2021

Transcutaneous Auricular Vagus Nerve Stimulation (TAVNS): Long-Term Effect on Neurodevelopment and Sensory Performance

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Recommended Citation

Aljuhani, Turki Khaild, "Transcutaneous Auricular Vagus Nerve Stimulation (TAVNS): Long-Term Effect on Neurodevelopment and Sensory Performance" (2021). *MUSC Theses and Dissertations*. 565.
https://medica-musc.researchcommons.org/theses/565

TRANSCUTANEOUS AURICULAR VAGUS NERVE STIMULATION (TAVNS): LONG-TERM EFFECT ON NEURODEVELOPMENT AND SENSORY PERFORMANCE

by

Turki Khaild Aljuhani, MA, BAppSc

A dissertation submitted to the faculty of the Medical University of South
Carolina in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in the College of Health Professions

Acknowledgments

My dissertation committee chaired by Dr. Craig Velozo including Dr. Patricia Coker-Bolt, and Dr. Dorothea Jenkins without their guidance continues support, expert advice, and critical feedback I would not have completed this wonderful journey without this support.

Dr. Patricia Coker-Bolt has and will always be my mentor, advisor, and supporter. Without her continues support, positive attitude, engorgement, and positive feedback this work would not been completed. Thank you for believing in me.

Dr. Dorothea Jenkins thank you for allowing me to be part of this novel and incredible research, the knowledge I gain from your mentoring is a highlight in my work and will reflect my future work.

Dr. Craig Velozo thank you for your advice and support, and helpful criticisms, without a doubt this work would not been finished without them.

Many Thanks to the children and their families without them this research would not happen.

This dissertation is dedicated to my mother, father, and all my friends who stood beside, belief in me and support me throughout this amazing journal of learning. Without my parents constant support, emotional help, none of this would be possible.

Abstract of dissertation presented to the
Doctor of Philosophy Program in Health and Rehabilitation Science
Medical University of South Carolina
In Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy

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Infants who do not succeed at early feeding are likely discharged from the nursery with a gastrostomy tube (G-tube), putting them at risk for worse neurodevelopmental and sensory outcomes than infants who achieve full oral feeds. This study aims to investigate the impact of Non-invasive transcutaneous auricular vagal nerve stimulation (taVNS) on infants' early motor development and long-term neurodevelopmental sensory performance at 18 months. Besides the observed feeding improvement using taVNS paired with bottle-feeding, we explore if pre-treatment total STEP scores' is able to predict response to taVNS intervention. The pre-treatment total STEP scores did not contribute to the prediction model significantly. Then, we looked at the long-term effect of early taVNS treatment in both neurodevelopmental and sensory outcomes at 18 months follow-up. We found that infants who responded to early taVNS treatment when paired with bottle-feeding had better overall neurodevelopmental outcomes than non-responders. We also found that responders had significantly better typical scores in the general sensory section, and had more typical average mean scores in almost all the sensory profile sections than non-responders. These preliminary results are encouraging of the use of taVNS. Future studies can include randomization of active and control taVNS intervention with larger sample size.

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CHAPTER 1: INTRODUCTION

1. INTRODUCTION

One of the first essential functions of a newborn is to successfully and safely take in enough nutrition to grow and thrive. Safe and successful early feeding requires the infant to demonstrate mastery of oromotor skills shortly after birth. However, it is estimated that between 25 to 45% of typically developing infants experience oral feeding difficulties (Bryant-Waugh, Markham, Kreipe, & Walsh, 2010; Ramsay, Gisel, McCusker, Bellavance, & Platt, 2002). This percentage increases to 80% in infants with developmental delays (Bryant-Waugh et al., 2010; Reilly, Skuse, Wolke, & Stevenson, 1999). The American Academy of Pediatrics regards successful oral feeding as one of the significant criteria for hospital discharge, especially with high-risk infants, including preterm infants, infants with special health needs, and infants with anticipated early death ("Hospital Discharge of the High-Risk Neonate," 2008). Infant feeding difficulties are known to increase hospital stay, add stress to the parents, and impact the infant's typical developmental trajectory (Aagaard, Uhrenfeldt, Spliid, & Fegran, 2015; Adamkin, 2006; Jackson, Kelly, McCann, & Purdy, 2016).

Gastrostomy tube (g-tube) placement is an invasive solution used for infants with feeding difficulties, and in recent years, the percentage of g-tube placement has increased significantly. For example, between the years 2000 and 2012, g-tube placement incidence in children doubled (Hatch et al., 2018). The g-tube placement rate increased the most for infants <1 year of age by 32% from the years 1997 to 2009 (Fox et al., 2014).

However, studies have reported that infants discharged with a g-tube within the first year of life are more prone to neurodevelopment and sensory problems (Mason, Harris, & Blissett, 2005). For instance, when comparing infants who were discharged after full oral feeding, Jadcherla et al. (2017) found that infants with a g-tube were more likely to have lower scores in all neurodevelopment outcomes (cognitive, language, and motor). The authors also noted that the majority of infants did not require g-tube placement in the first place (Jadcherla et al., 2017). Another recent study found that 61% of infants with a g-tube had neurodevelopmental delays at 18 to 22 months follow-up (Warren et al., 2019).

Studies have shown that the head and neck's fundamental movements are essential for successful early oral feeding (da Costa et al., 2010). While many studies have found an association between early oromotor sucking behavior in infants and later developmental delays, no studies have been able to predict infants’ feeding performance based on early motor performance, especially movements related to the head and neck (Tsai, Chen, & Lin, 2010; Wolthuis-Stigter et al., 2017; Wolthuis-Stigter et al., 2015; Zhang, Zhou, Yin, Dai, & Li, 2017). In fact, existing evidence is unable to determine the relationship between early sucking behaviors and neonatal brain injury or how early oromotor and feeding behavior relates to later developmental skills (Slattery, Morgan, & Douglas, 2012).

Children with cerebral palsy (CP) are at a higher risk of oral feeding problems and swallowing deficits (Arvedson, 2013). Generally, the more severe the motor issues, the more severe the swallowing and oral feeding problem (Calis et al., 2008; Parkes, Hill, Platt, & Donnelly, 2010). Yet, feeding difficulties are prevalent even in children with mild CP. This may lead to inadequate consumption of food and liquid, nutrition issues, and slow advancement of oral motor skills (Arvedson, 2013; Benfer et al., 2013).

Recently, an international consensus recommended the use of early motor assessment to detect CP at a very early stage of the infant's life (>3 months corrected age (CA)) (Novak et al., 2017). The application of this consensus can be extended to other motor-based delays. However, current assessments make implementing the consensus findings difficult as they a) require lengthy and rigorous training that makes it hard to use a large number of trainers (Maitre, 2018), or b) early infant motor assessments lack strong psychometric properties (Campbell, Swanlund, Smith, Liao, & Zawacki, 2008; Kim, Lee, & Lee, 2011).

The Specific Test of Early Infant Motor Performance (STEP) can provide a solution to these challenges. The STEP is a novel, quick, and easy-to-learn developmental screening test. The test consists of ten movement items; the STEP can be administered at term and three months for CA infants to establish and validate cutoff scores for both time points (Gower, Jenkins, Fraser, Ramakrishnan, & Coker-Bolt, 2019).

Lately, non-invasive neuromodulation techniques are successfully delivered more in pediatric populations. Specifically, neuromodulations interventions have been used to boost motor rehabilitation in children with movement disorders. For example, in a randomized pilot study, repetitive Transcranial Magnetic Stimulation (rTMS) was used in conjunction with CIMT in children with congenital hemiparesis aged between 8 and 17 years; all children received an equal dosage of CIMT. The result demonstrates significant improvement in the active tDCS group's affected hand compared to the sham tDCS group (Gillick et al., 2014). Furthermore, rTMS found to be safe and successful to implement in infants aged 3-12 months (Nemanich et al., 2019).

Another form of neuromodulation is Transcutaneous auricular vagus nerve stimulation (taVNS). taVNS is a new non-invasive method of treatment that stimulates the vagus nerve. Preliminary studies have investigated the effects of paring taVNS with bottle-feeding in infants with feeding difficulties. The initial results also showed that taVNS could improve infants' oromotor skills and help infants avoid g-tube placement (Badran et al., 2020; Badran et al., 2018).

Interestingly, vagus nerve stimulation (VNS) has been shown to improve motor abilities in adults recovering from a stroke when used in conjunction with intensive task practice (Dawson et al., 2016). Although preliminary and immediate effects of taVNS as a potential valid intervention for oromotor dysfunction are evident, the long-term effects are still unknown. This study will also address the knowledge gap between the impact of taVNS on an infant's early motor performance and examine the association between them.

Specifically, this study will investigate:

a) The association between early motor performance in infants enrolled in a taVNS oromotor study and their ability to achieve full oral feed independently upon hospital discharge
b) The long-term effects of (taVNS) intervention on an infant’s neurodevelopmental performance and sensory preferences between 18-24 months of age

1.2. Aims and Study Rationale

Aim 1:

To explore how the infant's initial motor abilities, as measured by the STEP, before receiving taVNS intervention, contribute to the predictive model for identifying which infants can benefit versus not benefit from taVNS intervention.

Hypothesis:

Infants with lower STEP scores before taVNS intervention benefitg more from taVNS intervention when compared with infants with high STEP scores. Our hypothesis was built on the assumption that infants with lower STEP total scores would be more likely to have a more extensive brain injury in comparison to those with high STEP total scores. Thus, infants with a higher total STEP score before the start of taVNS intervention (pre-STEP) would be more likely to respond to the intervention (achieve full oral feed).

Study Rationale:

It is essential to understand the relationship of an infant's motor ability, especially neck and head control, on early infant feeding to establish an early biomarker for successful independent feeding.

Aim 2:

To explore the difference in Bayley-III motor and language scores at 18 months between infants who respond to the taVNS intervention (achieve full bottle feed) and non-responders to taVNS (receive g-tube prior to discharge from hospital).

Hypothesis:

Responders' children at 18 months will have significantly higher scores in the Bayley-III motor and language sections.

Study Rationale:

TaVNS is a novel intervention that has been shown to improve infants' oromotor skills (Bashar W. Badran et al., 2020; B. W. Badran, Jenkins, et al., 2018), but the potential long-term effect is still unknown. Additional study of the impact of taVNS treatment in infants is needed to understand the long-term effects of this treatment.

Aim 3:

To explore the difference in the Toddler Sensory Profile-II caregiver questionnaires at 18 months between infants who respond to the taVNS intervention (achieve full bottle feed) and non-responders to taVNS (receive g-tube prior to discharge from hospital).

Hypothesis:

Responders' children will exhibit fewer sensory issues measured by the Toddler Sensory Profile-II caregiver questionnaires.

Study Rationale:

TaVNS is a novel intervention that has been shown to improve infants' oromotor skills (Bashar W. Badran et al., 2020; B. W. Badran, Jenkins, et al., 2018), but the potential long-term effect is still unknown. Additional study of the impact of taVNS treatment in infants' sensory performance at 18-24 months.

CHAPTER 2: REVIEW OF LITERATURE

2.1. Feeding Difficulties: Prevalence and Oral Motor Skills

Feeding difficulties have a high prevalence among neonates, with 25% to 45% of typically developing infants at risk of feeding difficulties (Bryant-Waugh et al., 2010; Lindberg, Bohlin, & Hagekull, 1991). The percentage increases to 80% in infants with developmental delays (Bryant-Waugh et al., 2010; Reilly, Skuse, Wolke, & Stevenson, 1999). Infants first require mastery of oromotor skills to safely and completely complete the feeding process; these skills involve coordinating the complex and rapid function of suck, swallow, and breathing (Mason, Harris, & Blissett, 2005). Additionally, the integration of sensorimotor and neck and head muscles are essential for successful early feeding (da Costa et al., 2010; Greene, O'Donnell, & Walshe, 2016). For example, early hypotonia is strongly associated with infant feeding difficulties caused by the infant's weak head and neck muscles (Crapnell et al., 2013). One hypothesis is that difficulties in learning oral motor sequences for feeding are the result of brain injuries that may be due to infection, ischemia, and/or brain dysmaturation (Huang et al., 2015; Ismail, Fatemi, & Johnston, 2017). These brain injuries may be why learning complex motor tasks such as oral motor skills becomes more difficult for some infants (Bashar W. Badran et al., 2020).

