Clinical Trials Study Protocol

Examining Transcutaneous Vagal Nerve Stimulation as a Facilitator of Social Bonding (NCT05899413)

Version 1

March 1, 2025

This study was approved by the Institutional Review Board at the University of California, San Francisco (#23-39189). We conducted a power analysis prior to the data collection to determine the power for the Level-2 direct effect (stimulation condition) based on 1,000 simulations using the package *simr* in R (R Core Team). Given the nature of our dyad and the outcomes (i.e., questions about the same interaction), we assumed a large intraclass coefficient (0.5). Following Arend and Schäfer's (2019) recommendations, the standardized Level-1 direct effect was set to small ($\gamma_{std.} = .10$) in our power estimation for the Level-2 effect. Our analysis suggested that 70 dyads will provide > 82% power (α set to .05) to detect a medium-to-large standardized Level-2 direct effect ($\gamma_{std.} = .40$).

Data collection for the study took place between February and June 2024. Participants were recruited in the San Francisco Bay Area through various community and university newsletters and listservs, flyers around the city, Reddit, and word of mouth. We tried to reach diverse populations by promoting the study through various community centers (e.g., SF LGBT Center) and universities in the city (e.g., San Francisco State University, UC Berkeley).

Eligibility was confirmed using a screening questionnaire. Participants were eligible for the study if both they and their partner were between 18 and 35 years old, fluent in English, had no major chronic diseases (e.g., cardiovascular disease) or untreated psychiatric diagnoses, were not taking any confounding medications (e.g., antihypertensive medications), and did not have unremovable piercings on the tragus. We restricted the age range to control for age-related differences in physiological responses. As HRV tends to decrease with age, we attempted to recruit a more homogenous group on one of our primary outcome variables (HRV level and reactivity), so we capped the age range to mid-adulthood. Participants were also required to have

been in a relationship with their partner for at least one year and be able to complete the in-person laboratory visit together.

Tasks and Measures

Pre-visit survey. Prior to the lab visit, participants completed pre-visit surveys that collected information on basic sociodemographic characteristics, relationship characteristics, and physical attributes, including height and weight. Self-reported height and weight were used to compute Body Mass Index (BMI; weight (*lb*) ÷ height^2 (inches) * 703), which we used as a covariate. Participants also responded to a 5-item measure of relationship satisfaction (e.g., "I feel satisfied with our relationship"; $\alpha = .81$) and a 7-item measure of commitment (e.g., "I want our relationship to last for a very long time"; $\alpha = .79$) from the Investment Model Scale (Rusbult et al., 1988), which we used to test differences between participants in the experimental and control conditions. Additionally, they rated 24 relationship issues (e.g., communication, family and in-laws; adapted from Geiss & O'Leary, 1981) on a scale ranging from 0 (*issues that rarely if ever raise conflict or disagreement*) to 10 (*issues that raise frequent or intense conflict or disagreement*). Participants' responses to this scale were used in the conflict interaction described below.

Gratitude interaction. The procedures were based on Algoe and colleagues (2013). Couples were told to think about a positive thing their partner did for them recently for which they felt grateful. They were given three minutes to prepare, then took turns to express their gratitude for three minutes each. While one partner was speaking, the listening partner was told to listen with minimal verbal reactions.

Conflict interaction. The procedures were based on Levenson and Gottman (1983) and previous studies that examined physiological reactivity during conflicts (e.g., Godfrey & Babcock, 2020; Raby et al., 2015). Couples were told that they would discuss a conflict topic

relevant to their relationship and try to resolve it. The interaction would involve the following steps: 1) couples selecting a topic to discuss together, 2) reflecting on the topic independently for three minutes, 3) each partner taking turns expressing their thoughts and feelings about the topic for one minute (the speaking order was predetermined based on the alphabetical order of their first names), and 4) couples engaging in free discussion for six minutes, with the goal of resolving the issue.

After receiving the instructions, couples were given a list of three topics to choose from. Unknown to the couples, this list was generated using participants' responses regarding the frequent topics of arguments that they provided in the pre-visit survey. The three topics were chosen based on the highest average ratings of the two partners. If no topics averaged higher than 3, the list included an option of "any topic that recently triggered a conflict or disagreement in the relationship."

