Official Title: Somatosensory Modulation of Salivary Gene Expression and Oral Feeding in Preterm Infants
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Statistical Analysis Plan.

Primary Outcome: Gene Expression

In this randomized controlled clinical trial, we analyzed salivary developmental gene expression changes in extreme and very preterm infants randomized to receive either therapy with the NTrainer feeding system or a 'Sham' mode (blind pacifier) to assess the effect of therapy on the developing infant. Salivary samples were collected at a series of timepoints, beginning with the initiation of therapy through the learning process of oral feeding or to transfer to an outlying hospital. The primary goal of this study was to assess and compare differences in gene expression patterns over time between groups. The expression of six genes involved in 1.) memory: *CDH13;* oral development*: FOXP2* and *WNT3;* 3.) sensory integration*: NPHP4,* and *PLXAN1*; and hunger signaling: *NPY2R,* along with two reference genes for proper normalization were measured via RT-qPCR for each subject at 6 timepoints. The raw CT values were normalized for each subject and timepoint by subtracting the geometric mean of the reference genes from the mean expression of each gene (deltaCT). These deltaCT values were used for the remainder of the analysis. A positive deltaCT value indicates that the target gene had lower expression than the reference gene; conversely increasing deltaCTs are indicative of decreasing expression. In order to determine changes in gene expression, one way and two-way ANOVA analysis in R [1] was used. Initially, the analysis was done to access expression pattern changes over time for 1.) the cohort as a whole, 2.) based on treatment status, and 3.) by sex. Given that the timepoint measurement are done on the same subject, repeated measure ANOVA and subject for which all time point were not measured were excluded from the analysis. Subsequently, the analysis was extended by grouping subjects by bronchopulmonary dysplasia (BPD) status, looking at the effect over time, the effect of treatment status and the interaction of the two. BPD was defined based on NIH criteria.

Secondary Outcomes: Non-nutritive Suck Dynamics and Growth in Daily Oral Feeds

A-priori power calculation indicated that a sample size of 110 was required for 80% power, with an assumed correlation of 0.60 among repeated measures and a small effect over time (Cohen's f = 0.10). This effect was considerably smaller than those observed in our previous empirical data (f = 0.22-0.24), suggesting adequate power for the final sample of 114 neonates in this study.

Linear mixed modeling (LMM) analysis was performed for non-nutritive suck (NNS) variables including NNS Bursts/minute, NNS Suck Cycles/minute, NNS Amplitude, NNS Spatiotemporal Index, and the transition to independent oral feeds based on daily measures of percent oral intake (%PO) to examine the effects of GA, PMA, sex (male, female), respiratory diagnosis (non-BPD, BPD), treatment (NTrainer, Sham), intervention phase (0, baseline), (1, low-dose), (2, high-dose), (3, post intervention), and potential interactions. The models

accounted for nesting of repeated measurements within infants (i.e., intraclass correlation), and a proper error covariance structure was determined based on model fit (i.e., adjusted Akaike Information Criterion, Bayesian Information Criterion). In addition, locally estimated scatterplot smoothing (LOESS) analysis was conducted to estimate the trajectory of %PO growth over PMA. All statistical analyses were conducted using R [**1**] and SAS 9.4 [**2**].

References

1. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing (2019) http://www.R-project.org/
2. SAS Institute. SAS/STAT 9.4 user's guide. (2002-2012).