nAE) exercise will have a 30 min-duration, but the workload intensity will be set within 5-10% baseline heart rate. We also used this method in our preliminary study [29]. The warm-up and cool down periods will consist of 3-minutes walking on the treadmill at a comfortable self-selected speed.

Conditioning exercise program:

The two groups will participate in a different conditioning exercise program according to their group assignment. Stimulation will only start after the conditioning exercise program.

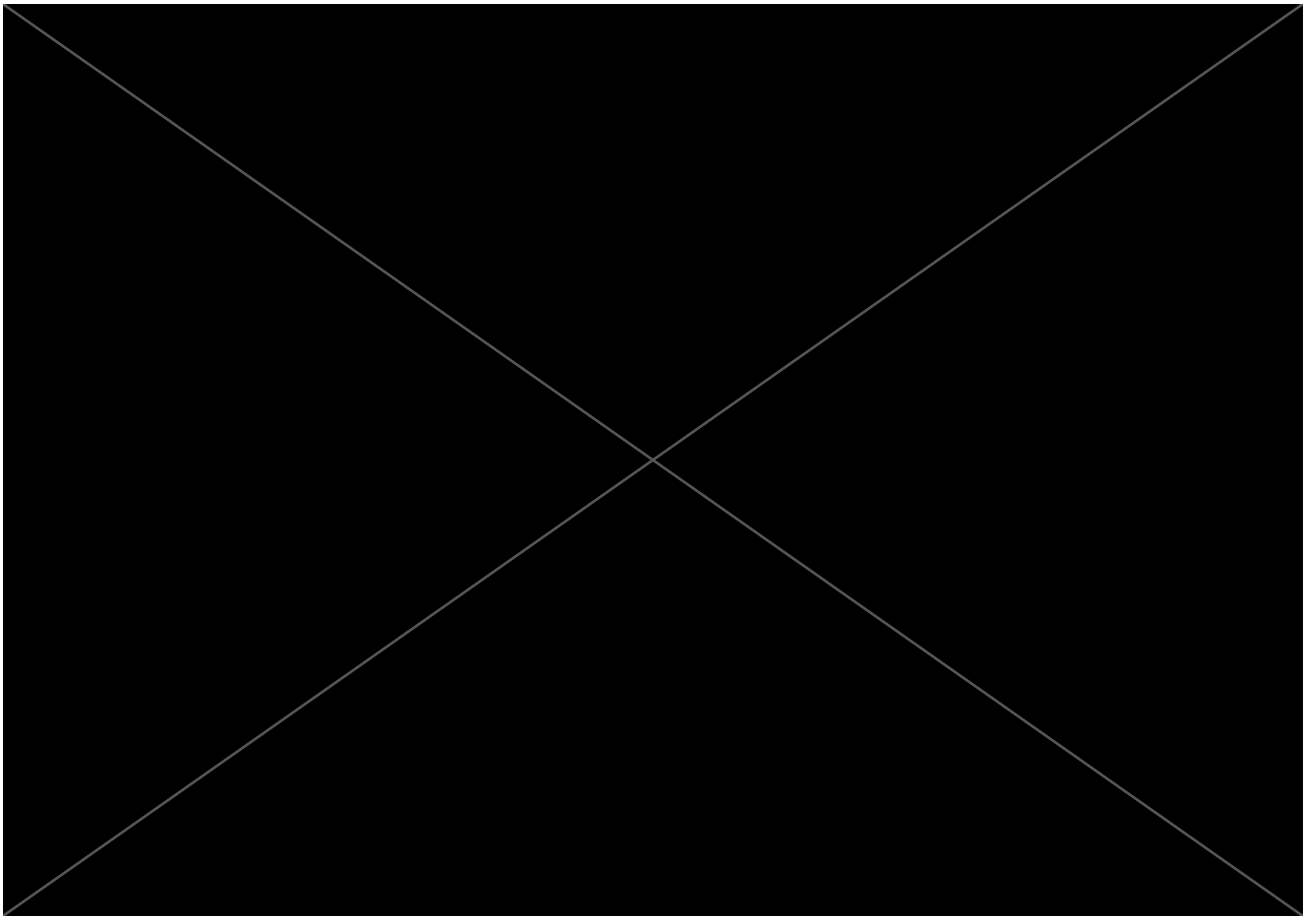
Group of Aerobic exercise: in this group participants will exercise (brisk walking in the treadmill – 60-70% of HRmax) following the schedule below (3 sessions within 1 week):

- Week 1: up to 15 min on the Monday (minimum 10 min), up to 25 min on Wednesday and up to 30 min on Friday (days of the week can change to adjust to subject schedule)

If a subject from this group cannot progress beyond 20 min at 60-70% HR max over the initial 1-week period, they will be excluded from further involvement in the study. If they reach 20 min at 60-70% of their HR max, he/she can progress to 30min during the second phase and this will be noted.

Group of Non-aerobic exercise: in this group participants will walk at within 5-10% of their baseline heart rate following the schedule below (3 sessions within 1 week):

- Week 1: up to 15 min on the Monday (minimum 10 min), up to 25 min on Wednesday and up to 30 min on Friday (days of the week can change to adjust to subject schedule)



Pre-screening and Visit Description

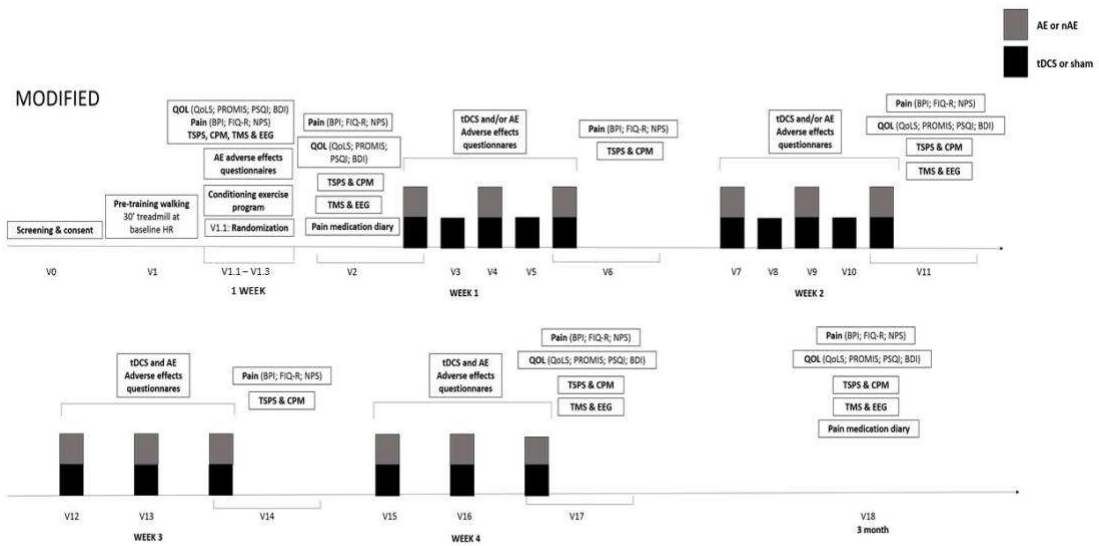


Figure 2 - Schematic representation of the intervention. Subjects will be assigned to either: Aerobic or anaerobic exercise (AE or nAE – grey squares) and active or sham transcranial direct current stimulation (tDCS - black squares).

Pre-screening Procedures

During the pre-screening process, the subject will contact a co-investigator usually via a phone call. During this call, the co-investigator will discuss in greater depth the details of the study, explain the study procedures and encourage the subject to ask questions. The co-investigator will ask the subject questions from the phone screening questionnaire, or if the subject desires, this can be arranged to be done in the privacy of the laboratory. We will follow partner's institutional policies regarding communication with study subjects including use of encryption.

Once this information is collected, the co-investigator will consult with the PI regarding the eligibility of the subject, who will then give approval for the subject to come to our laboratory for the screening procedure.

Visit 0 – Consent and Screening Visit

At screening visit the PI and/or a co-investigator will conduct once more a review of inclusion/exclusion criteria to determine the subject's eligibility for enrollment. Study procedures will be reviewed with the subject, and documentation of informed consent will be obtained.

At Screening, the following procedures will be completed:

- Discuss study-specific procedures with the subject
- Obtain a signed and dated consent form
- Review inclusion and exclusion criteria
- Pregnancy test (if applicable)
- Conduct a Demographics Survey, including self-reported hand dominance
- Conduct a Recruitment Survey

Visit 1 – Pre-training walking

*If the subject passes the screening for exercise (see section IV)) as reviewed by PI, subject will undergo the pre-training walking (*30' treadmill at baseline HR). If he/she complete the pre-training walking successfully as described above, then he/she will be randomized during visit 1.1. (subjects will also complete the adverse effects for aerobic exercise questionnaire)

- Pregnancy test (if applicable and wasn't done in visit 0 in the case of eConsent)

**Visits 0 and 1 may be completed in the same day or broken up into two separate visits as the subject prefers*

Daily pain reports will be collected for the two weeks between V1 and V2.

VISITS 1.1 – 1.3: Subjects will be randomized during visit 1.1. The two groups will participate in a different conditioning exercise program for two weeks according to their group assignment (see section VI). At visits 1.1, we will conduct assessments of pain, quality of life, TPSP, CPM, TMS and EEG acquisition (we will offer the possibility of splitting this visit into two visits if the subject gets too tired). At visit 1.3, we will conduct assessments of pain and quality of life, TPSP and CPM. In all these visits we will conduce AE adverse effects questionnaires.

VISITS 2, 11, 17: assessments of pain (except visit 2), quality of life (except visit 2), TSPS, CPM, TMS and EEG acquisition. At the same visit, tDCS and AE will be performed. Also, tDCS and AE adverse effects questionnaire.