Following the interaction, participants completed a short questionnaire that included measures of **positive affect** ("During the conversation, overall, I felt…"; 1: not at all positive, 7: extremely positive) and **negative affect** ("During the conversation, overall, I felt…"; 1: not at all negative, 7: extremely negative).

tVNS device and stimulation. We used a research edition of an auricular vagus nerve stimulator (tVNS RE, Vagus.net™, UK) that sends current-controlled stimulation in symmetric biphasic waveforms. The tVNS RE conforms to EU directive 93/42/EEC medical device standards and ISO/IEC 17050-1. This device consists of a handheld stimulator and an electrode clip. Following previous research (Kraus et al., 2007), couples in the sham stimulation condition had the electrode clip placed on the center of their left earlobe (free of cutaneous vagal innervation; Peuker & Filler, 2002) while those in the tVNS condition had the electrodes placed

on their tragus. While cymba concha has also been used for tVNS, there is no evidence that psychophysiological effects of tVNS depend on the stimulation site (Borges et al., 2021). In fact, tVNS effects on executive functioning may be larger when stimulation is applied at the tragus rather than cymba concha (Ridgewell et al., 2021). We stimulated the left ear, which has been a common practice as the efferent vagal fibers to the heart are located on the right side (Kim et al., 2022) but note that the side effects are considered negligible and studies have also used right-sided stimulation (see Kaduk et al., 2023).

The experimenter first cleaned the stimulation loci with alcohol cotton swabs to reduce skin resistance. To identify the lowest stimulation intensity that participants could perceive, we adopted the staircase procedures used in previous research (e.g., Schuerman et al., 2021). Specifically, the experimenter placed the electrode clip on the participant's left ear (tragus or earlobe) and increased the stimulation levels by 0.1mA, starting from zero, until the participant reported feeling a tingling sensation. Experimenters confirmed the threshold twice, and the stimulation level was set 0.2mA below each participant's perceptual threshold (Schuerman et al., 2021) to allow couples to converse as naturally as possible and to attempt to keep participants unaware of their condition assignment. We delivered stimulation at the individually calibrated intensity continuously during the interaction, and the default device settings we adopted were as follows: frequency = 30Hz, phase duration = 250μs, interphase interval = 50μs, and max voltage = 65V.

Physiological data acquisition. Participants' physiological responses were continuously recorded during the baseline and all interactions. We collected electrocardiography (ECG) and impedance cardiography, which were integrated using an MP150 system (Biopac Systems Inc., Goleta, CA). Spot sensors were placed in a modified lead II configuration on the torso (near the

right clavicle and below the left rib cage) to measure ECG (Mendes, 2009). Data were acquired using Acqknowledge software at a sampling rate of 1000Hz (Biopac Systems Inc, Goleta, CA, USA). RSA was edited and scored in 30-second intervals using the HRV module from Mindware Technologies (Gahanna, OH), which identified the R spikes of each heartbeat in the ECG. Although 60-second epochs are generally recommended, 30-second epochs have been reliably used in previous dyadic studies (Caldwell et al., 2018; Oshri et al., 2023; Qaiser et al., 2023) and may sufficiently capture high-frequency activity especially when the heart rate is elevated (i.e., shorter heart period; Quigley et al., 2024), as in conflict settings. After data collection, trained research assistants blind to the condition visually inspected all data for artifacts and edited them as needed (e.g., removal of misplaced R-peaks).

Post-study survey. At the end of the study, before debriefing and being informed of the condition they were assigned to, participants answered a few questions about their experience of the stimulation and the tVNS device. Specifically, we asked about **awareness of the stimulation** ("How much, if at all, could you feel the tingling sensation during the conversations?"), **general discomfort** ("How uncomfortable was wearing the tVNS device [i.e., the clip you had on your ear] throughout the study?"), **perceived influence of discomfort on interactions** ("How much, if at all, did the discomfort [from wearing the clip] affect your conversations?"), and **perceived influence of tVNS on interactions** ("How much, if at all, do you think the device changed the way you felt or interacted with your partner during the conversations?"). All items were rated on a 5-point scale.

Study Procedure

Interested participants first completed a screening questionnaire to confirm eligibility. Eligible participants were then contacted via email to schedule a lab visit. Two days prior to the

scheduled visit, participating couples received a link to a consent form and a questionnaire assessing sociodemographic information and relationship characteristics. Couples were randomly assigned to either tVNS or sham stimulation condition when they both completed the pre-visit survey. Each couple had an equal chance of being in either group.