Evidence suggests a sensitive critical period for the introduction of feeding during early infancy (Harris & Mason, 2017). For instance, in an experiment, Hubel and Wiesel deprived cats of visual stimulation in one eye for various periods of time at different ages. This leads to ocular dominance plasticity, in which this plasticity is most robust during a specific developmental age and fades once the cats become older (Hubel &

Wiesel, 1970). This work established the phrase “critical periods” during development. Critical periods can be defined as the time during normal development when input is necessary for a normal outcome to occur (Lewis & Maurer, 2005). One hypothesis suggested that critical periods of different regions of the brain happen at different times and are activated and managed by unique mechanisms (Hensch, 2004). For example, the American Academy of Opthalmology recommends that amblyopia be treated for up to 17 years (Wallace et al., 2018). Yet, because the critical period for visual development of visual acuity end when the child is 6 to 7 years, with the rapid development of visual acuity in the first six months (Lewis & Maurer, 2005; von Noorden & Crawford, 1979). This makes the success rates of optical correction treatment significantly decline with increasing age (Holmes et al., 2011; Mohan, Saroha, & Sharma, 2004; Scheiman et al., 2005). Therefore, there is the belief that early feeding interventions and rehabilitation should be introduced within the first year of life to help infants improve their feeding behaviors.

2.2. Gastrostomy tube (g-tube), Incidences, and Impact on Feeding and Development

The number of g-tube placements in at-risk infants continues to increase at a dramatic rate. This trend of g-tube placements has risen steadily over the last two decades (Horton, Atwood, Gnagi, Teufel, & Clemmens, 2018). Although g-tube placement is responsible for improving infants' survival rates, especially in low and very low birth weight infants, there are risks associated with g-tube placement (Hatch et al., 2018). Because a g-tube placement is an invasive procedure, the risk of mortality and morbidity is high (McSweeney, Jiang, Deutsch, Atmadja, & Lightdale, 2013; Nelson, Rosella, Mahant, Cohen, & Guttmann, 2019). The high mortality rate in infants who are candidates for the g-tube may be high since they are more fragile and have many underlying risk factors. Still, studies report that infants with g-tubes are at a greater risk to gag, choke, and/or vomit when the process for g-tube weaning starts (Blackman & Nelson, 1985). Specifically to CP, Gantasala et al., (2013) systematic review noted that the benefit and risk of g-tube for children with cerebral palsy are still uncertain (Gantasala, Sullivan, & Thomas, 2013). Moreover, there are shreds of evidence of a positive correlation between an increase in g-tube time duration and future feeding difficulties in preterm infants. The longer the infants were on g-tube feeding, the more complicated their transition was to oral feeding (Borowitz & Borowitz, 2018; Cerro, Zeunert, Simmer, & Daniels, 2002; Hawdon, Beauregard, Slattery, & Kennedy, 2000). It has been suggested that a lack of oromotor skills in infants with g-tube placement is due to the lack of experience sensing food in the mouth; thus, this may result in feeding and communication difficulties (Mason et al., 2005). Furthermore, studies have shown that g-tube placement in infants is associated with higher rates of emergency department and hospital readmission in the first 30 days after discharge, with most of the events caused by infections that are related to the g-tube placement site (Arca et al., 2017; Goldin et al., 2016).

Few studies directly compare long-term neurodevelopment performance between infants who are discharged from the hospital with a g-tube and infants who achieve full oral feeds. One study found that infants with a g-tube were more likely to have lower scores in all neurodevelopment outcomes (cognitive, language, and motor) than infants who achieve full oral feeds (Jadcherla et al., 2017). However, with a subgroup from this study, the authors found no difference between the two groups (g-tube vs. full feed) in terms of severity of brain lesions using conventional magnetic resonance imaging (MRI) (Kashou et al., 2017).

One limitation of this study is the use of conventional MRI, which may not include clear microstructure imaging, such as in Diffusional Kurtosis Imaging (DKI). Studies illustrate that DKI is qualitatively sensitive in identifying brain lesions in infants; DKI has been shown to help in the diagnosis of developmental delays well before clinical deficits are apparent (Coker-Bolt et al., 2016; Duerden et al., 2015; van Kooij et al., 2012). Another form of microstructure imaging is Diffusion tensor imaging (DTI) which, allows quantitative analysis of brain microstructure based on directional patterns of water diffusion in the brain. Fractional Anisotropy (FA) and Mean diffusivity (MD) are the most common values to report. Axonal membrane maturation and myelination lead to increasing white matter FA values with gestational age, decrease FA values mean less axonal maturation of specific tracks, While, increase MD values means more axonal maturation of specific tracks (Arzoumanian et al., 2003; Rose et al., 2014; van Kooij et al., 2012).

In a larger sample size study in infants with g-tube placements, the result revealed that 61% of infants with g-tube placements had neurodevelopmental impairments (Warren et al., 2019). With the significant increase of infants receiving g-tube placements (Fox et al., 2014), some studies argue that the majority of infants referred for g-tube, in fact, did not require g-tube placement and may have benefitted from infant-driven, cue-based feeding that targets quality of oral feeding sessions as opposed to increasing quantity of feeds (Jadcherla et al., 2017; Jadcherla et al., 2012).

2.3. Early Motor Biomarkers for Feeding and Sucking Issues

Fundamental movements of the head and neck are essential for successful early oral feeding (da Costa, van den Engel-Hoek, & Bos, 2008; da Costa & van der Schans, 2008). Only a few studies have investigated the link between infant early motor performance and feeding and sucking behavior. For example, Nieuwenhuis et al. (2012) used the General Movements Assessment (GMA) to show that infants who were characterized as having uncoordinated sucking patterns on the Neonatal Oral-Motor Assessment Scale (NOMAS) had a high rate of abnormal fidgety movements (FM) at 14 weeks post-term (Nieuwenhuis et al., 2012). The GMA assesses the quality of spontaneous movements, namely FM, in the first six months of life for predicting CP (Morgan et al., 2019; Prechtl, 1990). When combined with MRI, the GMA has been shown to have excellent sensitivity (98%) and specificity (91%) to detect CP early in life for high-risk infants (Kwong, Fitzgerald, Doyle, Cheong, & Spittle, 2018; Novak et al., 2017). However, the GMA's ability to detect mild motor impairments is limited (Einspieler et al., 2019; Morgan et al., 2019). One study found that 71% of infants with a mild disability had normal GMAs (Morgan et al., 2019).

Interestingly, the other study used the GMA in conjunction with the Hammersmith Neonatal Neurological Examination (HNNE) and the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) to predict oral motor feeding impairments. The results showed that both the HNNE and NNNS improved the accuracy of predicting oral motor feeding impairments at 12 months of age more than the GMA alone (Sanchez et al., 2017). Therefore, the GMA has a poor ability to predict feeding difficulties without combining it with another neuromotor examination or MRI.

Currently, these are the only two studies that looked at early motor biomarkers in infants with feeding difficulties. Only one study measured both feeding and motor performance at the same time (Nieuwenhuis, Verhagen, Bos, & van Dijk, 2016; Sanchez et al., 2017). Yet, the evidence is still inconclusive about whether there is a relationship between early sucking behaviors, neonatal brain injury, and early motor skills (Slattery et al., 2012).

Nfant® is a new device that recoded infant's tongue movement and measure nutritive sucking. When combined with microstructure diffusion tensor imaging (DTI). The results showed that lack of smoothness was correlated significantly with low FA of motor tracks. High-sucking irregularity and low-smoothness variability correlated significantly with high-mean diffusivity in sensory tracks in infants with confirmed brain injury (Tamilia et al., 2019).

This current study will use the STEP, a reliable and quick motor assessment that can detect mild disabilities (Gower et al., 2019), in combination with an advanced MRI technique DKI, to identify early motor movements (especially head and neck) that may be an indicator of feeding and sucking problems shortly after birth.

2.4. The Long-Term Effect of Early Feeding and Sucking Problems

2.4.1 Long-Term Effect on Neurodevelopment

Many studies have demonstrated that early feeding difficulties can predict neurodevelopment delays at different ages. For example, infants with feeding difficulties are more likely to have both receptive and expressive language delays at the 18 months follow-up (Adams-Chapman et al., 2013). Likewise, there is an association between poor language, cognitive performance, and feeding difficulties in infancy and delays by primary school age (5 years ago), negatively impacting the children's school performance (Wolthuis-Stigter et al., 2017). Tsai et al. (2010) showed a significant difference in infants' neurodevelopmental outcomes with feeding difficulties compared to infants without feeding difficulties using the Bayley-II at six and 12 months. Also, in a relatively large sample size of moderately and late preterm infants, Zhang et al. (2017) showed that feeding difficulties could be predictive of neurodevelopmental delays at six months (Zhang et al., 2017). Furthermore, multiple studies confirm that neurodevelopmental delays can persist to early childhood up to five years of age in infants with feeding difficulties (Crapnell, Woodward, Rogers, Inder, & Pineda, 2015; Wolthuis-Stigter et al., 2017; Wolthuis-Stigter et al., 2015). In fact, evidence suggests that adolescents born preterm have an abnormality in their oromotor and motor tracks (corticospinal tract and speech motor corticobulbar tract), which have been linked to difficulties in oromotor control and speech development (Northam et al., 2012). Nevertheless, some studies have reported a similar rate of feeding difficulties between preterm and full-term infants at three years of age (Nieuwenhuis et al., 2016; Sanchez, Boyce, Morgan, & Spittle, 2018).

In their systematic review, Slattery et al. (2012) investigated the link between early sucking and swallowing problems and neonatal brain injury and neurodevelopmental outcomes (Slattery et al., 2012). Five studies explore the concurrent relationship between early sucking and swallowing problems and neonatal brain injury. In a sample of 84 neonates with arterial ischemic stroke, 48.8% of the infants had sucking or swallowing problems (Barkat-Masih, Saha, Hamby, Ofner, &

Golomb, 2010). A similar percentage (42%) was reported in a sample of 43 infants with neonatal hypoxic-ischaemic encephalopathy (HIE), while 35% of the neonates with a mixed diagnosis of brian injury experience moderate to severe sucking difficulties (Mizuno & Ueda, 2005; Quattrocchi et al., 2010). However, further studies are needed to determine if there is a relationship between early feeding problems and neonatal brain injury, including more accurate diagnosis tools of sucking and swallowing difficulties and neuroimaging biomarkers (Slattery et al., 2012).

2.4.2 Long-Term Effect on Sensory Processing

Oral aversion or defensiveness is a frequent and severe issue in infants. Still, it is uncertain if this is a primary sensory disorder or secondary to developmental delay and/or is a result of early negative oral sensory and feeding experiences (Dobbelsteyn, Marche, Blake, & Rashid, 2005). A few studies have examined the influence of infants' and toddlers' feeding difficulties with sensory processing. Toddlers with feeding difficulties demonstrated more atypical sensory processing than healthy toddlers; specifically, they scored statistically significant differences in oral, vestibular, and tactile sensory processing (Yi, Joung, Choe, Kim, & Kwon, 2015). In a more detailed study using the Infant/Toddler Sensory Profile (TISP), Tauman et al. (2017) found that infants with feeding difficulties scored significantly lower in oral and auditory processing than healthy controls. They also scored lower in three out of the four sensory quadrants (low registration, sensory sensitivity, and sensory avoidance). The authors noted that infant feeding difficulties are also associated with a higher incidence of behavioral insomnia (Tauman et al., 2017). DTI allows quantitative analysis of brain microstructure based on directional patterns of water diffusion in the brain as measured by Fractional Anisotropy (FA) values.

Axonal membrane maturation and myelination lead to increasing white matter FA values with gestational age, decrease FA values mean less axonal maturation of specific tracks (Arzoumanian et al., 2003; Rose et al., 2014; van Kooij et al., 2012). Children with sensory processing problems reported using the sensory profiles had shown a significant decrease in FA than healthy control children using DTI. This is especially true in the posterior corpus callosum, posterior corona radiata, and posterior thalamic radiations tracks (Owen et al., 2013; Payabvash et al., 2019). And, there was a strong correlation of FA with both sensory profiles and abnormal auditory processing, multisensory integration, and attention across children with atypical sensory profiles (Chang et al., 2015; Narayan et al., 2020). These studies illustrate an association between abnormal white matter brain microstructure and atypical sensory performance.

2.5. Use of Neuromodulation in pediatric

Recent studies on adult neurologic and psychiatric disorders have resulted in active research in neuromodulation techniques in the pediatric population over the last decade. In particular, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) have shown early and potentially promising results in children's various disorders, including ADHD and autism by demonstrated some therapeutic benefits (stereotyped behaviors, social behavior, and executive function)(Barahona-Corrêa, Velosa, Chainho, Lopes, & Oliveira-Maia, 2018). Pertinently, these neuromodulations have shown even greater benefit in children with stroke and hemiplegic CP (Malone & Sun, 2019; Rubio et al., 2016).