VISITS 6 and 14: tDCS, AE, tDCS and AE adverse effects questionnaire, CPM, TSPS, assessments of Pain

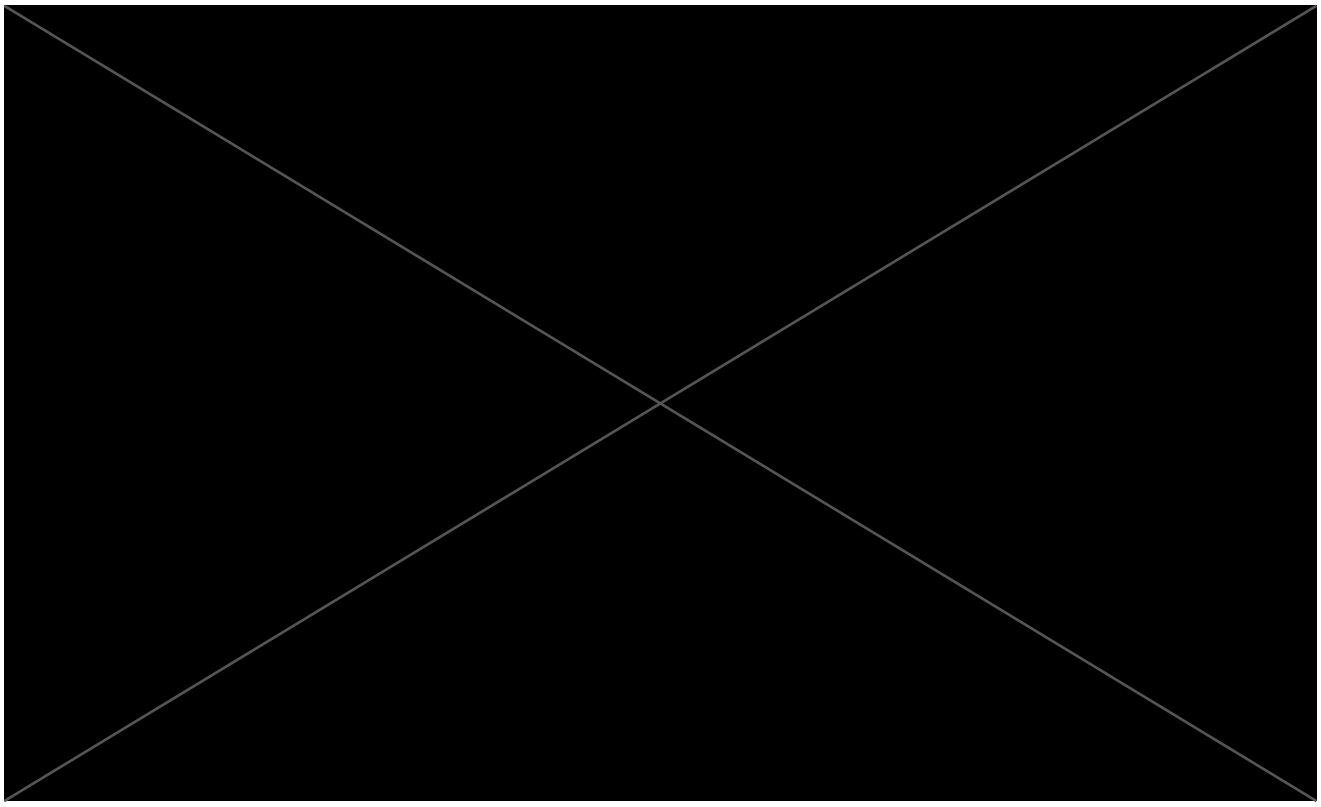
VISITS 3, 5, 8, 10: tDCS only and tDCS adverse effect questionnaire. The subjects will have the option of choosing to do the visits remotely home-based tDCS (Soterix Medical 1X1 tDCS mini-CT stimulator device), in order to decrease the burn of daily visits at our site.

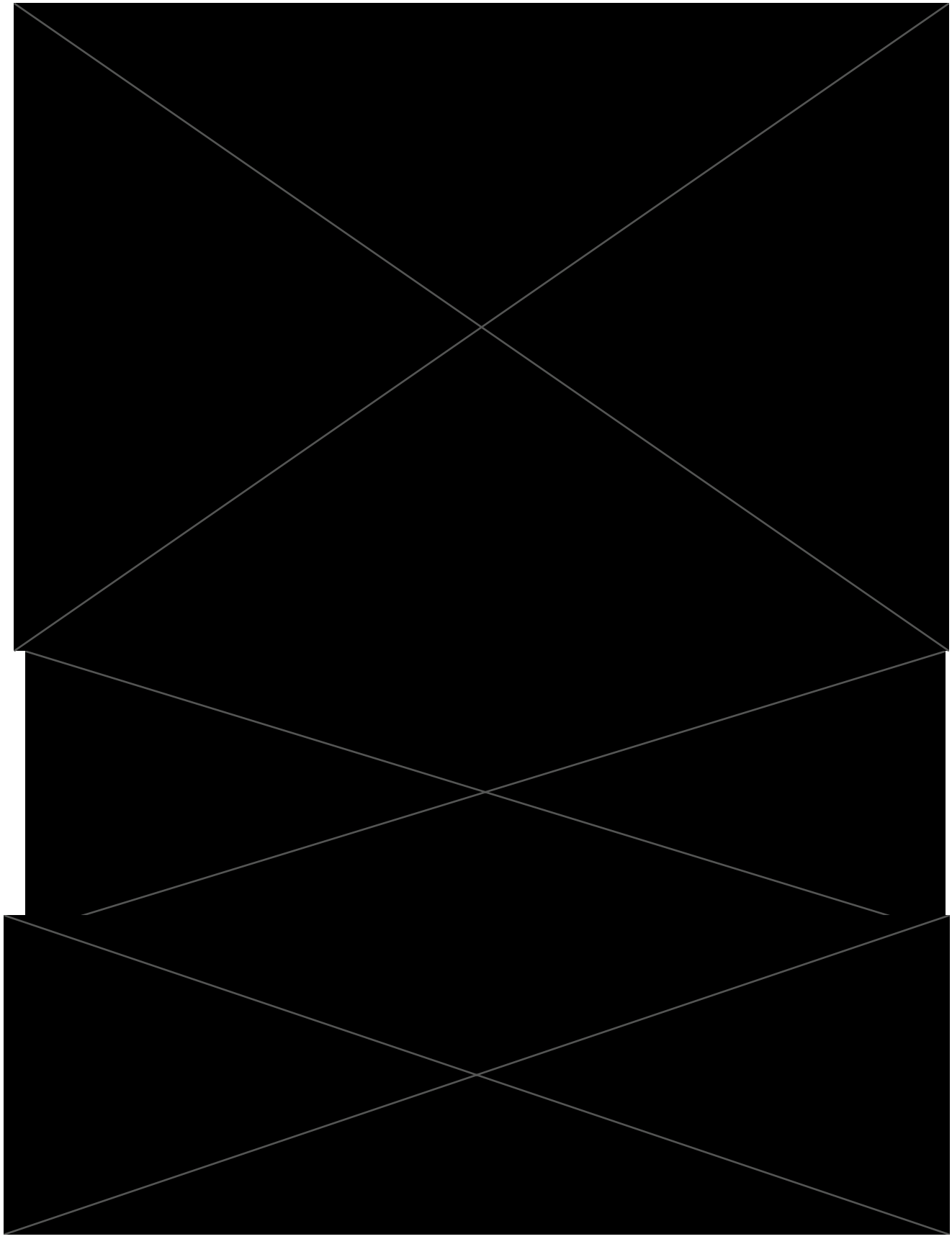
VISITS: 4, 7, 9, 12, 13, 15, 16: tDCS, AE and tDCS and AE adverse effects questionnaire.

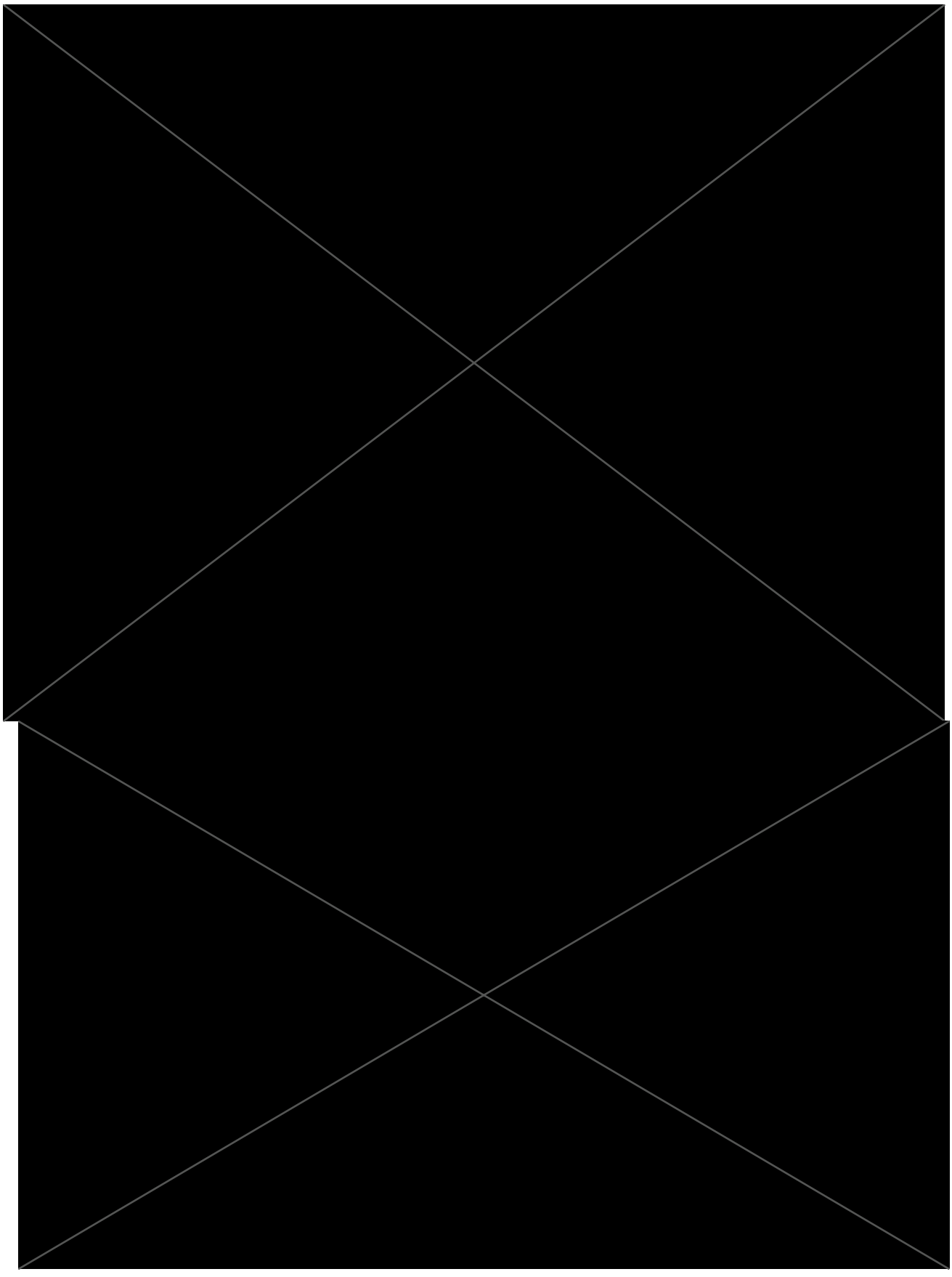
VISIT: 18: Pain and quality of life assessments, TMS, EEG, CPM, TSPS.