Upon arrival at the lab, partners were escorted to separate rooms where they each received a full overview of the study and underwent threshold testing for stimulation. Then partners reunited in the same room, were connected to physiological sensors, and spent five minutes, resting in a comfortable seated position (baseline). As shown in Figure 1, they were seated at an angle, partially facing each other, with their bodies slightly tilted toward the front where a fixed wall-mounted camera was positioned. Participants were recorded during gratitude and conflict interactions. Full study procedure, including the timing of the stimulation is illustrated in Figure 2. Following a cool-down period, participants completed a brief questionnaire about their experience with the stimulation and were debriefed. They also signed an additional consent form for the use of their recorded videos.

Figure 1. Example Positioning of the Couple During the Study

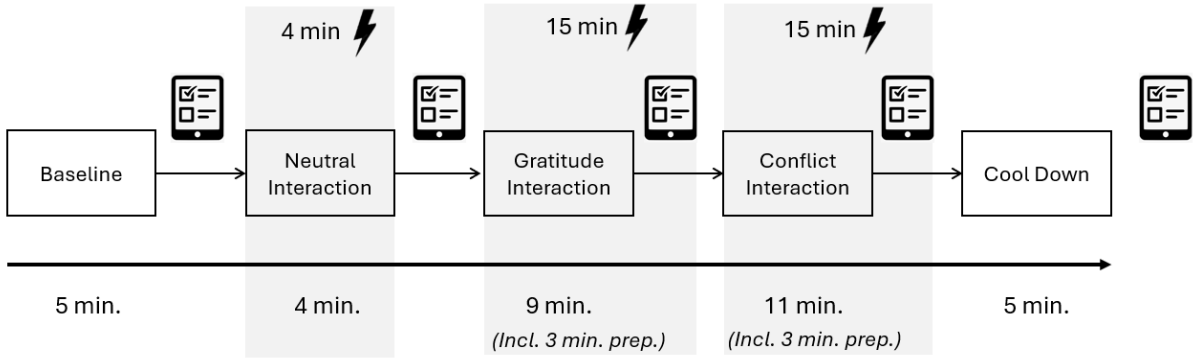

Figure 2. Overview of Study Procedure

Notes. The time next to the lightning icon indicates the duration of the (active or sham) stimulation. The time at the bottom represents the duration of each study segment described within the rectangles. The survey icon indicates when participants complete short questionnaires.

Statistical Analysis Plan

All analyses will be conducted in R (R Core Team, 2022). Multilevel model analyses will be conducted using the packages *nlme* and *lmer*. All tests will be two-tailed, with a significance threshold of $\alpha = .05$.

Randomization check. We will first examine whether there are condition differences in personal and relational characteristics at baseline, including age, relationship duration, global relationship satisfaction, and commitment. To account for the interdependence of the dyadic data, we will fit a series of multilevel models with a random intercept included for participants nested within couples and condition included as a fixed effect. Restricted Maximum Likelihood will be used to fit the model parameters. For relationship duration, which is a couple-level variable, Welch's t-test will be conducted.

Study artifact check. We will examine if there are condition differences in participants' experiences related to the stimulation and the tVNS device reported at the end of the study, including awareness of the stimulation, general discomfort, perceived influence of discomfort on interactions, and perceived influence of tVNS on interactions. We will run a parallel set of multilevel models as described above.

tVNS effects on emotional experience of the conflict. We will examine if there are condition differences in how couples self-report their experience of the conflict interaction, specifically in terms of positive and negative affect during the interaction. We will run a parallel set of multilevel models as described above.

tVNS effects on physiological reactivity. As in previous research, we will calculate reactivity scores by subtracting the raw RSA score during the last 30 seconds of baseline from every segment of the interaction. We will fit a multilevel model, first estimating the effect of condition (coded as -0.5 for sham and 0.5 for tVNS) on RSA reactivity with no covariates. As in previously described multilevel models, we will include a random intercept for participants nested within couples, and as recommended for physiological data, with autoregressive covariance structure of the residuals. In our follow-up models, we will control for sex (male/female/other), age, BMI, and relationship duration, which can influence physiological reactivity to conflicts. Then, to rule out the possibility that any condition effects are reducible to differences in the experience of the stimulation, the next model will additionally control for stimulation intensity and discomfort reported at the end of the study. We will repeat these analyses using IBI in place of RSA.

References

Algoe, S. B., Fredrickson, B. L., & Gable, S. L. (2013). The social functions of the emotion of gratitude via expression. *Emotion, 13*(4), 605–609.