Applying low frequency of repetitive transcranial magnetic stimulation (rTMS) in children with subcortical ischemic stroke between the ages 8 to 20 resulted in improved grip strength compared with shame control (Kirton et al., 2008). A larger subsequent 2014 study randomized 45 children ages 8 to 17 to receive active or sham (control) rTMS (Kirton et al., 2016). All children in the study also received an equal amount of constraint-induced movement therapy (CIMT); the aim was to investigate if rTMS could boost the active rTMS + CIMT group results compared with sham rTMS + CIMT. The active rTMS + CIMT group showed the most significant improvement measured by the Assisting Hand Assessment (AHA) at six months follow-up. However, there were no significant differences between the sham rTMS + CIMT and the active rTMS + CIMT. It should be noted that the CIMT-only group showed improvement in week one and at two months post-intervention and not at six months follow-up (Kirton et al., 2016). Another study demonstrated an immediate improvement of the children affected hand when using active rTMS and CIMT (Gillick et al., 2014). Furthermore, in a double-blind randomized control study brain structural changes using magnetic resonance spectroscopy (MRS) were only observed when using active tDCS vs. sham tDCS with both groups receiving an intensive motor therapy in children with hemiplegic CP (Carlson, Ciechanski, Harris, MacMaster, & Kirton, 2018).

However, the long-term effect of rTMS and tDCS is still unclear; for example, at two months follow-up, there was no effect of tDCS alone on the affected had (Kirton et al., 2017). Kirston et al. (2016) found that the rTMS only group demonstrated gains at one-week post-intervention only (Kirton et al., 2016). Other studies also found inconsistent results regarding the long-term impact of tDCS and rTMS in hand function with small sample size randomized studies (Nemanich, Rich, et al., 2019; Rich, Menk, Krach, Feyma, & Gillick, 2016).

2.6. TaVNS Treatment and Rationale

The vagus nerve delivers an extensive afferent and efferent network of innervation for the internal organs. Furthermore, the vagus nerve plays a key role as an interface between the higher central nervous system (CNS) circuits and the brain stem's autonomic control circuitry. It is a mixed autonomic nerve originating at the medulla oblongata and projecting from the brain stem bilaterally along the neck and esophagus before branching diffusely to innervate the internal organs (Hulsey et al., 2016). Also, the vagus consists of ~80% sensory afferent and 20% motor efferent fibers (Yu, Weller, Sandidge, & Weller, 2008).

The vagus nerve sends afferent fibers to a number of nuclei that are identified to release neuromodulators associated with cortical plasticity, including the locus coeruleus, raphe nuclei, and the basal forebrain; all are important for neuroplasticity (Dorr & Debonnel, 2006; Hassert, Miyashita, & Williams, 2004; Henry, 2002). Vagus Nerve Stimulation (VNS) studies have shown that this electronic stimulation could involve the noradrenergic locus coeruleus (LC) and cholinergic nucleus basalis (NB) in the central nervous system (Groves & Brown, 2005). In fact, a low current electrical stimulation of the vagus nerve has shown to drive activity in both the LC and basal cholinergic forebrain (Detari, Juhasz, & Kukorelli, 1983; Groves, Bowman, & Brown, 2005; Manta, Dong, Debonnel, & Blier, 2009).

This low current electrical stimulation, in turn, can enhance the releases of norepinephrine and acetylcholine throughout the brain (Follesa et al., 2007; Landau et al., 2015; Roosevelt, Smith, Clough, Jensen, & Browning, 2006). Both norepinephrine and acetylcholine act independently and synergistically to facilitate plasticity in the brain by increasing the synaptic activity of specific brain regions (Bear & Singer, 1986; Seol et al., 2007).

In animal studies, when VNS is paired with intensive task practice (forelimb training), a reorganization of the rat's primary cortex resulted in more representation of proximal forelimb activity than the untrained controls. In addition, the primary cortex reorganization was depending on the task. For example, the wheel spin training resulted in more distal forelimb representation while, lever press training resulted in more proximal forelimb representation (Porter et al., 2012). Furthermore, Hulsey et al. (2016) illustrated that VNS reorganizes the motor cortex via cholinergic nucleus basalis in the animals' brains. Remarkably, in one animal study, Meyers et al. (2018) showed that the VNS effect could be generalized to other similar tasks (from supination task to isometric pull task) and that it has a long-lasting up to ten weeks post-training impact on motor performance (Meyers et al., 2018).

When VNS was tested in humans in small-randomized control studies, evidence from these studies suggested that the use of VNS, in conjunction with intensive task practice, could improve upper-motor function in the adult with stroke (Dawson et al., 2016; Engineer et al., 2019; Kimberley et al., 2018). However, because VNS is a surgically implanted device, adverse events were reported, such as wound infection for device implantation, vocal cord palsy, hoarseness, and fatigue, these side effects may make the treatment less desirable (Ben-Menachem, Revesz, Simon, & Silberstein, 2015; Dawson et al., 2016).

To avoid these side effects, non-invasive methods to use VNS was developed. The auricular branch of the vagus nerve (taVNS) proved to activate the afferent and efferent vagus nerve network without needing the surgically implanted device. (B. W. Badran, Dowdle, et al., 2018; B. W. Badran, Mithoefer, et al., 2018; Garcia et al., 2017; Kraus et al., 2013; Yakunina, Kim, & Nam, 2017).

A recent study demonstrated that the use of taVNS in conjunction with intensive task practice is safe and can also improve hand motor function in adult stroke survivors (Redgrave et al., 2018). A more recent randomized control trial study found that taVNS was safe and more effective than conventional rehabilitation in subacute stroke patients (Wu et al., 2020). Additionally, the improvement lasted for 12 weeks in the taVNS group. Yet, more studies are needed to investigate the additive effect of taVNS in the adult stroke population with larger sample sizes.

TaVNS intervention has also been shown to improve infants' oromotor skills in infants with feeding issues when paired with bottle-feeding (Bashar W. Badran et al., 2020; B. W. Badran, Jenkins, et al., 2018).

2.7. The need and gap of knowledge

This study aims to investigate a relatively new area of research with the first use of taVNS in pediatrics, specifically neonates. Concurrently, the original study seeks to improve infants' feeding performance with feeding difficulties by pairing taVNS with bottle-feeding. However, in this sub-study, we aim to measure early motor performance after taVNS in the short-term and the long-term impact on neurodevelopmental and sensory outcomes. The use of taVNS as neuromodulation in the neonate and pairing it with a specific task (bottle-feeding) is unique and has never been done before.

Previous neuromodulation studies recruited older children ages 7 to 17 years, with limited application of paring the neuromodulation with a specific functional task. This may lead to mild or inconsistent long-term implications of the findings. It is also very challenging to pair current neuromodulation (TMS & tDCS) with a functional task due to TMS and tDCS unit size and use. Yet, our original study seems to solve these two problems by including infants (less than one year of age) and pairing taVNS with a functional task (bottle-feeding) using a specially designed bottling system to deliver taVNS called ‘BabySTRONG system’ (**Figure.1**).

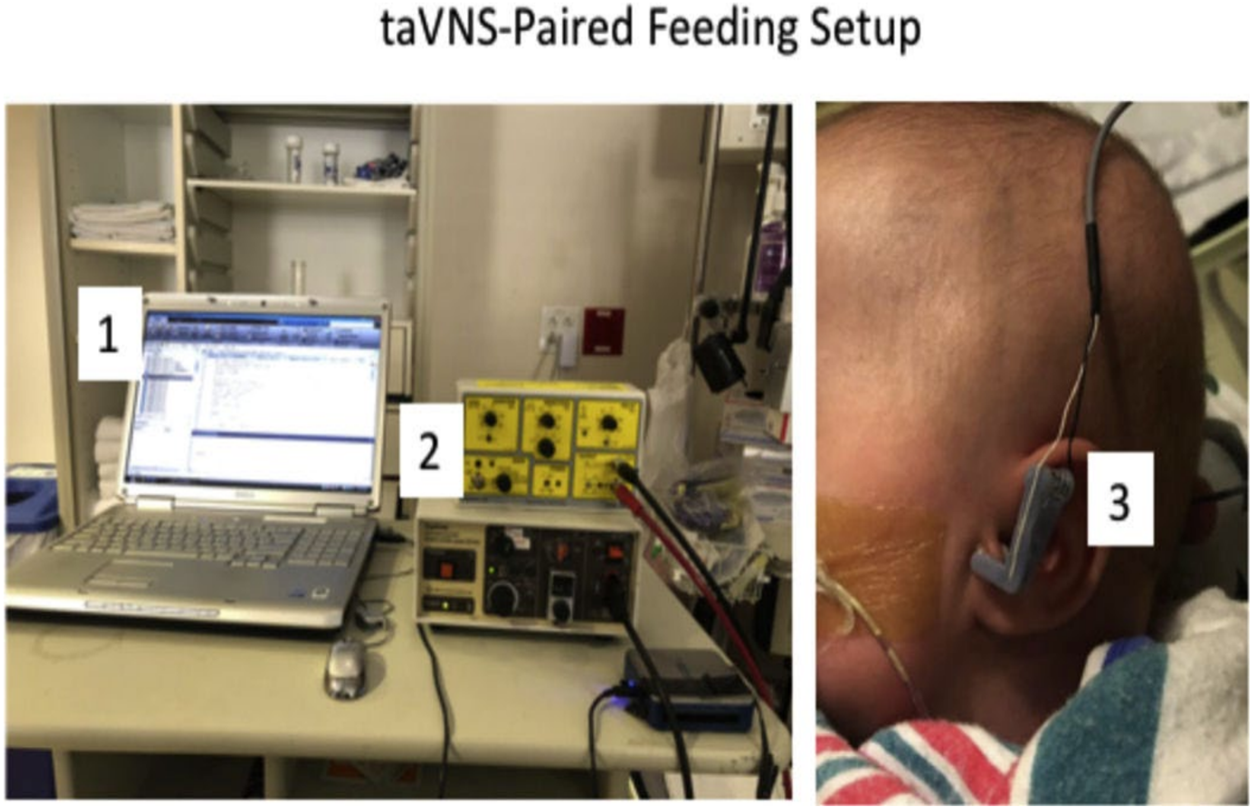

Fig 1: TaVNS electrode positioning on left tragus, and equipment setup.
A computerized script (1) is used to communicate with a constant current simulator (2) Stimulator delivers taVNS via custom ear electors (3) attached to the left ear of the neonate.
Source: Badran BW, Jenkins DD, DeVries WH, Dancy M, Summers PM, Mappin GM, et al. Transcutaneous auricular vagus nerve stimulation (taVNS) for improving oromotor function in newborns. Brain stimulation. 2018;11(5):1198-200.

2.8. International Classification of Function (ICF) Model

The current research has two major points:

a. First, explore if the Specific Test of Early Infant Motor Performance (STEP) total score is a significant factor in a modal to predict later developmental delays for infants who received taVNS intervention.
b. Second, explore the difference in neurodevelopmental performance and sensory preferences at 18 months between infants who respond to the taVNS intervention (achieve full bottle feed) and infants who do non-respond (g-tube placement). Our hypothesis is that responders would have better neurodevelopmental performance and more typical sensory profiles than non-responders.

Using the ICF model **(Figure. 2)** illustrates the current research plan would fit. This research will most likely fit the activity and body function, and structure concepts in the ICF model with a bidirectional link between the two.