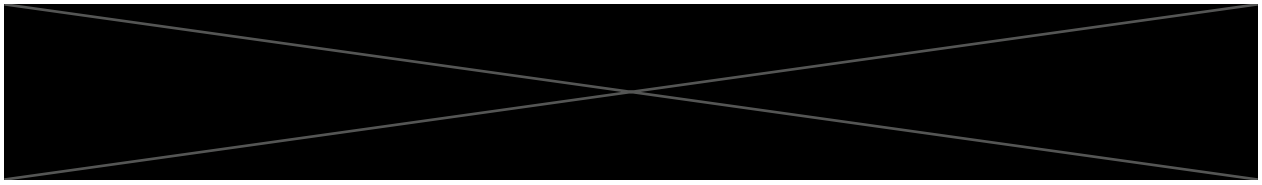
Replacement of missed sessions (though all the effort will be made to avoid missing sessions) will be allowed to up to 20% of missed sessions (up to 4 total visits) according to the treatment schedule. Follow-up and assessments will be encouraged even if more than 20% of missed sessions given data will be analyzed as ITT (though we expect this to be low). We will allow the following visit windows:

- *between visit 1 and 1.1: 2 weeks*
- *between visits during the conditioning period (from visit 1.1 to 1.3): 2 weekdays*
- *between visit 1.3 and visit 2: up to 1 week*
- *between visit 17 and visit 18: up to 2 weeks (before or after the final follow-up, meaning visit 18 could happen 2.5 months to 3.5 months from visit 17)*
- *between Visit 2 and Visit 17: up to 6 weeks*









VII. DESCRIPTION OF ASSESSMENTS

TSPS Protocol – Heat pulses will be generated by a TSA-II Stimulator (Medoc Advanced Medical Systems, Ramat Yishai, Israel) delivered to the right dominant proximal volar forearm using an appropriate size embedded HP-thermode. We will follow the adapted protocol suggested by [43], in which the HP-thermode was programmed to deliver pulses rising/fall of 1-2-s, depending on subject's heat-evoked pain threshold, from adapting temperatures to peak temperatures, with a plateau of .7-s. Subjects will be trained to determine the temperature necessary to elicit pain-60 (see CPM protocol below) . Subsequently, they will receive 1 train of 15 repetitive heat stimuli at 0.4 Hz to the same area, in which by being suitable for eliciting TSPS in most subjects, allows the rating of individual pain stimuli and is unlikely to induce peripheral sensitization [44]. . TSPS will be calculated as the difference between heat pain rating after the 15^{th} stimulus minus the 1^{st} stimulus.

CPM Protocol – The protocol involves two conditions, the *test-stimulus* and the *conditioned-stimulus*. We will follow the adapted protocol suggested by Granot, 2008 [84] and Nirl, 2011[85]. We will first determine the pain-60 test temperature (which is the temperature that induces pain experience at a magnitude of 60 on a 60-100 NPS) by applying a Peltier thermode (Medoc Advanced Medical Systems, Ramat Yishai, Israel) on the right forearm of subjects and delivering three short heat stimuli (43, 44, and 45 °C), each lasting for 7 s starting from the time the stimulus intensity reaches the destination temperature. Subjects will be asked to rate the level of pain intensity using a numerical pain scale (NPS) ranging from 0 = ''no pain'' to 100 = ''the worst pain imaginable''. If the first temperature of 43 °C is considered too painful (>60), we will stop the series and will provide additional stimuli at lower temperatures of 41 and 42 °C. If the three temperatures of (43, 44, and 45 °C), were not able to achieve pain-60, we will deliver additional stimuli at 46, 47, and 48 °C until the desired pain level of 60 (in the unlikely event that neither of those temperatures were sufficient to elicit pain-60, we will consider it to be 48°C). Once determined the pain-60 temperature, we will administer the test stimulus, applied for 30 s at that temperature and subjects will be asked to rate their levels of pain intensity 3 times: at 10, 20 and 30s after the thermode reaches the pain-60 temperature (mean scores of the the three pain rating will be calculated). Five minutes after delivering the test stimulus, for the *conditioned-stimulus,* the left hand of the subject will be immersed in a bath of water set at 10 to 12°C for 30 seconds. Then, the same pain-60 temperature will be applied on the right forearm of the subject (left hand will still be immersed), for 30 s and subject will again be asked to rate their levels of pain intensity 3 times after the thermode reaches the pain-60 temperature: at 10, 20 and 30s (mean scores of the three pain rating will be calculated). CPM response will be calculated as the difference between the average of pain ratings from the test stimulus minus the average of pain ratings during the conditioned stimulus.

TMS – Single and Paired Pulse TMS to measure cortical mapping and cortical excitability. Subjects will undergo several sessions of TMS to assess cortical excitability. We will study the first dorsal interosseous (FDI). We will initially investigate changes in cortical excitability evaluating the motor evoked potential (MEP) and the resting motor threshold (MT); we will use the same methods as in our previous study [47], as well as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) using the paired-pulse technique.

We will investigate the resting motor threshold (MT), measured following the technique as described by Rossini et al., where resting MT will be defined as the lowest stimulus intensity to evoke a MEP of 100 μV in three of five trials in the relaxed muscle (50% of attempts) [48]. We will record this in both primary motor cortices. For the MEP study, we will initially adjust TMS intensity to achieve a baseline MEP in the selected muscle of 1 mV peak-to-peak amplitude before intervention. Stimulation intensity will be kept constant for each subject throughout the evaluation sessions. The MEPs will be recorded and stored in a computer for off-line analysis. We will record 10 MEPs for each time point and average their peak-to-peak amplitude and area-under the-curve.

The short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) will be measured using the paired-pulse technique [49]. A supra-threshold test stimulus adjusted to an MEP amplitude of 1 mV will be preceded by a subthreshold conditioning stimulus (CS; 80% MT) at interstimulus intervals for inhibitory (2ms, SICI), excitatory (10 ms, ICF) and MEP windows, respectively. Ten stimuli will be applied at each interval in a randomized order. The percentage of inhibition or facilitation for each ISI before and after treatment will be calculated. The intensity of MEP among the paired pulse is the MEP- intensity the day (machine output intensity to elicit 1 mv in the day of the assessment not the baseline MEP-intensity used for the single pulse). In the case we are not able to locate the motor threshold, we will not perform MEP's or Paired Pulse.

EEG – EEG is a reliable tool to measure electrical activity in the brain [50]. The use of EEG can identify features of cortical excitability and inhibitory activities, as well as thalamocortical rhythm's abnormalities. This is particularly important for understanding the processing of nociception, given that the power of different EEG bandwidths have been shown to be associated with the intensity of pain experience. EEG activity will be assessed in all participants using standardized procedures [51]. The use of EEG in this protocol is exploratory and will be sampled using a 64 channel EGI system (EGI, Eugene, United States of America,). This is a portable, comfortable system that requires the placement of a cap with active electrodes. In total, this component of the visit is expected to take about 30 min typically. EEG will be collected with eyes open, eyes closed during rest and also with motor tasks (mental imagery, movement observation and actual movement).

Average Pain Intensity as Assessed by Modified Brief Pain Inventory (BPI) – short form: The BPI is a short self-assessment questionnaire that provides information on various dimensions of pain including how pain developed, the types of pain a patient experiences, time of day pain is experienced, as well as current ways of alleviating pain [52]. The BPI also includes the VAS Pain scale, a simple 10- point scale (0 = ''no pain'', 10 = ''pain as bad as you can imagine'') measuring patients' worst pain and least pain, on average and at present time. The BPI provides information

on the intensity of pain (the sensory dimension) as well as the degree to which pain interferes with function (the reactive dimension). According to several studies on pain in spinal cord injury, the BPI is an effective measure, as shown by both our group [20] and other studies [53]. BPI will be collected as shown the diagram above.

Revised Fibromyalgia Impact Questionnaire (FIQ-R): A 21-item, multiple-choice questionnaire will be administered at the beginning of the study and at follow-up, in order to assess function, overall impact and symptoms [54].

Beck Depression Inventory (BDI): A 21-item, multiple-choice questionnaire will be administered at the beginning of the study and at follow-up to assess the presence and degree of depression in adults, as studies in chronic pain have found that depression can modulate pain perception [55].

Medication Use Questionnaire and Diary: We will obtain a medication use history at study entry using a standardized questionnaire similar to that used in our prior studies [56, 57], and update this information at each subsequent visit. We will also monitor patient medication throughout the course of the study using a subject Medication Diary. Participants will be required to record medications daily in a pain medication diary. Participants will be instructed to keep the pain medication diary throughout the baseline, treatment, and follow-up period. This diary will be maintained until completion of the study. This pain medication diary will be given in a weekly basis. If the patient refuses to receive the daily pain medication diary, we will make a note in the binder.