Arend, M. G., & Schäfer, T. (2019). Statistical power in two-level models: A tutorial based on Monte Carlo simulation. *Psychological Methods, 24*(1), 1-19.

Borges, U., Laborde, S., & Raab, M. (2019). Influence of transcutaneous vagus nerve stimulation on cardiac vagal activity: Not different from sham stimulation and no effect of stimulation intensity. *PloS one, 14*(10), e0223848.

Caldwell, W., da Estrela, C., MacNeil, S., & Gouin, J. P. (2019). Association between romantic partners' rumination and couples' conflict is moderated by respiratory sinus arrhythmia. *Journal of Family Psychology, 33*(6), 640-648.

Geiss, S. K., & O'Leary, K. D. (1981). Therapist ratings of frequency and severity of marital problems: Implications for research. *Journal of Marital and Family Therapy, 7*(4), 515-520.

Godfrey, D. A., & Babcock, J. C. (2020). Facial affect recognition moderates the relation between autonomic nervous system reactivity and aggression during dyadic conflict. *Psychophysiology*, *57*(9), e13588.

Kaduk, K., Petrella, A., Müller, S. J., Koenig, J., & Kroemer, N. B. (2023). Non-invasive vagus nerve stimulation decreases vagally mediated heart rate variability. *bioRxiv*, https://doi.org/10.1101/2023.05.30.542695

Kim, A. Y., Marduy, A., de Melo, P. S., Gianlorenco, A. C., Kim, C. K., Choi, H., Song, J.J., & Fregni, F. (2022). Safety of transcutaneous auricular vagus nerve stimulation (taVNS): A systematic review and meta-analysis. *Scientific Reports*, *12*(1), 22055.

Kraus, T., Hösl, K., Kiess, O., Schanze, A., Kornhuber, J., & Forster, C. B. O. L. D. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of Neural Transmission, 114*, 1485-1493.

Levenson, R. W., & Gottman, J. M. (1983). Marital interaction: Physiological linkage and affective exchange. *Journal of Personality and Social Psychology*, *45*(3), 587-597.

Mendes, W. B. (2009). Assessing autonomic nervous system activity. In E. Harmon-Jones, & J. S. Beer (Eds.), *Methods in social neuroscience* (pp. 118–147). New York, NY: The Guilford Press.

Oshri, A., Liu, S., Suveg, C. M., Caughy, M. O. B., & Huffman, L. G. (2023). Biological sensitivity to context as a dyadic construct: An investigation of child–parent RSA synchrony among low-SES youth. *Development and Psychopathology, 35*(1), 95-108.

Peuker, E. T., & Filler, T. J. (2002) The nerve supply of the human auricle. *Clinical Anatomy, 15,* 35–37.

Qaiser, J., Leonhardt, N. D., Le, B. M., Gordon, A. M., Impett, E. A., & Stellar, J. E. (2023). Shared hearts and minds: Physiological synchrony during empathy. *Affective Science, 4*(4), 711-721.

Quigley, K. S., Gianaros, P. J., Norman, G. J., Jennings, J. R., Berntson, G. G., & de Geus, E. J. (2024). Publication guidelines for human heart rate and heart rate variability studies in psychophysiology—Part 1: Physiological underpinnings and foundations of measurement. *Psychophysiology*, e14604.

Raby, K. L., Roisman, G. I., Simpson, J. A., Collins, W. A., & Steele, R. D. (2015). Greater maternal insensitivity in childhood predicts greater electrodermal reactivity during

conflict discussions with romantic partners in adulthood. *Psychological Science, 26*(3), 348-353.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ridgewell, C., Heaton, K. J., Hildebrandt, A., Couse, J., Leeder, T., & Neumeier, W. H. (2021). The effects of transcutaneous auricular vagal nerve stimulation on cognition in healthy individuals: A meta-analysis. *Neuropsychology*, *35*(4), 352.

Rusbult, C. E., Martz, J. M., & Agnew, C. R. (1998). The investment model scale: Measuring commitment level, satisfaction level, quality of alternatives, and investment size. *Personal Relationships, 5*(4), 357-387.

Schuerman, W. L., Nourski, K. V., Rhone, A. E., Howard, M. A., Chang, E. F., & Leonard, M. K. (2021). Human intracranial recordings reveal distinct cortical activity patterns during invasive and non-invasive vagus nerve stimulation. *Scientific Reports, 11*(1), 22780.