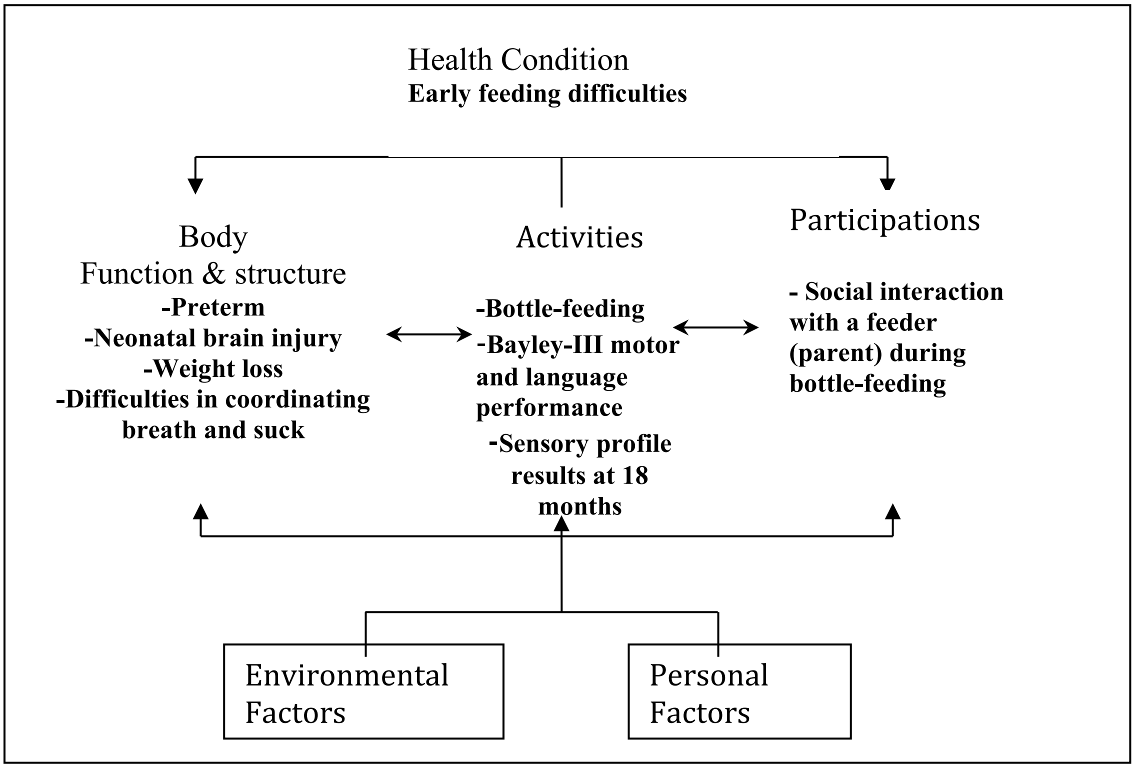

Fig. 2: Research plan based on the ICF model. Based on the World Health Organization (2001) International classification of functioning, disability, and health: ICF. Geneva: World Health Organization, pg. 18. Accessed online: http://www.disabilitaincifre.it/documenti/ICF_18.pdf

Feeding is considered an activity, but it is influenced by the infant's body function and structures (i.e., dysmaturiton). When an infant is unable to take an adequate amount of food, this can result in slow growth, increase the length of stay at the hospital, and can cause additional potential long-term sensory and neurodevelopment effects. While there is a participation component in bottle-feeding and parent interaction with the newborn, the current study does not examine this factor. The comprehensive assessments (Bayley-III and Sensory Profile-II caregiver questionnaire) at 18 months will help determine the body structure and function factors that mostly affect the child's performance and any possible restriction on participation.

The current study would not explore the personal and environmental factors, and this may consider as a limitation to our findings. Personal and environmental factors are known to influence the child's outcome and are believed to be core factors of the child's early development (Birch & Davison, 2001; Loth, Mohamed, Trofholz, Tate, & Berge, 2021; Shankar et al., 2018). For instance, in a randomized control study, Law et al. (2011) demonstrated that context-focused interventions are equal in effectiveness to child-focused interventions (Law et al., 2011).

CHAPTER 3: METHODOLOGY

3.1. Research Questions

Question 1: **Pre-STEP total scores as a predicted respond to taVNS**

Can the Specific Test of Early Infant Motor Performance (STEP) be a significant factor in a model that predicts infants with early feeding difficulties who would benefit, versus not benefit, from taVNS intervention?

Aim 1:

To explore how the infant's initial motor abilities, as measured by the STEP before receiving taVNS intervention (pre-STEP), contribute to the predictive model for identifying which infants could benefit, versus not benefit, from taVNS intervention.

Hypothesis 1*:*

Our hypothesis was built on the assumption that infants with lower STEP total scores would be more likely to have a more extensive brain injury in comparison to those with high STEP total scores. Thus, infants with a higher total STEP score before the start of taVNS intervention (pre-STEP) would be more likely to respond to the intervention (achieve full oral feed).

Question 2: **Long-term effect of taVNS in neurodevelopmental performance**

Will there be a significant change in neurodevelopmental performance between infants who responded to taVNS versus infants who did not respond?

Aim 2:

To explore the differences in neurodevelopmental performance (language and motor skills) at 18 months between infants who respond to the taVNS intervention (achieve full bottle feeding) and infants who are non-responders (require g-tube placement).

Hypothesis 2:

Responders to early taVNS intervention would have a higher Bayley-III scores at 18 months when compared to non-responders. Infants who responded to early taVNS treatment and achieved full bottle feeds will continue to show good progress in developmental skills compared to non-responders.

Question 3: **Long-term effect of taVNS in sensory performance**

Will there be a significant change in neurodevelopmental and sensory performance between infants who responded to taVNS versus infants who did not respond?

Aim 3**:**

To explore if infants who responded to taVNS intervention and non-responder have significant differences in the sensory preferences at 18 months of age using the Toddler Sensory Profile-II (SP-II) caregiver questionnaires.

Hypothesis 3*:*

Infants who are responders after receiving taVNS intervention are more likely to have typical sensory preferences based on the Toddler SP-II caregiver questionnaires at 18 months in normed rang scores to specific aged. In contrast, infants who are non-responders will be more likely to have atypical sensory preferences.

3.2. Research Strategy

3.2.1. Screening

Prospective participants will be identified by the Primary Investigator (Dr. Jenkins) at the MUSC neonatal intensive care units (Level II and III) and checked for potential inclusion. Other clinicians based in the nursery may also mention the study to parents and, if they are interested in participating, refer them to Dr. Jenkins.

3.2.2. Participants

Inclusion criteria:

Infants must be clinically stable, on minimal respiratory support (nasal cannula or room air) and

1) Be premature (>33 weeks gestational age at enrollment) and currently working on oral feeding;

or

2) Have had greater than or equal to 35 weeks gestation, with significant medical issues that have precluded oral feeding and oromotor development, such as Hypoxic-Iscameic Encephalopathy (HIE).

Exclusion criteria: Infants who

1) Are unstable or require respiratory support involving positive pressure
2) Have < 33 weeks gestation at enrollment

3) Have major unrepaired congenital anomalies or anomalies that limit feeding volumes
4) Have cardiomyopathy
5) Have repeated episodes of autonomic instability (apnea or bradycardia) that are not self-resolving

Neonates who are beginning oral feeding after medical treatment for critical illnesses, such as HIE brain injury, will be included because these neonates represent a population in which taVNS-paired feeding could achieve the greatest success in overcoming impaired brain development. Congenital syndromes may be included if the infants do not have major, unrepaired anomalies or anomalies that limit feeding volumes.

Written informed consent will be obtained from the mother if available and if she has custody of the infant; otherwise, consent will be obtained from a parent or legal guardian prior to the child’s participation in the experimental paradigm.

3.2.3. Study design

Up to 30 preterm neonates were enrolled in this prospective, open-label, safety, and feasibility trial. The experimental paradigm consisted of two to three weeks of daily taVNS-paired feeding. All consented participants received the active stimulation condition. Additionally, all enrolled neonates had neurodevelopmental and sensory assessments at 18 months of age. The follow-up assessments were completed at MUSC high-risk clinic or the parent's home.

3.2.4. Sample size

For Aim 1 and 2, we estimate a sample size of 30 infants, allowing for a dropout rate of 15% and thus lost data; therefore, the sample size for Aim 1 will be 20 infants. For Aim 2 and 3, a sample size of 20 infants, allowing for a 15% dropout, each aim 2 and 3 groups will have an estimated sample size of 10 children.

3.3. Measures

3.3.1 Study outcomes selection and rationale

In addition to neuroimaging before and after taVNS dosing, the following three assessments will be completed:

a. The Specific Test of Early Infant Motor Performance (STEP) pre and post-intervention
b. The Bayley-III Assessment of language and motor skills at 18 months adjusted age
c. The Toddler Sensory Profile-II (SP-II) questionnaire at 18 months adjusted age

Diffusion Kurtosis Imaging (DKI) and magnetic resonance spectroscopic (MRS) imaging:

Un-sedated MRS and DKI will be obtained before initiating the taVNS protocol. These sequences will take approximately 40 minutes and will be performed after feeding to ensure sleepiness during the procedure. The sequences will be performed on the clinical scanner, usually at night, before initiating the taVNS, and at two to three weeks after the protocol is completed, and at 2 -3 months corrected age, which may be done as an in- or outpatient. Studies have illustrated that DKI is qualitatively sensitive in identifying brain lesions in infants who are eventually diagnosed with developmental delays but well before clinical deficits are apparent (Coker-Bolt et al., 2016; Duerden et al., 2015; van Kooij et al., 2012).

Specific Test of Early Infant Motor Performance (STEP):

STEP is a novel developmental screening test consisting of ten movement items, include; anti-gravity flexion and extension of the head and neck, movement in the arms and legs, and tone in the shoulder girdle and pelvis (Bentzley et al., 2015; Shehee et al., 2016). STEP ten movements' items with a total score of 30 showed excellent discrimination between preterm infants of different motor abilities. Each item can be scored 0, 1, 2, or 3 with a higher score resulting in better motor performance (Coker-Bolt et al., 2014). Moreover, the STEP intra-rater and inter-rater reliability were excellent for expert raters and good to excellent for novice raters; the time to administer the STEP is up to ten minutes for a novice examination (Gower et al., 2019).

The STEP can be administered at term and at three months with established cutoff scores for both times (Gower et al., 2019). STEP term cutoff (≤ 16) sensitivity and specificity to predict the Bayley-III gross motor performance were excellent (sensitivity = 1.00, specificity = 0.909). Similar results were found with three months STEP cutoff scores (≤ 22) (sensitivity = 0.75, specificity = 0.909). Compared with the Test of Infant Motor Performance (TIMP), the STEP showed better predictability of delays at 12 months (Gower et al., 2019).

Bayley-III:

The Bayley-III is considered the most widely used standardized measure of early development for clinical and research purposes (Anderson & Burnett, 2017).

The Bayley Scales' primary objective is "to identify children with developmental delay and provide information for intervention planning" (Bayley, 2006, p.8) through individually administered assessment of children aged 1–42 months. The Bayley-III has fair to good reliability and excellent validity (Bayley, 2006; Griffiths, Toovey, Morgan, & Spittle, 2018; Visser, Ruiter, Van der Meulen, Ruijssenaars, & Timmerman, 2015). Bayley-III with <1 standard deviation (S.D.) at 4 years has a sensitivity = 0.83 specificity = 0.94; at <2 S.D., the sensitivity = 0.67, and specificity =1.00 when predicting motor outcome in very preterm children (Spittle et al., 2013). One SD below the mean in any of the Bayley-III sub-sections is defined as <9 scaled scores and is considered below normal (Duncan et al., 2015; Vohr et al., 2012).

Toddler Sensory Profile-2 caregiver questionnaire:

The Toddler SP-2 was selected to identify sensory sensitivities that have been reported as factors in infants with feeding difficulties (Dunn, 2014; Tauman et al., 2017). The questionnaire can be administered to toddlers between 7-35 months with a normed range of typical sensory performance, more than or less than the typical ranged scores are considered atypical sensory performance (Dunn, 2014).

Three domains were found to play an essential part in how easy an infant will accept or refuse food: taste and smell, and respond to tactile stimulation, texture, and visual appearance (Harris & Mason, 2017). SP-2 covers all three domains and auditory, vestibular, and behavior (Dunn & Brown, 1997). SP-2 has good test-retest reliability and high to moderate internal consistency (Ohl et al., 2012). The SP-2 assesses four sensory quadrants (registration, sensitivity, avoiding, and seeking) that help identify the child's atypical behavior.

3.4. Data analysis plan

Statistical analysis will be performed using SAS statistical software (version 9.4, released 2016, SAS Institute, Inc., Cary, N.C., USA) and SPSS software (IBM, version 26 SPSS Inc, Chicago, IL).

- Aim 1: The analysis will be conducted with logistic regression modeling of the pre-STEP total score (n=26) as a function of response / non-response adjusted for gestational age at birth and the infant's birth weight. The adjusted odds ratio and 95% CI will be reported for the response/non-respond with the Pre-STEP score. Proc Logistic statement will be used to run the analysis.