Concomitant medications questionnaire: We will collect concomitant medications taken by the subject using a validated NCCIH tool, this includes the name of medication, start date, dosage, frequency, route of administration, stop date, and indication for use. This questionnaire will be only completed during the baseline visit (visit 1.1) in order to describe further medications.

Quality of Life Scale (QoLS): A 16-item, multi-purpose questionnaire that yields a profile of functional health and well-being scores. The aim is to compare the relative burden of the disease, and to differentiate health benefits produced by different treatments.

Patient Reported Outcomes Measurement Information System (PROMIS): A self-report measure of patient–reported physical, mental, and social well–being. The aim is to assess what subjects are able to do and how they feel, and thus is an additional measure of the effectiveness of treatment.

Pittsburgh Sleep Quality Index (PSQI): A self-report measure of the quality and patterns of sleep in adults. It assesses 7 components of sleep quality: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month. Scoring of the answers is based on a Likert scale from "0" (not during the past month) to "3" (3 or more times week). A total sum of "5" or greater indicates a "poor" sleeper. This instrument can be used to assess quality of sleep (i.e., poor or good sleep quality) in several time points of a given intervention (from baseline assessment to follow ups) [58].

Physical performance: running speed, HR range and distance that have been ran for the 30 minutes of exercise will be collected during exercise.

tDCS Adverse Effects Questionnaire: At the end of each stimulation session, subjects will complete a 5-point scale questionnaire that evaluates potential common adverse effects of tDCS (headache, neck pain, scalp pain, scalp burning sensation, tingling, skin redness, sleepiness, concentration, and acute mood change).

Exercise Adverse Effects: During each AE session, subjects will be monitored (HR variability). The session will be stopped if the HR is superior to 80% of HRmax [36], or if the patient presents any signs of discomfort. Also if the subject requests, the intensity can be reduced up to 50% of HR max or the duration can be reduced up to 15min. To evaluate the adverse effects of AE during the training, we will record any musculoskeletal symptoms, such as pain, fatigue, tingling or cardiovascular symptoms, such as shortness of breath [29]. As recommended by the ACSM guidelines for AE in patients with FM, we will monitor the level of pain and fatigue continuously throughout the tests using the VAS. We will also follow the emergency procedure guidelines from the ACSM.

If a subject is not comfortable to perform one of the described assessments, we will make a note in the study binder and proceed to the next one.

VIII. BIOSTATISTICAL ANALYSIS

Sample Size Calculation: This study's original sample size calculation was based on an expected effect size of 0.78, with 85% statistical power and a significance level of 0.05. However, after 6 years of the original sample size calculation (it was done in 2017 upon grant submission, see appendix 2), we have observed in our recent study in collaboration with other colleagues and recent meta-analyses effect sizes ranging from 0.9 to 1.26, which are larger than we initially anticipated. Considering this new information, we re-estimated the sample size to ensure we do not expose subjects to the burden of participating in a study that involves a placebo arm. Also, this would make the study feasible to be completed in the 5-year period of this grant, given our recruitment was significantly affected by the COVID pandemic, especially in the years 2020 and 2021. We have recalculated the sample size using the smallest reported effect size (0.90) using the t-test calculation for the difference between two independent means (to test for the main hypothesis) – this is the same method used in the original sample size calculation; and determined that we need a total of 116 participants (29 in each group) to achieve 85% power (as planned before). We assumed a type I error of 5% (alpha), and a type 2 error of 15% (beta). We also made this calculation considering the same parameters used in the original sample size calculation, such as dropout rates of 20%. The sensitivity analysis for the sample size calculation is summarized in the table below.

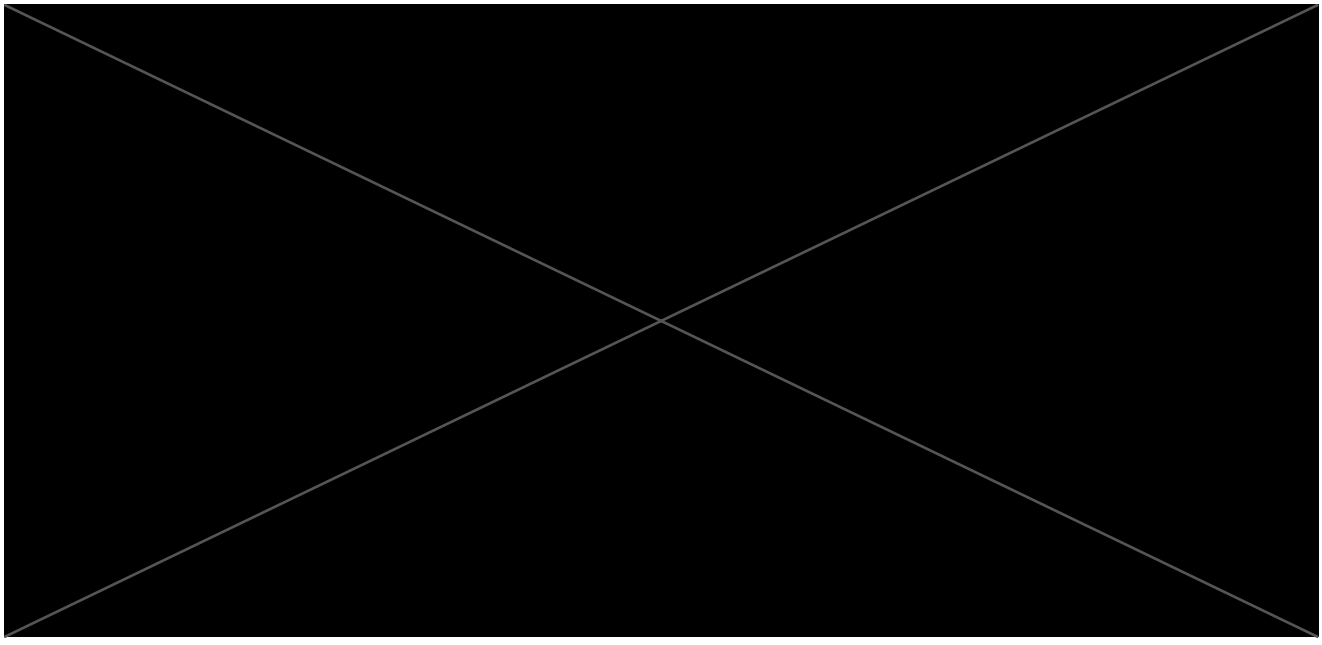
Therefore, we reduced the sample size from 148 to 116 participants, which will maintain the same planned statistical power (85%). This revised sample size will allow us to detect the more precise effect sizes currently reported in the literature and will also make the study feasible to be completed on time, given the large impact of the COVID pandemic on our study.

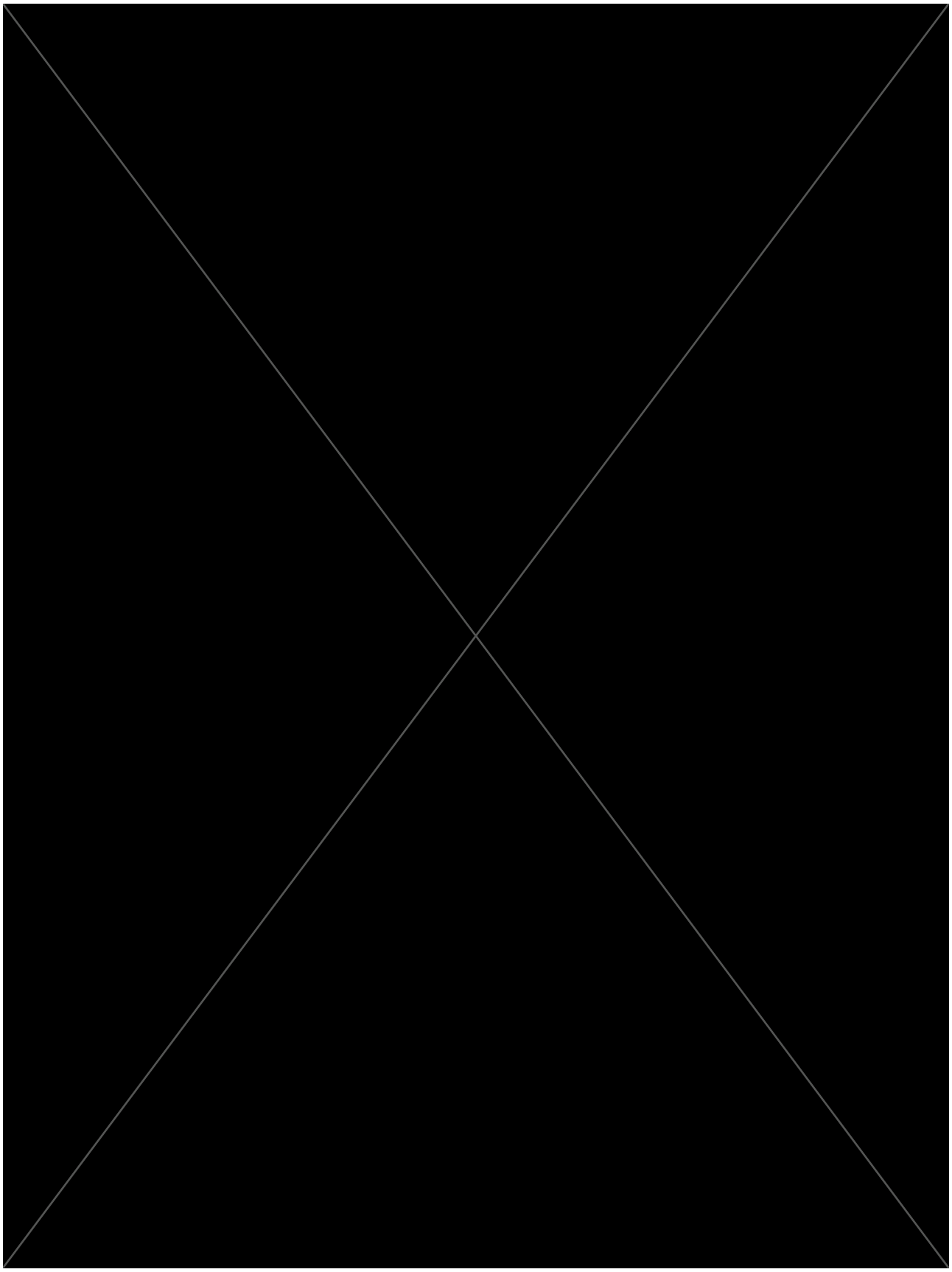
Planned Analysis:

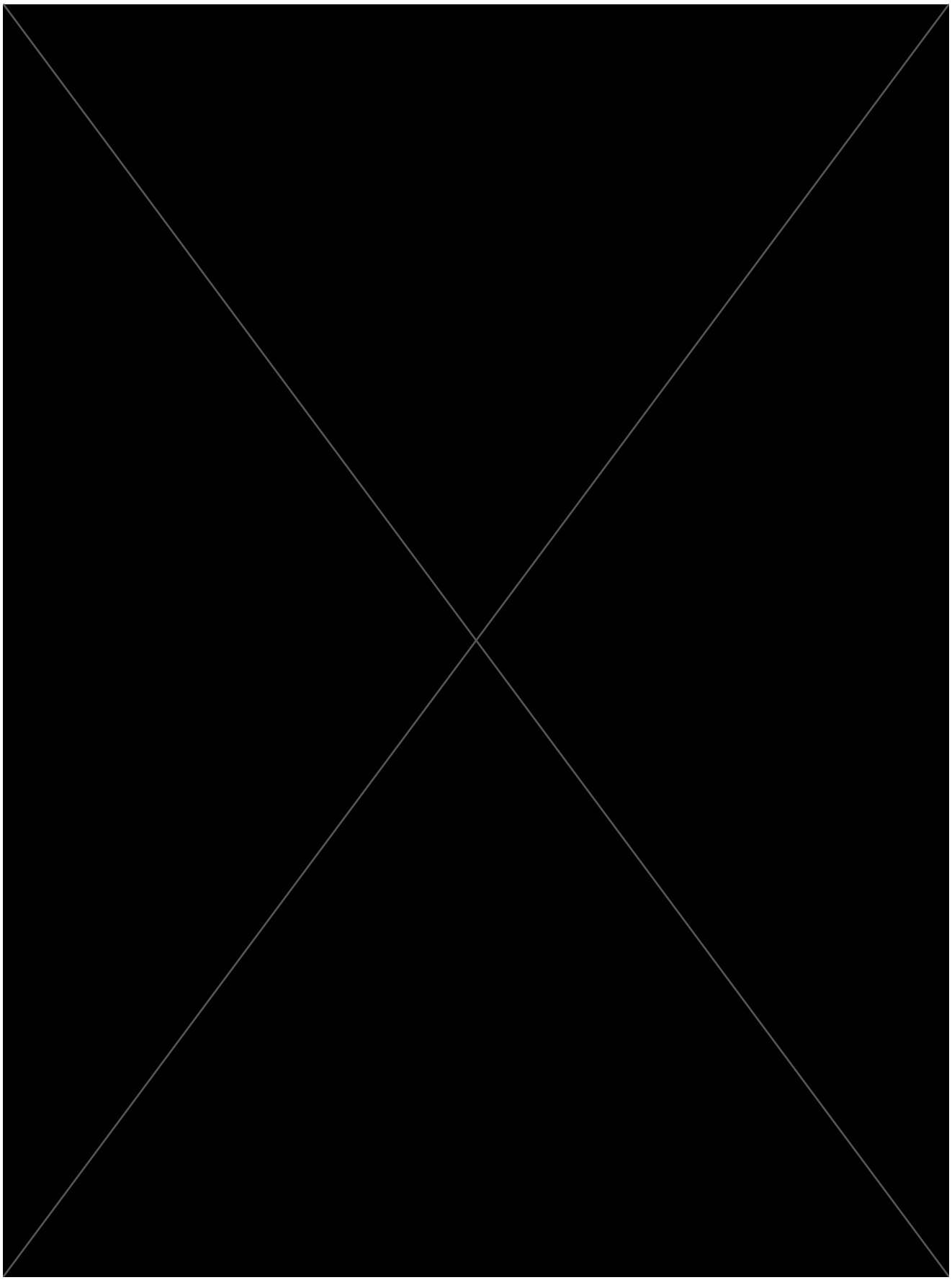
The primary analysis: All the analyses will be performed as intention-to-treat in which all subjects undergoing randomization who receive at least one intervention session (randomization will take place immediately before the first intervention) will be included. We will conduct sensitivity analyses and test different models of handling missing data (LOFC and MI). The change in the primary efficacy endpoint, CPM (and also TSPS), from baseline to week 4 will be tested with a mixed linear model. This model will be adjusted for important demographic variables (i.e., gender) and also baseline clinical parameters where appropriate. All tests will be two-sided (alpha level, 0.05). We will initially test our main hypothesis that the combined treatment induces a greater increase in CPM (and decrease in TSPS) as compared to the control condition. If the effect is significant, we will then test differences between the combined group vs these two interventions alone.

In order to understand the time effect of this intervention (using the secondary endpoints added in this model (week1 and follow-up)), we will run a secondary mixed linear model to estimate the rate of change over time by also including an interaction between treatment and time as to detect whether treatment effect changes differently according time. If the interaction is not significant we will then test whether there is a main effect of time that is independent of treatment level (interaction will be then removed from the model). We will adjust this model for important covariates such as age, gender, pain levels (numeric pain scale – NPS), and other baseline clinical outcomes where appropriate. For the secondary clinical variables with a significant effect we will also test whether they moderate the interventions' effects on our mechanistic outcomes, thereby gaining additional mechanistic insights.

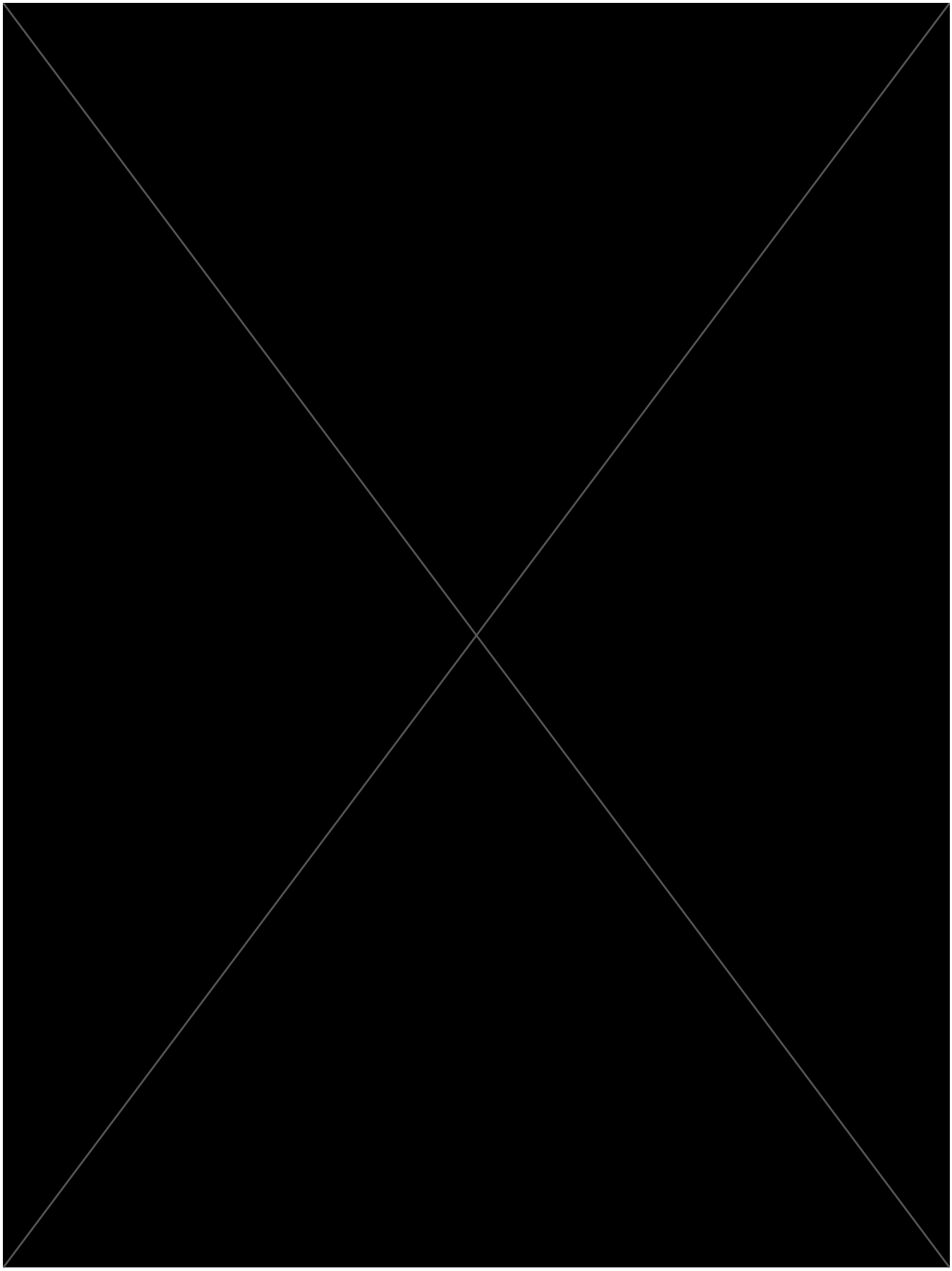
To complete our analysis, we will apply a path analysis [1, 2] to the outcome data (CPM and TSPS) to determine if endogenous pain modulation changes (as indexed by CPM and TSPS) associated with the combined intervention (tDCS and AE) is related to direct effects versus indirect effects through improvements in secondary outcomes. We propose that a direct effect of tDCS and AE on the endogenous pain inhibitory system can be inferred if the treatment effect cannot be explained by changes in psychological or functional outcomes.