  Sample size justification: A logistic regression of response to taVNS predicted by the pre-STEP total score will be used. The outcome will be treated as a continuous, normally distributed variable with 80% power at a 5% significance level. A sample size of 26 would allow detecting a change in probability of responding at the mean STEP score 13.5 unite increased to 17.5 unite. In other words, the sample size of 20 infants is enough to detect an odds ratio of 3.5, for a change in STEP scores from 13.5 units to 17.5 units is considered significant. This sample size estimation is based on pre-STEP scores prediction of the best responders to taVNS. Potential weaknesses to this analysis are the small sample size; also, this current estimation is not powered for the other predictors such as; infant's medical condition feeding volume, number of taVNS sessions, and days of trying oral feed prior to taVNS. It has been suggested that the data should contain at least ten subjects for each variable entered into a logistic regression model (Peduzzi, Concato, Kemper, Holford, & Feinstein, 1996). However, other researchers have questioned the rule of thumb's validity that ten subjects are needed for each variable. Since this study is exploratory, we expected a small sample size (Vittinghoff & McCulloch, 2007).

- Aim 2: We will use the ANCOVA test to analyze the difference between two groups (responders versus non-responders) and Bayley-III at 18 months (n=10) adjusted for gestational age at birth.

  Sample size justification: An expected sample size of 10 subjects will allow us to estimate the difference between the Bayley-III mean score in the two taVNS groups within a margin of error of 17.5, with a 95% confidence level, assuming S.D. of 15 (Bayley, 2006).
- Aim 3: Fisher's exact test will measure the difference in proportions (frequency) between responders versus non-responders and infants with typical versus non-typical sensory preferences at 18 months of age (n=12). A Fisher’s exact test was selected as the expected test as the cell numbers will be very small (fewer than 5 in some cells).

3.5. Predicted Outcomes and Potential Pitfalls

This proposed study addresses a novel treatment approach (taVNS), exploring its short and long-term potential impacts on at-risk infants' neurodevelopment and movement. This knowledge gap impedes the diagnosis and treatment of feeding in neonates who are at particularly high risk for adverse outcomes, including poor nutritional status, feeding tube dependence, prolonged length of hospital stay, and poor neurodevelopmental performance.

Consequently, this study's successful completion will provide foundational knowledge about the influence of the neuromodulator, taVNS, on oral feeding in infants, with the long-term goal of improving the neurodevelopmental and sensory performance of infants with feeding difficulties.

<u>Potential Pitfall 1</u>: Hypotheses are not confirmed.

- Alternative Strategy 1: If our study results fail to confirm our hypotheses, we will interpret those results, explain what can be learned from our findings, and develop new hypotheses to answer our research questions. For example, even if our findings reveal that the pre-STEP total scores did not significantly predict infants who are more likely to benefit from taVNS (Hypothesis 1), we will still have valuable information because there is currently limited data in the literature about the role of motor assessment in infants with feeding difficulties. The results will add to the knowledge gap and allow us to conduct future studies on specific STEP items (related to head and neck movement) if required.
- Our findings will also provide a foundation for future incidence studies with larger participant samples. Also, suppose the results of this proposed study reveal that responders to taVNS treatment did not differ significantly from non-responders (*Hypothesis 2*). In that case, we will devise a more extensive study with the control (sham) taVNS group. Furthermore, we will explore the

other factors, including assessments at long-term effect (school performance), with large and more heterogeneous sample size.

CHAPTER 4: RESULTS

The results chapter will be divided into two main sections based on the three study aims. The first section will show the results related to the pre-treatment scores and the ability of the scores to influence the infants' outcome (responders vs. non-responded). There are a total of 26 infants in this section that are split into 11 in the responders' group and 15 in the non-responder group. The second section of the results chapter will present the long-term performance of taVNS on both neurodevelopment and sensory performance at 18 months of age. The neurodevelopment results have ten toddlers in total (six responders, four non-responders), while the sensory performance results have 12 toddlers (seven responders and five non-responders). Both the responder and non-responder participants share common medical and birth characteristics. Additionally in both groups infants:

- Tried oral feed (po) prior the start of the taVNS treatment
- Were scheduled for G-tube procedure as the solution to the feeding difficulties
- Received taVNS intervention

4.1. Pre-STEP total scores as a predicted respond to taVNS

One of this study aims to explore the STEP test's ability to help predict infants who may benefit from the taVNS intervention after enrollment. This aim hypothesized that infants with a higher total STEP score before the start of taVNS intervention (pre-STEP) would be more likely to respond to the intervention (achieve full oral feed).

Twenty-six infants had pre-total STEP scores (pre-intervention); of those, 15 were non-responders, and 11 responded to taVNS. (**Table.1**) illustrates infants’ medical history, birth characteristics, STEP scores, and taVNS data. No significant differences were found between the responders and non-responders regarding medical history, birth characteristics, and STEP information. Moreover, there were no differences in days trying oral feed, the total number of days, and the sessions of taVNS between the groups. The STEP information shows that at term, four non-responders (26.7%) participants out of the 15 were categorized as low risk of developmental delays (STEP score >16). Yet, all of the responders' participants, 11 (100%), scored in the high-risk category (STEP score ≤16).

Table 1: Demographic information of participants (n=26) with pre-treatment STEP scores included in the model and divided into responders vs. non-responders.

		Responders (Full po Feed)	**Non-Responders (g-tube)**	**Total**	**P-value***
	Total number	11	15	26	
	Gender:				
	Male	5	5	10	
	Female	6	10	16	
Medical History	Clinical Sepsis	2	7	9	0.14
	Persistent pulmonary hypertension of the newborn (PPHN)	2	2	4	0.57
	Patent Ductus Arteriosus (PDA)	3	4	7	0.65
	Intraventricular hemorrhage (IVH)	Grade I (2) Grade II (1) Grade III (2)	Grade I (4) Grade II (1) Grade IV (1)	11	0.55
	Hypoxic Ischemic Encephalopathy (HIE)	3	2	5	0.35
	Periventricular Leukomalacia (PVL)	1	3	4	0.43
	Lenticulostriate vasculopathy (LSV)	0	3	3	0.17
	Neonatal abstinence syndrome (NAS)	1	2	3	0.62

Birth	GA at birth (weeks)	30.3 ± 3.9	31.0 ± 4.1	30.7 ± 4.0	0.67
	GA at taVNS start (weeks)	42.0 ± 1.7	42.3 ± 3.5	42.1 ± 2.8	0.78
	Birth weight (grams)	1442.7 ± 831.2	1725.3 ± 1021.7	1605.7 ± 938.7	0.46
taVNS information	Days trying po, prior to taVNS[†]	37.9 ± 14.5	38.1 ± 10.8	38.0 ± 12.2	0.97
	Total number of taVNS sessions	26.0 ± 15.4	26.2 ± 11.5	26.1 ± 13.0	0.97
	Total number of taVNS days	15.6 ± 6.4	18.9 ± 7.3	17.5 ± 7.0	0.24
	GA at the end of taVNS (weeks)	44.2 ± 2.1	45.0 ± 3.7	44.7 ± 3.0	0.60
STEP Information	GA at STEP assessment (weeks)	41.9 ± 1.7	41.7 ± 3.0	41.8 ± 2.5	0.87
	Performance on STEP at term:				
	Low risk >16	0	4	4	0.91
	High risk ≤16	11	11	22	

Mean ± Standard deviation

[†] Po= Oral feed

* Independent t-test and Fisher's Exact test

A predictor logistic model was fitted to the data to examine the hypothesis that a high score of the pre-treatment STEP would likely predict responders to taVNS intervention. According to the overall model evaluation, none of the variables in the model are significant, therefore, the hypothesis was not supported (**Appendix A, Table. 1**). The exploratory model showed that a while higher pre-treatment STEP score was negatively related to the infant's responding to the taVNS treatment course, it was not significant (*p* > .05, 95% CI 0.69-1.79). Neither infant's GA nor birth weight was statically significant (**Table 2**).

Infants in the responder group had a median STEP total score of 13 (range, 9-15), and those in the non-responder group had a median STEP total score of 14 (range, 9-19). This difference was not significant (*p*= 0.06, Mann-Whitney U test) (**Appendix B, Figure. 1**).

Table 2: Logistic regression analysis of pre-treatment STEP scores for the responders and non-responders.

Predictor	**β**	**SE β**	**Wald χ^2**	***df***	**P-value**	**95% CI**
Constant	1.40	6.96	0.04	1	0.83	NA
GA at birth	0.10	0.24	0.19	1	0.66	0.69,1.79
Birth weight	-0.00	0.00	0.51	1	0.47	0.99,1.00
Pre-treatment STEP scores	-0.00	0.18	2.33	1	0.12	0.52,1.08

χ^2: Chi-square, *df*: degree of freedom, β: estimate, SE: standard error, CI: confidence limits

4.2. Long-term effect of taVNS in neurodevelopment performance

This study aimed to establish if early responses to taVNS intervention significantly improve neurodevelopment outcomes at 18 months compared to the non-responders. This aim's exploratory hypothesis stated that responders would perform better than non-responders in long-term neurodevelopment assessment (Bayley-III).

A total of ten toddlers completed the Bayley-III follow-up. All sections of the Bayley-III were applied (cognitive, language, and motor). There were no significant differences between the responders (n=6) and non-responders (n=4) in age at the follow-up assessment, birth information, medical history, or taVNS information.

Moreover, in the medical history of both the responders' and non-responders' groups, three infants were diagnosed with Intraventricular hemorrhage (IVH). However, only in the responder's group were their infants with Patent Ductus Arteriosus (PDA) n=3. (**Table. 3**) highlights the demographic and medical information of the participants.

Table 3: Demographics information participants (n=10) who completed the 18-month follow-up Bayley-III split into responders vs. non-responders

		Responders (Full po Feed)	**Non-Responders (g-tube)**	**Total**	**P-value***
Medical History	Total number	6	4	10	[redacted]
	Gender: Male	2	2	4	[redacted]
	Female	4	2	6	[redacted]
	Clinical Sepsis	4	3	7	0.66
	Persistent pulmonary hypertension of the newborn (PPHN)	3	1	4	0.45
	Patent Ductus Arteriosus (PDA)	3	0	3	0.16
	Intraventricular hemorrhage (IVH)	Grade I (1) Grade II (1) Grade III (1)	Grade I (2) Grade II (1)	6	0.45
	Hypoxic Ischemic Encephalopathy (HIE)	2	2	4	0.66
	Periventricular Leukomalacia (PVL)	0	1	1	0.54
	Lenticulostriate vasculopathy (LSV)	0	1	1	0.40
	Neonatal abstinence syndrome (NAS)	1	1	2	0.40
Birth Characteristics	GA at birth (weeks)	29.81 ± 5.3	28.21 ± 1.8	29.17 ± 4.2	0.58
	GA at taVNS start (weeks)	45.5 ± 6.4	44.5 ± 3.5	45.10 ± 5.2	0.79

	Birth weight (grams)	1495 ± 1072.9	965 ± 204.5	1283 ± 953.4	0.37
taVNS information	Days trying po, prior to taVNS[†]	50.5 ± 33.7	53.25 ± 16.2	51.6 ± 26.9	0.88
	Total number of taVNS sessions	16.3 ± 6.7	17 ± 3.4	16.6 ± 5.2	0.86
	GA at the end of taVNS (weeks)	48.2 ± 6.7	45.1 ± 2.7	46.9 ± 5.5	0.42
	Age at follow-up assessment (months)	19.95 ± 2.2	18.7 ± 0.3	19.44 ± 1.8	0.31

Mean ± Standard deviation
[†] Po= Oral feed
* Independent t-test and Fisher's Exact test

To confirm that there are no differences between responders and non-responders, we tested the correlation between the pre-STEP total scores and Bayley-III scaled scores. We found no significant correlation (**Table.4**)

Table 4: Person correlation between Pre-STEP total score and Bayley-III 18 month's performance.

Factors	Responders vs. non-responders	Cognitive	Receptive language	Expressive language	Fine motor	Gross motor
Pre-STEP total score	0.65	0.07	0.48	0.50	0.05	0.13

Analysis of covariance, controlling for GA, showed no differences between responders and non responders across cognition, receptive language, fine motor and gross motor (p>0.5) (**Table. 5**).

Table 5: Results of the Bayley-III assessment using ANCOVA between the responders and non-responders

Section (scaled scores)	Responders	Non-Responders	P-Value
Cognitive	8.6 ± 4.4	7.0 ± 5.3	0.6
Receptive language	8.8 ± 3.0	8.5 ± 4.5	0.9
Expressive language	5.5 ± 2.6	7.2 ± 5.4	0.5
Fine motor	7.2 ± 4.1	6.5 ± 4.6	0.8
Gross motor	7.3 ± 2.5	5.7 ± 4.3	0.5

Mean ± Standard deviation

Infants who responded to early taVNS feeding treatment showed greater average Bayley-III scaled scores than non-responders in the area of cognition (+1.6), receptive language (+0.3), fine motor (+0.7), and gross motor (+1.6), although differences were not statistically significant. However, the expressive language's scaled score was lower in the responders' group than the non-responders group (-1.7) (**Figure. 2 A-E.**) Illustrates the differences in the average scaled scores between the responders and non-responders. We have shown a small but noticeable improvement in the responders' group.

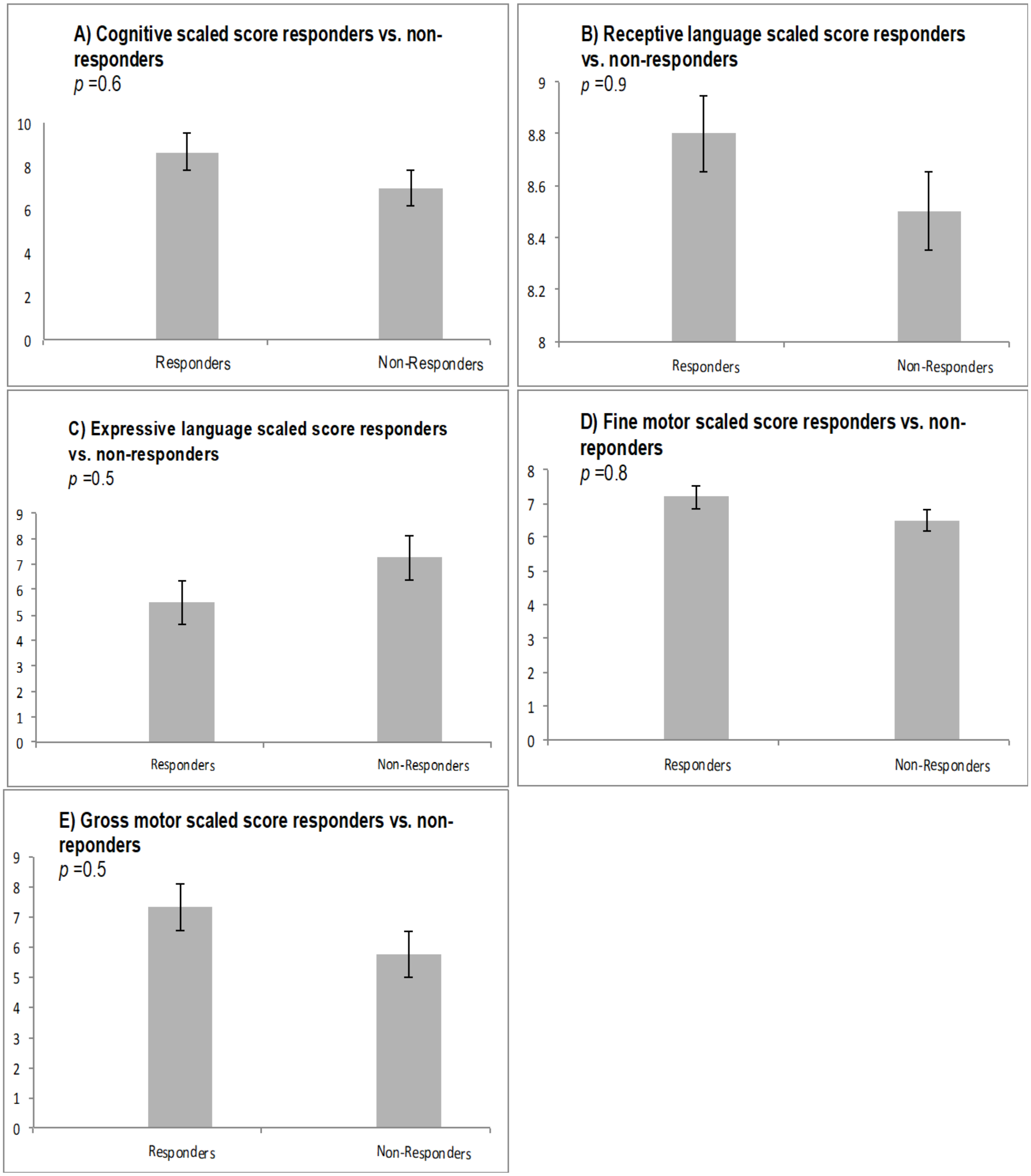

Fig 3: Average Bayle-III scaled scores mean differences between responders and non-responders with standard error.

4.3. Long-term effect of taVNS in sensory profile

This aim's exploratory hypothesis stated that responders would have more typical sensory scores than non-responders, as shown in the Toddler Sensory Profile-2 caregiver questionnaire (SP-2) at 18 months.

In the infants' medical history, all five participants in the non-responder group had IVH, while three out of the seven in the responder's group were diagnosed with IVH. On the other hand, three of the responder's participants PDA, while none of the non-responders had this diagnosis. Furthermore, only in the non-responders was a diagnosis of Lenticulostriate vasculopathy (LSV) (n=1) and Neonatal abstinence syndrome (NAS) (n=2) reported. Yet, none of the diagnoses reach statistical significance.

Twelve toddler parents were contacted and completed the SP-2 caregiver questionnaire, some during the Bayley-III assessment and others via phone interviews. There were seven responders and five non-responders in this sample. All sections of the SP-2 were completed (four quadrants and seven sensory and behavioral sections). (**Table. 6**) displays the dimorphic and medical information of the participants. There was a statistically significant difference between the responders and non-responders in the taVNS information section; days of trying oral feed prior to taVNS intervention initiation (*p*= 0.04). The non-responders, on average, spent more days trying oral feed than the responders' group. There were no other significant differences between the two groups: other taVNS information, medical history, birth characteristics or, age at the follow-up assessment.

Table 6: Demographics information participants (n=12) who completed the 18-month follow-up SP-2 questionnaire, divided into responders vs. non-responders

		Responders (Full po Feed) n=7	Non-Responders (g-tube) n=5	Total	P-value*
Medical History	Total number	7	5	12	[redacted]
	Gender:				[redacted]
	Male	4	3	7	[redacted]
	Female	3	2	5	[redacted]
	Clinical Sepsis	2	3	5	0.31
	Persistent pulmonary hypertension of the newborn (PPHN)	3	2	5	0.69
	Patent Ductus Arteriosus (PDA)	3	0	3	0.16
	Intraventricular hemorrhage (IVH)	Grade I (1) Grade II (1) Grade III (1)	Grade I (3) Grade II (2)	7	0.25
	Hypoxic Ischemic Encephalopathy (HIE)	4	2	6	0.50
	Periventricular Leukomalacia (PVL)	1	1	2	0.68
	Lenticulostriate vasculopathy (LSV)	0	1	1	0.42
	Neonatal abstinence syndrome (NAS)	0	2	2	0.15
Birth Characteristics	GA at birth (weeks)	32.1 ± 5.1	27.9 ± 3.0	30.3 ± 4.7	0.14
	GA at taVNS start (weeks)	41.8 ± 1.8	39.6 ± 9.1	40.8 ± 5.8	0.54
	Birth weight (grams)	1955.0 ± 1335.1	1359.0 ± 1175.6	1706.7 ± 1252.6	0.44
taVNS information	Days trying po, prior to taVNS[†]	28.4 ± 15.0	49.6 ± 15.5	37.25 ± 18.1	0.04
	Total number of taVNS sessions	16.6 ± 7.4	27.4 ± 16.3	21.1 ±	0.15

			12.5	
GA at the end of taVNS (weeks)	43.7 ± 1.7	45.8 ± 3.5	44.6 ± 2.7	0.20
Age at follow-up assessment (months)	19.0 ± 1.7	18.5 ± 0.3	18.8 ± 1.3	0.50

Mean ± Standard deviation
† Po= Oral feed
* Independent t-test and Fisher's Exact test

The results of the SP-2 were broken into two sections; the first section has the four quadrants (seeking, avoiding, sensitivity, and registration) using the Fisher's Exact test. There was no statically significant difference in any of the quadrants between the responders and non-responders (**Table. 7**). Of important note, all participants in the responders' group scored typically except for one responder who atypical score in the four quadrants.

Table 7: Results of the SP-2 quadrants section using Fisher's Exact between the responders and non-responders

Quadrants	Responders (full feed) n=7	Non-Responders (G-tube) n=5	P-value
Sensory seeking			
Typical	6	3	.56
Atypical	1	2	
Sensory avoiding			
Typical	6	3	.36
Atypical	1	2	
Sensory sensitivity			
Typical	6	3	.36
Atypical	1	2	
Sensory registration			
Typical	6	3	.36
Atypical	1	2	

The second section of the SP-2 includes all seven sensory behaviors (general, auditory, touch, movement, oral, and behavior). Of all sections, the sensory general showed a statistically significantly different (*p*= 0.04) (**Table. 8**). The result of the SP-2 has shown that responder to taVNS has more typical sensory scores than the non-responder group in all sections of the SP-2. Also, in the touch and behavior sensory sections, all responders showed typical behavior. Furthermore, the average means of the general, auditory, touch, behavior and all four sensory quadrants (expect seeking behavior) were atypical (much higher than average score) in the non-responders group. In contrast, no atypical average means were found in the responders group (**Appendix C, Table. 1**).

Table 8: Results of the SP-2 sensory behavior section using Fisher's Exact between the responders and non-responders

Sensory behavior	Responders (full feed) n=7	Non-Responders (G-tube) n=5	P-value
Sensory general			
Typical	6	1	.04
Atypical	1	4	
Sensory auditory			
Typical	6	3	.36
Atypical	1	2	
Sensory visual			
Typical	6	3	.36
Atypical	1	2	
Sensory touch			
Typical	7	4	.41
Atypical	0	1	
Sensory movement			
Typical	6	4	.68
Atypical	1	1	
Sensory oral			
Typical	5	4	.68
Atypical	2	1	
Sensory behavior			
Typical	7	4	.41
Atypical	0	1	

CHAPTER 5: DISCUSSION

This research examines the long-term impact of taVNS treatment on an infant's 18 months neurodevelopmental and sensory outcomes. More specifically, looking at the difference between the early responses to taVNS (responders vs. non-responders) on the long-term development of the toddler's life.

One objective of this research was to help predict potential responders to taVNS prior to the intervention. We examined the infant's motor performance using the pre-treatment total STEP score and included it in a predicted model. The other two objectives of this research were to investigate the difference between the responders and non-responders at 18 months follow-up using 1) neurodevelopmental assessment (Bayley-III) and 2) sensory assessment (SP-2).

There are three primary findings from this research. First, pre-treatment STEP total scores did not seem to predict whether an infant would respond to taVNS. Second, the neurodevelopmental outcome demonstrates a positive difference in favor of the response in (cognition, receptive language, fine and gross motor skills). However, these findings did not reach a statically significant difference. Third, findings related to the sensory profiles showed more typical sensory scores in the responder group when compared to the non-responder group at 18 months. Thus, resulting in a statistically significant difference in the general sensory processing section. These results and findings with the related issues are discussed below. Each topic is followed by limitations, future directions, and opportunities in this line of research.

5.1. Pre-STEP total scores as a predicted respond to taVNS

Prior to the study, we hypothesized that the STEP assessment's early motor movement could help predict which infants would respond to the taVNS treatment course. However, this hypothesis was not supported by our findings. Pre-treatment STEP total scores were not a predictor of taVNS responders when controlling for both GA and birth weight. In fact, four out of 15 non-responding infants had a low-risk total STEP score (i.e., high score); this likely explained the non-significant findings in the predicted model. Although, when we used a non-parametric test to examine the differences between the total pre-STEP scores between the responders and non-responders the result was close to significant (p= 0.06, Mann-Whitney U test). This demonstrated that taVNS *may* benefit infants with initial low STEP score to respond to taVNS treatment.

Our hypothesis was built on the assumption that infants with lower STEP total scores would be more likely to have a more extensive brain injury in comparison to those with high STEP total scores. It is known that early brain injuries impact the infant's oral motor functions, which include sucking and swallowing (Benfer et al., 2017; Reilly, Skuse, & Poblete, 1996). Early brain injury may also impact the head and neck's fundamental movements, which are essential components for successful early oral feeding (da Costa et al., 2010). Hence, a possible link between early motor performance and sucking and swallowing skills.