Detailed Protocol 24



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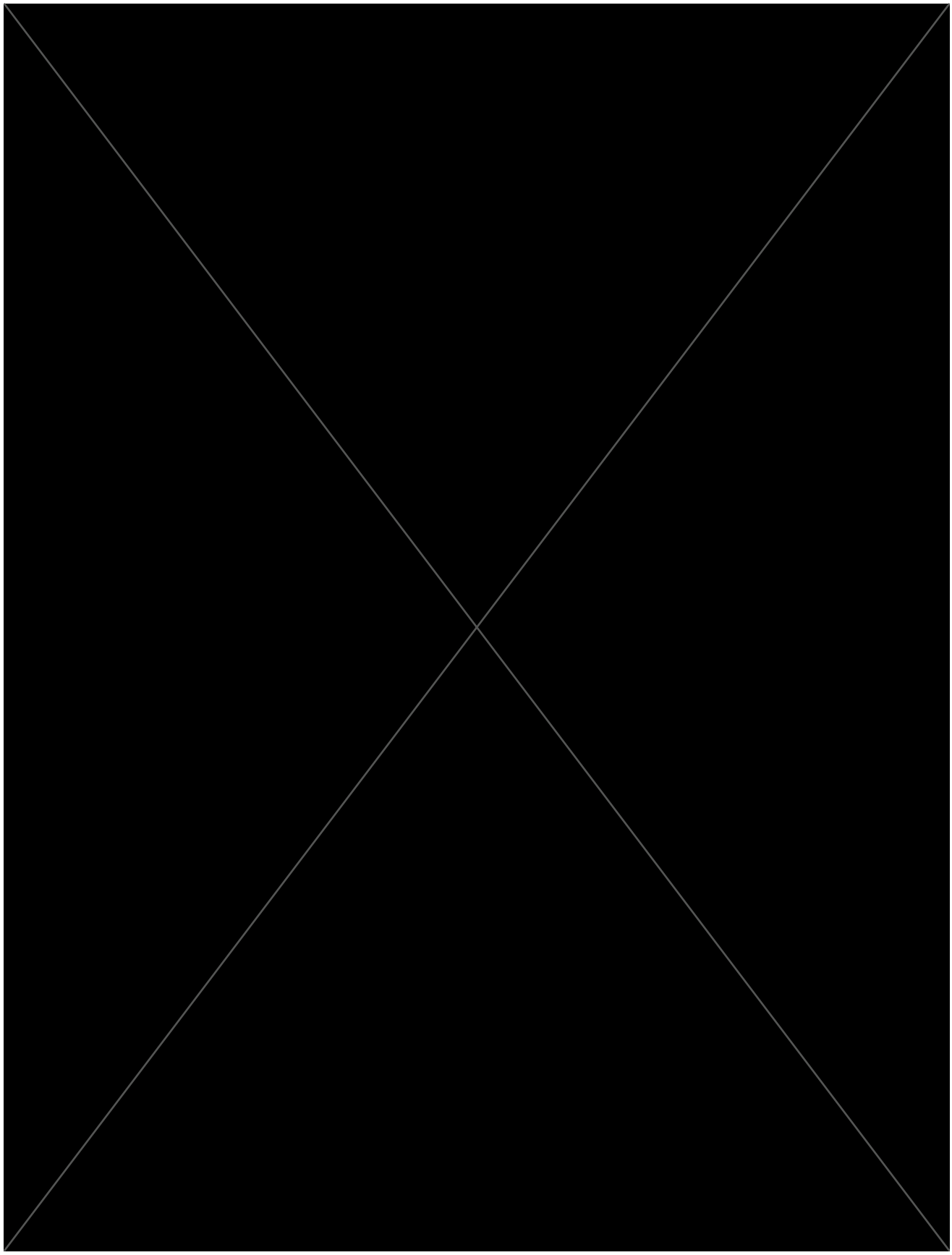

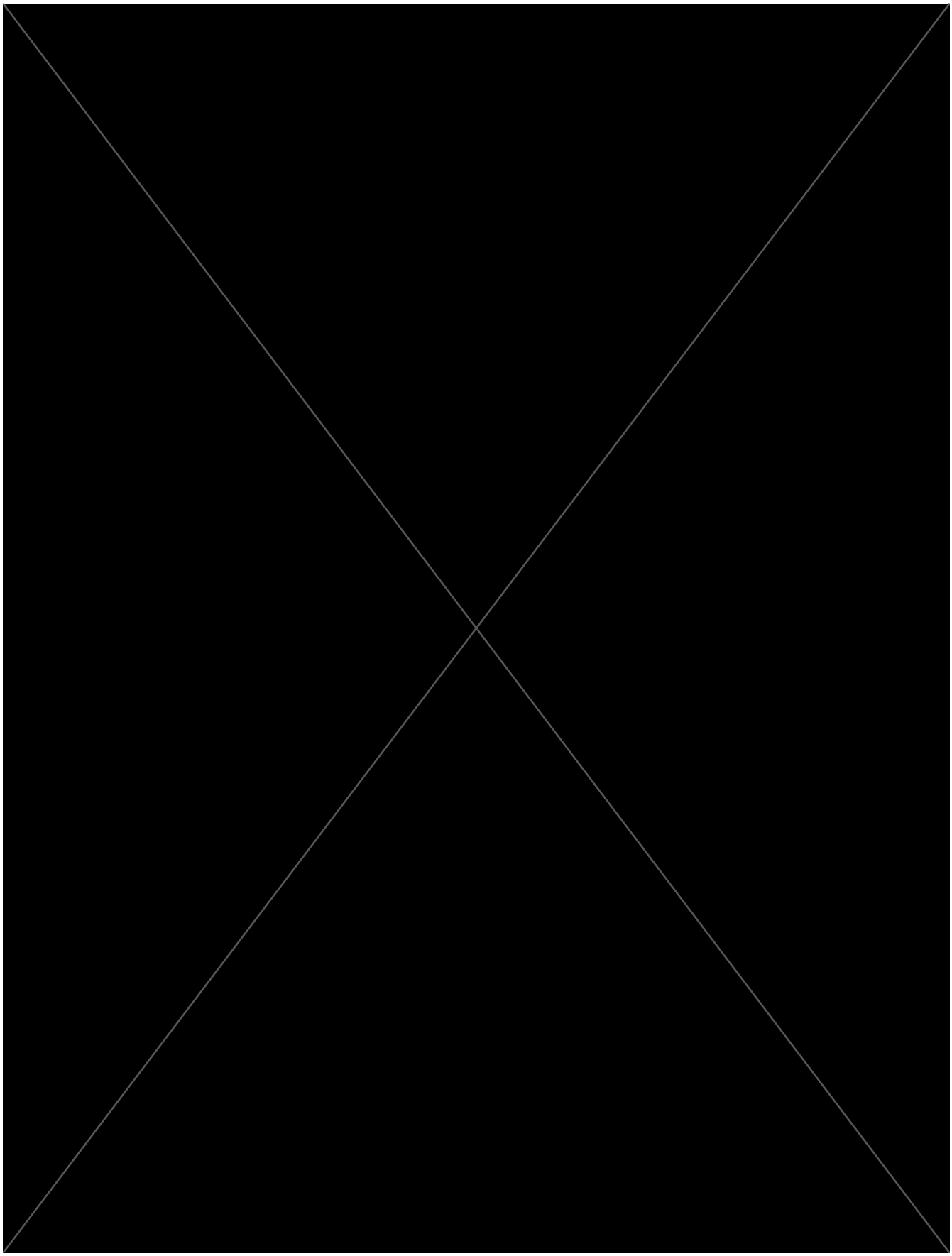

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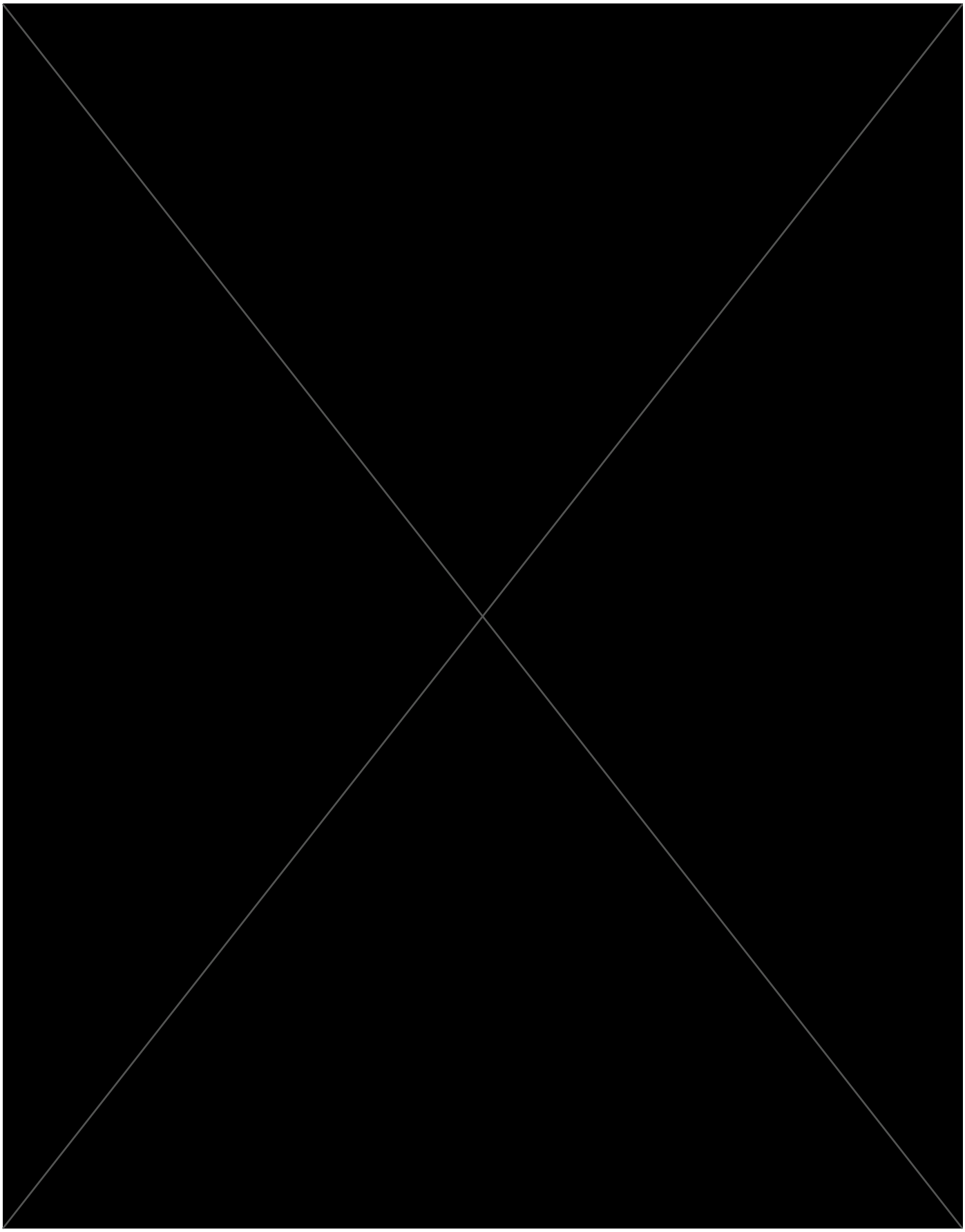