Our findings contribute to a gap in the literature exploring the association between early motor performances and early sucking skills. To date, only two studies have investigated this relationship, and both were observational studies. First, Nieuwenhuis et al. (2012) found a correlation between early sucking using Neonatal Oral-Motor Assessment Scale (NOMAS) and early motor movement measures by the General Movements Assessment (GMA). The GMA assesses the quality of spontaneous movements, namely fidgety movements (FM), in the first five-months to predict cerebral palsy. The authors reported an association between the FM and uncoordinated sucking (Nieuwenhuis et al., 2012). However, these conclusions were not supported by statistically significant data. Of the 42 preterm infants, 30 infants had normal fidgety movement, and ten infants had abnormal fidgety movement. The remaining two infants had absent fidgety movement, only one of which had a normal sucking pattern.

Compared to this study, our study found that the median STEP total score was 13 for the responders and 14 for the non-responders. Both are below the cut-off score (STEP score >16), indicating a high risk of developmental delays. In Nieuwenhuis et al. (2012) study, the authors used Motor Optimality Score (MOS) which, combined the fidgety movement with other motor movements; MOS optimal score is 28 points and a minimum score of 5 (Bruggink et al., 2009). They found no correlation between the MOS and normal and abnormal sucking pattern. In addition, the median MOS scores were 26 for the arrhythmic group and 20 for the uncoordinated group in sucking pattern. The MOS has no cut-off scores established at that time; only recently MOS cut-off scores were associated with Gross Motor Function Classification System for CP (GMFCS) (MOS > 14 GMFCS I or II, MOS > 8GMFCS III-V) (Einspieler et al., 2019).

Lastly, with one infant in the normal sucking pattern, the study claimed a positive correlation between normal sucking pattern and MOS.

Second, Sanchez et al. (2017) found an association between abnormal GMA and later 12 months oral-motor feeding impairment (Sanchez et al., 2017). The study also found evidence correlating abnormal MRI scores with later feeding impairment. Although when combined with MRI, the GMA has an excellent ability to predict CP early, its predictive accuracy is low for mild neurodevelopment conditions or when the GMA used by itself (Kwong, Fitzgerald, Doyle, Cheong, & Spittle, 2018; Morgan et al., 2019; Novak et al., 2017; Støen et al., 2019). Thus, these studies can only identify children with severe CP, who are at a very high risk of developing oral-motor dysfunction that causes later feeding issues. Lastly when quantifying sucking performance in infants with confirmed brain injury results demonstrate an association between motor and sensory brain tracks with sucking smoothness, variability, and irregularity (Tamilia et al., 2019). However, this was completed in a small sample size (n=10), in infants with established brain injury, who are able to orally feed.

Out of the two studies only one study examining infant's feeding and motor performance simultaneously, our study used an intervention (taVNS treatment paired with bottle-feeding). Also, neither of the previous studies reported placement of G-tube and used preterm as their primary inclusion. Our research's primary inclusion criteria were feeding behavior difficulties, and all participants tried oral feed prior to the start of taVNS for an average of 38 days. It is essential to appreciate that not all infants with neonatal brain injuries have early sucking and swallowing issues. In fact, the percentage of infants with early sucking and swallowing problems range between 35%-

48.8% in infants with neonatal brain injuries diagnosis (Barkat-Masih, Saha, Hamby, Ofner, & Golomb, 2010; Mizuno & Ueda, 2005; Quattrocchi et al., 2010). The prevalence of sucking and swallowing problems in infants diagnosed with CP were 57% and 38%, respectively (Reilly et al., 1996).

In our cohort of infants, we found that nine infants, all in the non-responders group, were born to diabetic mothers (IDM); this indicates a negative association between IDM and response to taVNS. Furthermore, some infants received taVNS treatment once a day while others received treatment twice a day; seven responders and nine non-responders were among the participants that received treatment twice a day. These factors may influence our results, making the pre-treatment STEP total scores unable to detect responders to taVNS treatment.

5.1.1. Aim 1 limitations

Our aim's limitations include a small sample size, less ability to control for more potential influence variables, and participant heterogeneity. Due to our small sample size, we were unable to have more variables in the model, such as days of trying oral feed prior to taVNS treatment or GA at the start of taVNS. Also, since this is the first study to examine taVNS in the neonate, our inclusion criteria were not very strict, which led to a heterogenic sample.

In another study with the same cohort, we demonstrated that scores of four STEP items involving head movements (*head in supine with visual stimulation, head in supine with no visual stimulation, rolling elicited by the arm,* and *head movements in supported sitting*) improved significantly in responders ($p<0.05$) in comparison to non-responders. All four items are related to fundamental head and neck movement and may explain why these infants responded to the taVNS intervention when paired with bottle-feeding. Furthermore, Diffusion MRI supports the improvement in the *rolling by arm* item (manuscript in preparation). These results suggest including pre-STEP items related to head and neck movements only in the model instead of the total pre-STEP scores in future research.

5.1.2. Future directions and opportunities aim 1

Our current aim did not show that the infant’s pre-treatment total STEP predicted responds to taVNS intervention. However, our finding did add to the knowledge of early motor performance and infant feeding ability. These findings can be added to further studies that use the novel taVNS treatment with bottle-feeding. A larger and more homogenous sample size, diffusion MRI results, focus on STEP's items related to head and neck movement, and other variables' inclusion are some recommendations for future studies.

The inclusion of a more homogenous sample will ensure that future results are not affected by other factors such as genetic abnormalities and type of brain injury. The use of pre-treatment diffusion MRI will help determine the infant's brain injury type and help stratify the sample size if required.

5.2. Long-term effect of taVNS in neurodevelopment performance

This is the first study demonstrating that infants who received early taVNS treatment for feeding delays also showed long-term better scaled scores outcomes in the responders in cognitive, receptive language, fine and gross motor skills at 18 months evaluation when compared to non-responder group. Although the results did not reach a significant level (*P* < 0.05), these preliminary results are encouraging and suggest some positive impact even with a small sample size. For example, when we compared our findings to Jadcherla et al. (2017) study we find very similar results between infants that discharge with g-tube and full oral feed and our full cohort (responders and non-responders) in all Bayley-III scaled scores at 18 months (**Appendix D, Table. 1**). Our cohort preformed better at receptive language and gross motor scaled scores. While, they scored slightly lower at expressive language and fine motor scaled scores. However, when compering our results with the full-feed group we found that our group scored lower in all scaled scores except for expressive language section.

Our current study is unique in two ways:

1) It is the first study to use a non-invasive taVNS intervention paired with a task (bottle-feeding) in the pediatric population, and

2) The current study is the second ever study that used non-invasive neuromodulation in neonates

To our knowledge, only one study prior to ours has investigated the use of non-invasive neuromodulation, Transcranial Magnetic Stimulation (TMS) in infants less than one year of age. The study showed that TMS could be safe and feasible to use (Nemanich, Chen, et al., 2019). However, the study did not pair the TMS with a task or show the long-term term impact of TMS in the follow-up.

There is increasing evidence that early intervention (in the first 12 months of age) leads to cognitive and motor improvements, and these improvements could lead to long-term gains (Finlayson et al., 2020; Harbourne et al., 2020; Spittle, Orton, Doyle, &

Boyd, 2007). That may describe the difference in performance in favor of the responder group in our study.

Previous studies demonstrated that the use of neurostimulation with a fictional task (i.e., Constraint-induced movement therapy (CIMT)) in older pediatric patients resulted in motor improvement of the targeted area. For example, significant improvements in the active repetitive Transcranial Magnetic Stimulation rTMS) group's affected hand compared to the sham rTMS group were reported (Gillick et al., 2014). This was supported by structural changes in the brain in a double-blind randomized study (Carlson, Ciechanski, Harris, MacMaster, & Kirton, 2018). While the immediate short-term impact of rTMS or tDCS in hand improvement is evident when used with intensive motor learning therapy in hemiplegic children, the long-term impact is still unclear (Kirton et al., 2017; Nemanich, Rich, et al., 2019; Rich, Menk, Krach, Feyma, & Gillick, 2016).

Contrary to our study that targeted very early development in neonates, these studies were completed in older children 7 to 17 years of age, which may be past the critical periods of optimum development. Thus, explain the mild or inconsistent long-term implications.

5.2.1. Aim 2 Limitations

Our study had two primary limitations. One limitation was the small sample size that may explain why we found no significant differences. In addition, we did not control for when and what type of early interventions the children in the two groups received as well as environmental factors. Another possible reason for our finding is that all the infants in our study were scheduled for G-tube placements. Also, the oral feed was tried for 51 days on average before infants were enrolled in the study. These reasons could suggest that our cohort study was at higher risk of neurodevelopmental impairments when compared to other infants with feeding difficulties issues only (Jadcherla et al., 2017; Lainwala et al., 2020; Warren et al., 2019). Without a lager sample size, it is hard to generalize our findings of neurodevelopmental outcome improvement.

5.2.2. Future directions and opportunities aim 2

These promising preliminary results lay a foundation for future research on the long-term positive impact of early taVNS on a child's neurodevelopment.

Additional investigation of the long-term taVNS effects is required to determine further potential benefits. Future studies should have a larger sample size of developmentally delayed children to validate the current results further.

The estimated sample size required to detect a significant difference should be a total of 30 infants, 15 in each group, is required for the power analysis (Faul, Erdfelder, Lang, & Buchner, 2007).

5.3. Long-term effect of taVNS in sensory profile

Our finding with the long-term impact of taVNS on the sensory profile shows that infants who responded to early taVNS treatment and achieved full oral feed before hospital discharge had more typical average mean scores in almost all sensory processing patterns than non-responders. There was a statistically significant difference between the two groups in the general processing sensory sections (p =0.04), items in the general processing section, measure the toddler's broad sensory processing (i.e., the child's sleeping, eating pattern, and adaptation to a new situation) (Dunn, 2014).

Typical sensory scores were more apparent in the responder group than the non-responders; this implies that responders to early taVNS intervention are more likely to react appropriately to typical everyday sensory stimuli in the environment of non-responders. For example, atypical scores were reported on average only in the non-responders group in the following sections: auditory, touch, behavior, and avoiding, sensitivity, registration quadrants. On overage all sensory behaviors and quadrants had a higher scores in the non-responder group this may shows that they were more likely to overreact to sensory stimulus.

Furthermore, there was a statistically significant difference between the responders and non-responders in the taVNS information section; days of trying oral feed prior to taVNS intervention initiation (*p*= 0.04). The non-responders, on average, spent more days trying oral feed than the responders' group. This significant difference is explained by the one extreme outlier participant in the non-responders who tried oral feeding for 77 days before being included in the study. (**Appendix E, Figure. 2**) demonstrate the extreme outlier in the non-responder group by using box and plot graph. The median (range) days of trying oral feed scores were 28 (22-59) days for the responder group and 44 (39-77) days for the non-responder group.

The vagus nerve's location and function may suggest an explanation as to why responding to early taVNS treatment would result in more typical SP-2 scores. The vagus nerve originates in the medulla oblongata and has bilateral projections (Yu, Weller, Sandidge, & Weller, 2008). Importantly, it contains around 80% sensory afferent and 20% motor efferent fibers (Yu et al., 2008). The vagus nerve plays a key role because it acts as an interface between the higher central nervous system circuits and autonomic control circuitry of the brain stem (Hulsey et al., 2016). Its projections end in the nuclei, including the locus coeruleus, raphe nuclei, and the basal forebrain (Dorr & Debonnel, 2006; Hassert, Miyashita, & Williams, 2004). Stimulation of the vagus nerve increases neurotransmitter release in the brain regions (Hassert et al., 2004; Henry, 2002).

There is evidence that VNS improves mood and is a treatment option for adults with mental conditions documented via clinical observation and neuroimaging results (Bajbouj et al., 2010; Elger, Hoppe, Falkai, Rush, & Elger, 2000; Harden et al., 2000). In the Neuroimaging results, VNS affects the bilateral thalami, hypothalami, inferior cerebellar hemispheres, and right postcentral gyrus (Chae et al., 2003; Henry, Bakay, Pennell, Epstein, & Votaw, 2004; Yakunina, Kim, & Nam, 2017). Thus, leading the US Food and Drug Administration (FDA) to approve of vagus nerve stimulation (VNS) as an intervention modality in treatment for adults with depression and children with epilepsy. While the mention evidence is driven from the adult population, it is possible to postulate that the responders' typical sensory profile scores are associated with taVNS treatment.

Observational studies investigated supported our study feeding that there is an association between infants' history feeding difficulties and their sensory profile scores (Davis et al., 2013; Rahkonen et al., 2015; Tauman et al., 2017; Wickremasinghe et al., 2013; Yi, Joung, Choe, Kim, & Kwon, 2015). The results suggested that toddlers with a history of feeding difficulties have statically more significant issues with tactile, movement, and oral sensory processing than toddlers with no history of feeding problems at infancy (Yi et al., 2015). Our study found a significant difference in the general processing sensory section at 18 months follow-up between toddlers who responded to early taVNS treatment in comparison to the non-responders.

In general, children born prematurely are at greater risk of having atypical sensory scores in all four quadrants, tactile, movement, and auditory sensory processing (Rahkonen et al., 2015; Wickremasinghe et al., 2013). Furthermore, toddlers with feeding disorders are significantly more likely to score atypical in oral sensory possessing, registration, sensitivity, and avoidance quadrants than healthy toddlers (Tauman et al., 2017). Overall, toddlers with feeding issues tend to score higher sensory processing scores (Davis et al., 2013).

In another study we found a significant association between the Bayley-III motor composite score (fine and gross scores) and the oral sensory performance (p= 0.03) (Manuscript in preparation). Lower Bayley-III motor scores were correlated with more atypical oral sensory issues.

These findings are aligned with other studies that found correlations between atypical sensory processing scores in the sensory profile and children's low neurodevelopmental motor, cognition, and language (Eeles et al., 2013; Flanagan, Schoen, & Miller, 2019; Yi et al., 2015). Children with feeding difficulties who score atypical in the sensory profile scored significantly lower in both mental and motor developmental index of the Bayley-II and the Sequenced Language Scale for Infants (SELSI) (Yi et al., 2015). More typical scores of auditory, touch, and oral sensory processing were associated with improved language composite scores, and more typical scores of touch and movement correlated with improving cognitive composite scores in the Bayley-III (Eeles et al., 2013). Also, atypical registration and avoidance quadrants were associated with low motor composite scores (Eeles et al., 2013). Our finding may be related to the early taVNS treatment when paired with bottle-feeding which may perhaps influence the oral sensory performance at 18 months. Further study about this potential related is needed.

5.3.1. Aim 1 Limitations

Our small sample size is a limitation to our finding due to the COVID-19 pandemic hindering our ability to complete more follow-up evolutions. Another limitation is that all infants were trying oral feed for an average of 37 days. All participants were scheduled for G-tube placement; this may influence the SP-2 results by not intervening early on. The wait while trying oral feed can significantly impact infants' transition ability to full oral feed and may cause oral aversion or defensiveness (Borowitz & Borowitz, 2018; Cerro et al., 2002; Dobbelsteyn et al., 2005).

5.3.2. Future directions and opportunities aim 3

Our findings are encouraging and demonstrate a significant difference in favor of the responder's group in the general processing sensory section. More typical scores were apparent in almost all sensory processing sections and quadrants in the responders to taVNS compared to the non-responders. However, to generalize our findings, additional studies with a larger sample size are necessary to investigate the long-term impact of taVNS and confirm our initial results about the sensory performance. There are a strong association between infants' neurobehavioral assessment and 12 months feeding impairment (Sanchez et al., 2017). Thus, we recommend the use neurobehavioral assessment such as the Neonatal Intensive Care

Unite Network Neurobehavioral Scale (NNNS) in our study particularly items related to infant’s stress and arousal level and examine the association to SP-2 at the follow-up.

In addition, it appears that days of trying oral feed maybe an influencer factor in the SP-2 results. Thus, further studies should control for the days of trying oral feed prior to taVNS or establish an inclusion criteria about the numbers of days allowed to try oral feed prior to enrollment.

CHAPTER 6: CONCLUSION

In the first part of this study, we used the pre-treatment total STEP scores as an early motor biomarker for this cohort of infants who all experienced feeding behavioral issues. The pre-treatment total STEP scores were used for both the infants who responded to taVNS treatment when paired with bottle-feeding and non-responders. We then examined if the pre-treatment total STEP scores predicted to respond to taVNS intervention.

We found that the pre-treatment total STEP scores failed to contribute to the prediction model significantly. Yet, our findings increased our knowledge of the possible association between neonatal brain injury, early feeding problems, and early motor outcomes. Initial result suggest that infants who started to taVNS with STEP total scores can be the once who responded better to the intervention.

In the second part of the study, we looked at the long-term effect of early taVNS treatment in neurodevelopmental and sensory outcomes at 18 months follow-up.

We found that infants who responded to early taVNS treatment when paired with bottle-feeding had better overall neurodevelopmental outcomes compared to non-responders, but was not statically significant. Furthermore, the average mean of the responders was within the typical range in all the behavioral and quadrants sections. On the other hand, the non-responders' average mean was atypical in auditory, touch, behavior, and avoiding, sensitivity, registration quadrants of the sensory profile.

These preliminary results are encouraging in support of the use of taVNS intervention. The results also suggest that early taVNS treatment for infants with feeding problems can have a long-term some positive impact on both neurodevelopmental and sensory performance. Specifically, in lowering hypersensitivity in all sensory behaviors and quadrants in the responder group, this was evident in general sensory processing.

These initial findings provide proof of concept to the use of neuromodulations in pediatric research in general, and more specifically to the use of neuromodulations with neonates. For our study future studies can include a larger sample size, utilization of DKI/DTI, controlling for more variables (days trying oral feed and GA at the assessment), and stratification based on type of brain injury, pre or full term participants.

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Appendix A:

Table 1: Global test of the predicated model

Test **(Overall model evaluation)**	χ^2	*df*	**P-value**
Likelihood Ratio	3.6	3	0.31
Score test	3.7	3	0.33
Wald test	2.9	3	0.39

χ^2: Chi-square, *df*: degree of freedom

Appendix B:

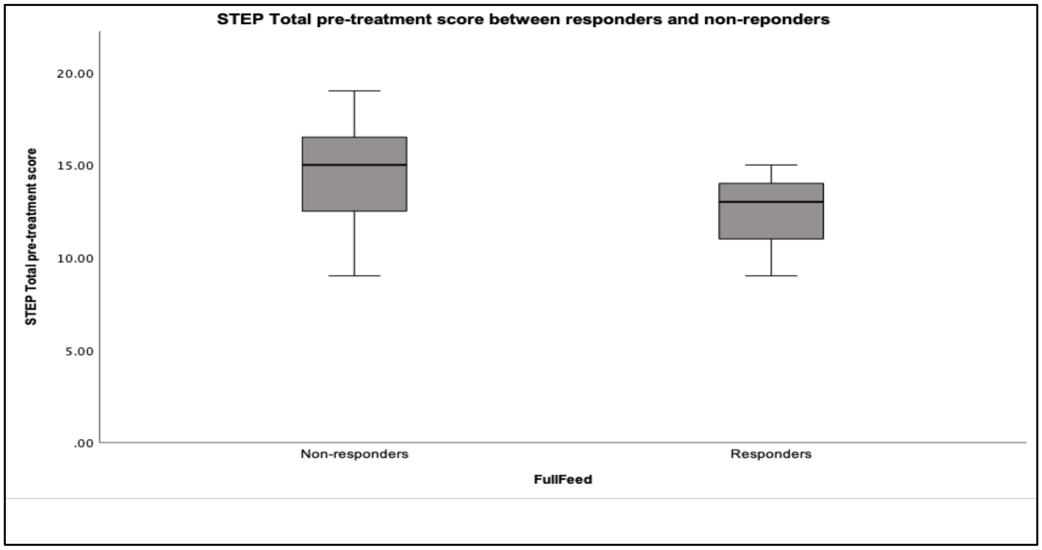

Fig 1: STEP total pre-taVNS scores between the responders and non-responders. The boxes represent the individual values between the 25th and 75th percentile (IQR), the whiskers represent the range of the values

Appendix C:

Table 1: Results of the SP-2 between the responders and non-responders.

SP-2 Sensory behavior	**Responders (n=7)**	**Non-responders (n=5)**
General	16.8 ± 5.1	24.0 ± 3.5
Auditory	10.1 ± 2.8	15.0 ± 5.7
Visual	16.6 ± 3.9	17.6 ± 4.7
Touch	8.1 ± 1.9	14.0 ± 9.2
Movement	16.4 ± 3.4	18.0 ± 4.2
Oral	13.1 ± 5.3	13.8 ± 3.8
Behavioral	9.4 ± 1.5	15.2 ± 8.3
SP-2 Quadrants		
Seeking	28.7 ± 4.9	31.2 ± 2.9
Avoiding	16.6 ± 4.9	22.0 ± 8.7
Sensitivity	21.1 ± 3.9	27.8 ± 7.5
Registration	17.4 ± 7.5	21.8 ± 10.7

Mean ± Standard deviation

Appendix D:

Table 1: A comparison of Bayley-III between Jadcheria et al. (2017) study and our cohort results. Values stated as mean ± SD and median (IQR)

Domain	Jadcheria et al., 2017, G-tube-Fed (n=77)	Jadcheria et al., 2017, full-PO-Fed (n=177)	Our findings (R + NR) (n=10)
Time of Bayley-III evaluation (months)	18.3 ± 1.3	18.4 ± 1.8	19.44 ± 1.8
Receptive scaled score	6 (5-9)	8 (6-9)	8 (4-12)
Expressive scaled score	6 (4-8)	7 (5-9)	5.5 (1-15)
Fine motor scaled score	7 (5-10)	9 (7-11)	6 (2-13)
Gross motor scaled score	5 (3-8)	8 (6-9)	6.5 (1-11)

* PO= Oral feed

Appendix E:

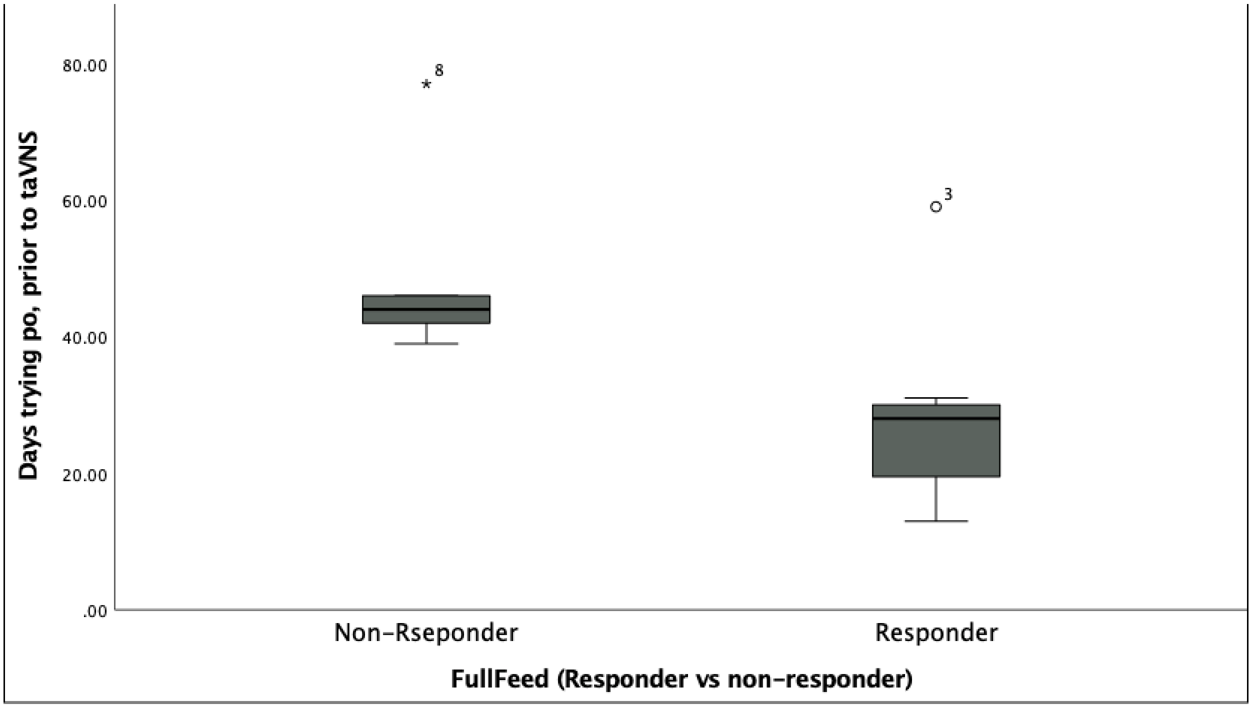

Fig2: Number of days of trying oral feed prior to the start of taVNS between the responders and non-responders. The boxes represent the days 25th and 75th percentile (IQR), the whiskers represent the range of the values.
* Extreme outlier