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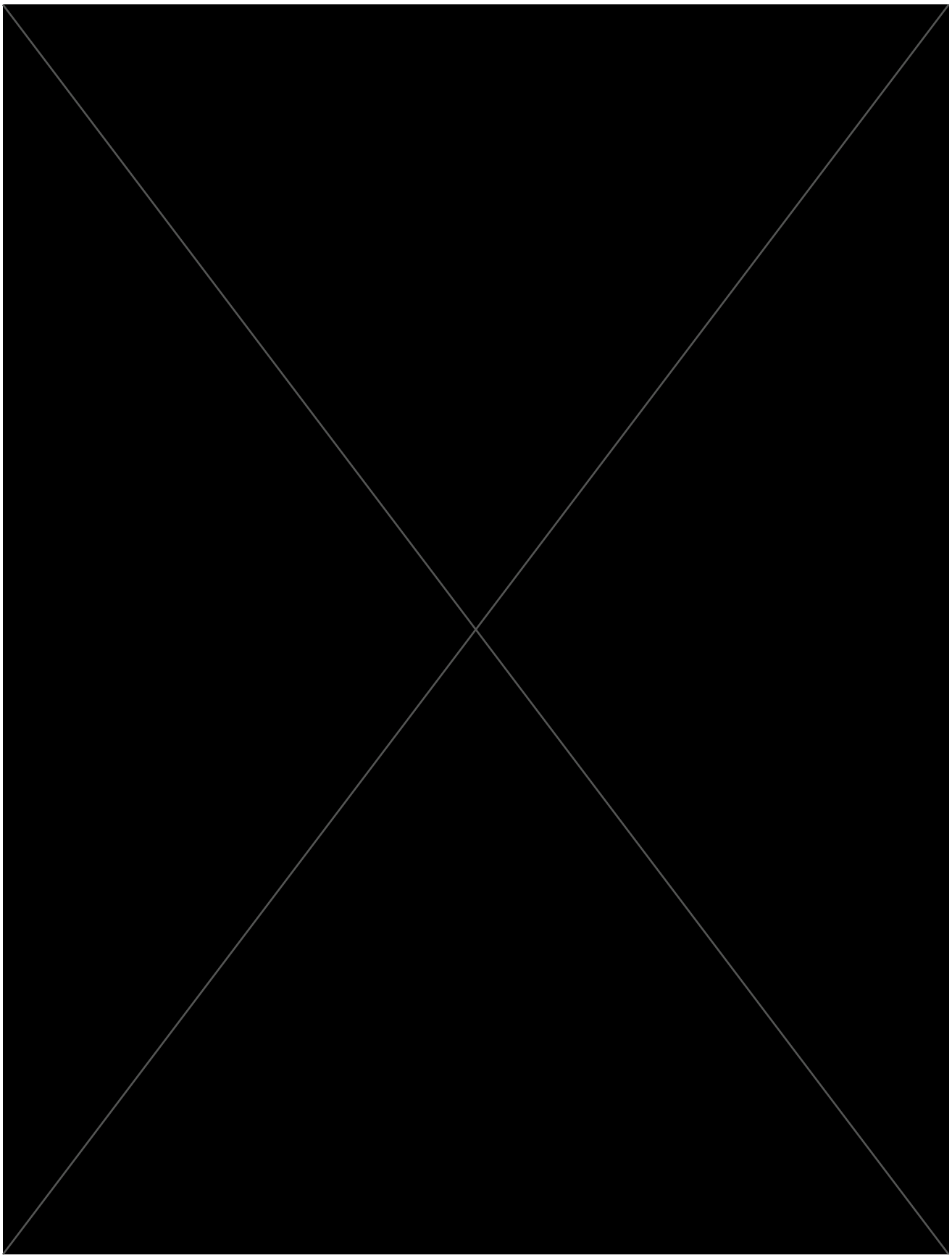

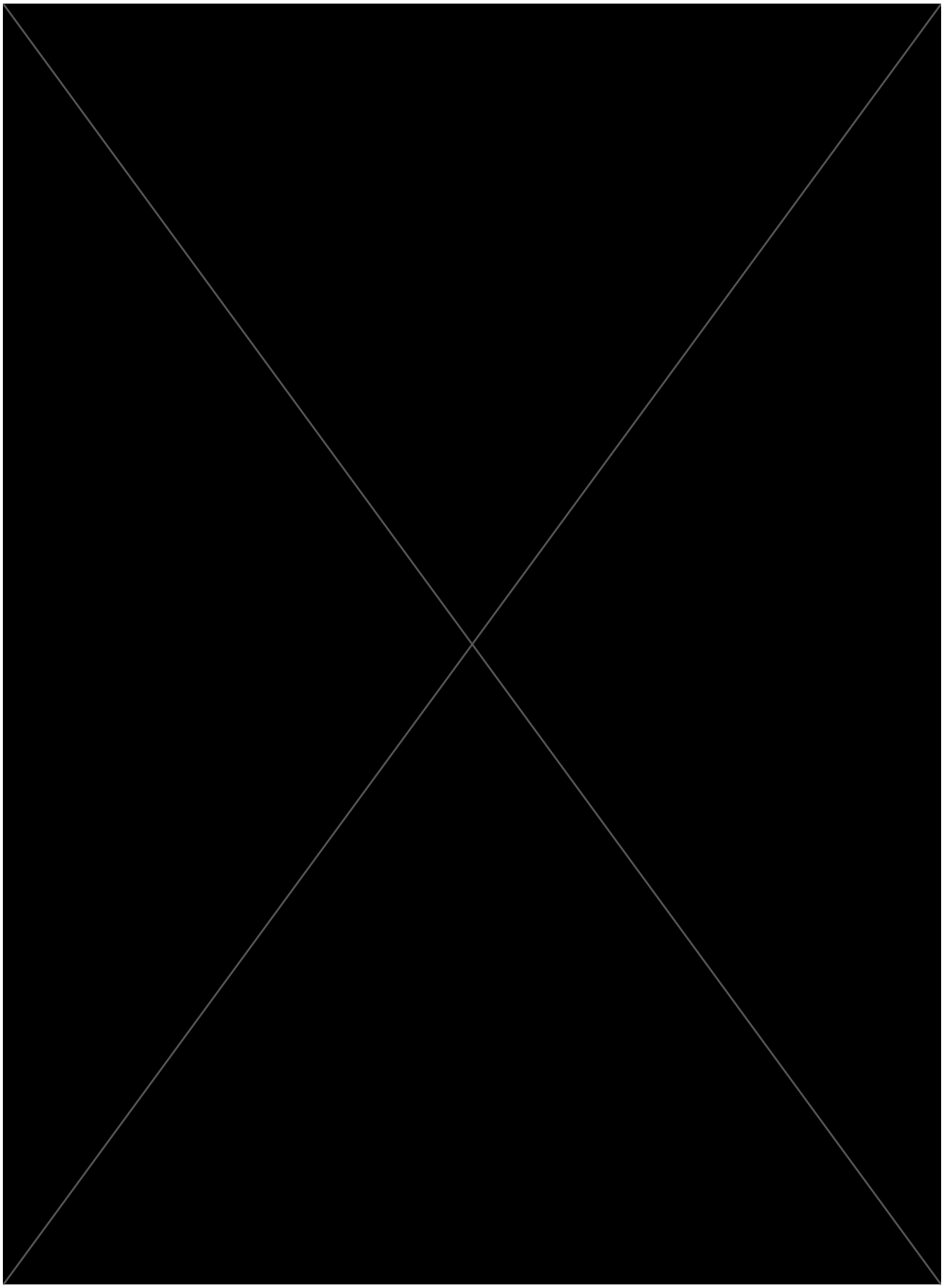

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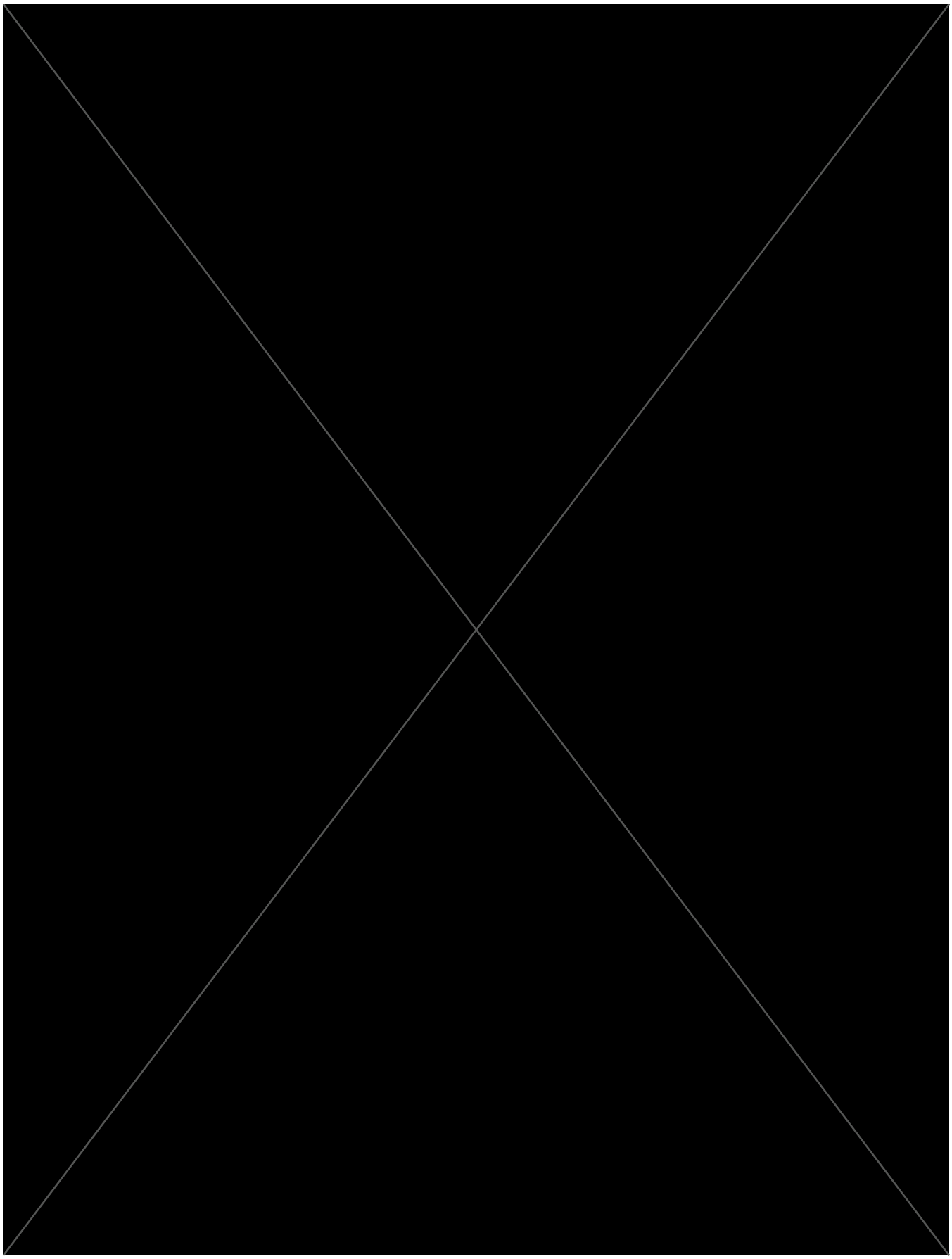

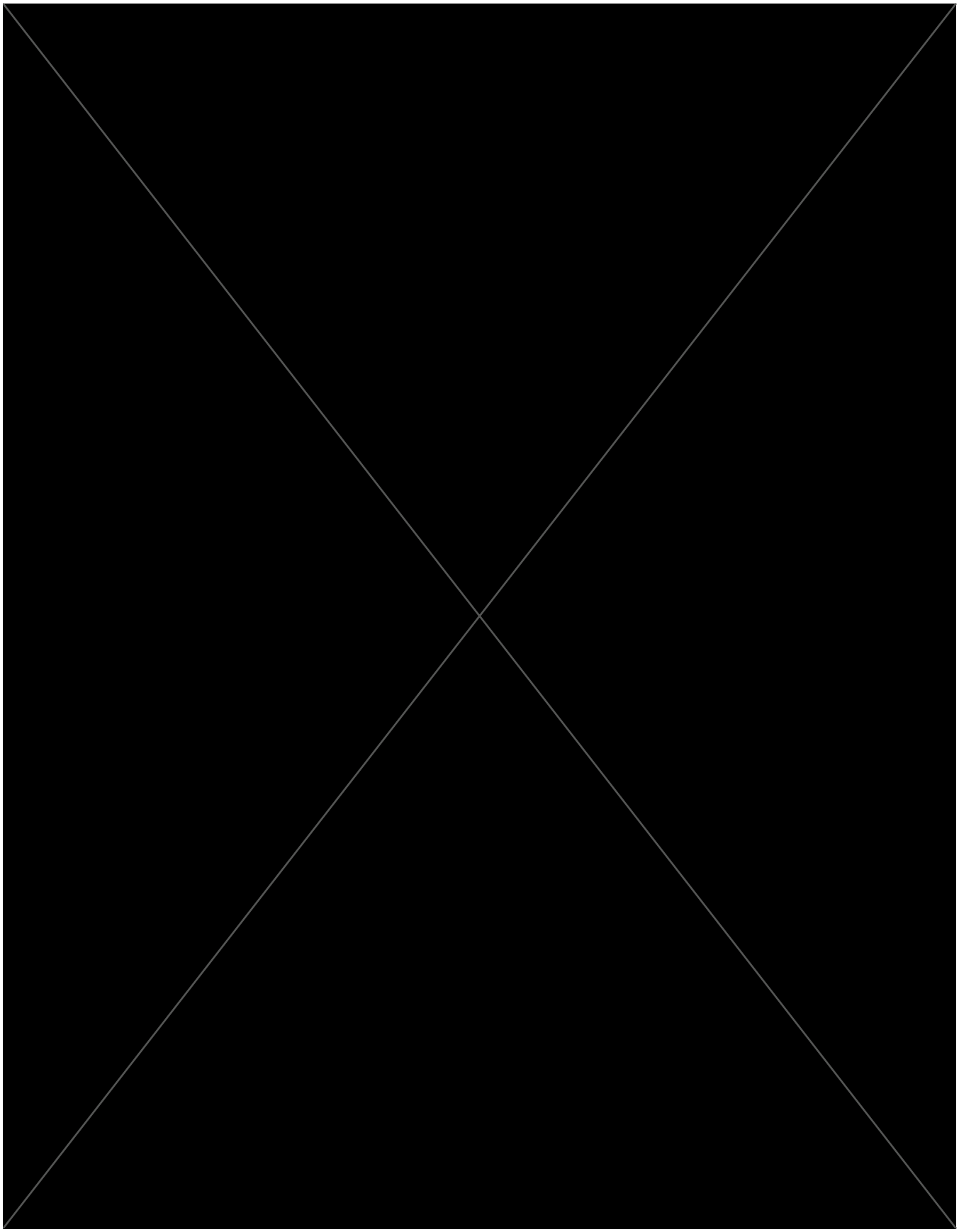

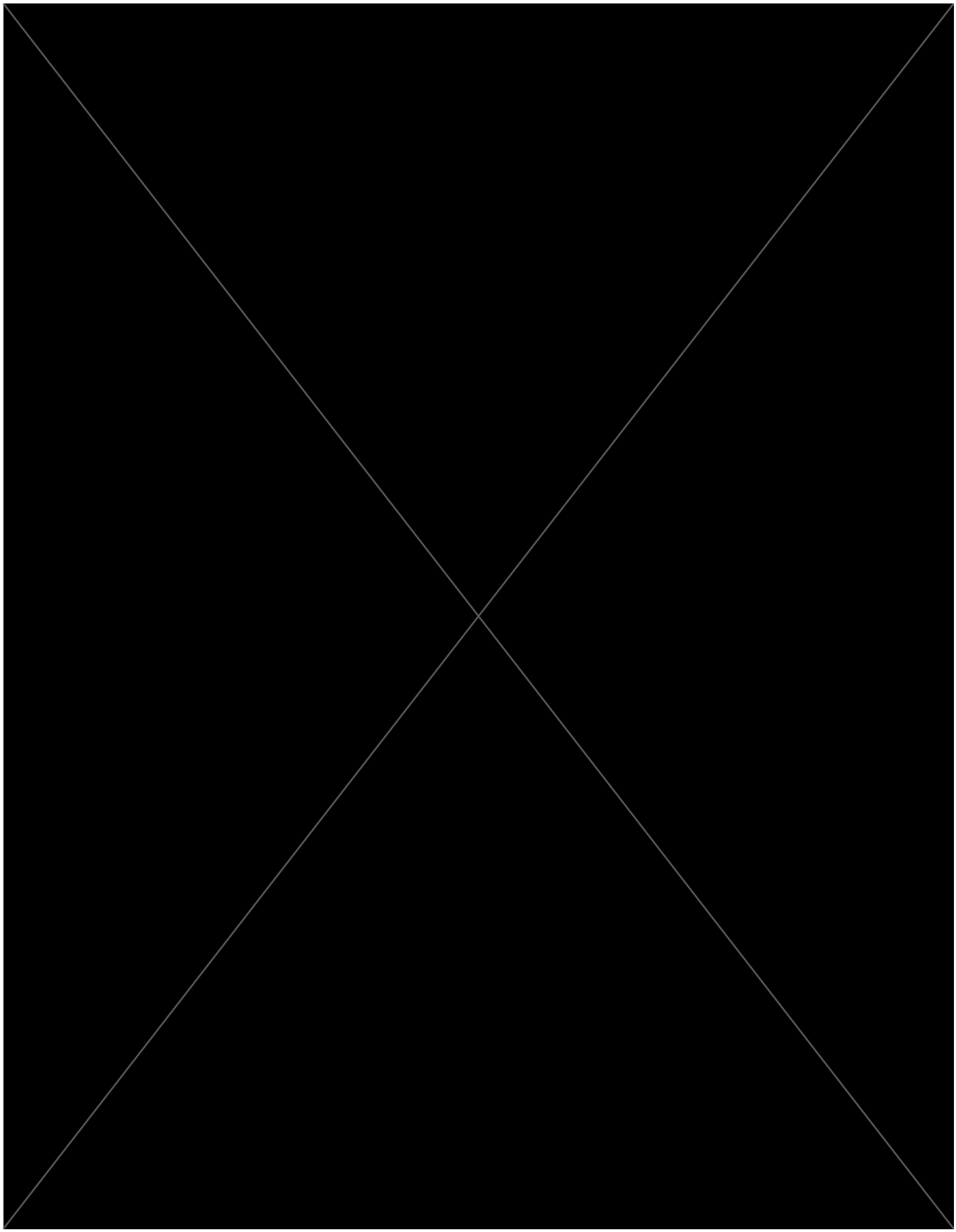

Detailed Protocol 33