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Title: Salivary α-Amylase as a Marker of Stress Reduction in Individuals with Intellectual Disability and Autism in Response to Occupational and Music Therapy

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Abstract

Background Though the benefits of a range of disability-centric therapies have been well studied, little remains known about how they work, let alone how to monitor these benefits in a precise and reliable way. **Methods** Here, in two independent studies, we examine how sessions consisting of occupational or music therapy, both widely recognised for their effectiveness, modulate levels of salivary α-amylase (sAA), a now time- and cost-efficient marker of stress, in individuals with intellectual disability (ID) and autism spectrum disorder (ASD). Pre- and post- session levels of sAA were compared in both groups in response to therapy and control sessions. **Results** In comparison to control sessions, occupational therapy significantly dampened rises in sAA levels while music therapy significantly decreased baseline sAA levels, highlighting the ability of both types of therapy to reduce

stress and by proxy contribute to enhancing overall wellbeing. **Conclusions** Not only do these results confirm the stress-reducing nature of two types of multisensory therapy, but they support the use of sAA as a potential tool for evaluating stress levels in individuals with ID and ASD, providing an important physiological lens which may guide strategies in clinical and non-clinical care for individuals with disabilities.

Introduction

ID and ASD – definitions, prevalence, and altered stress responsivities

Intellectual disability (ID) is a generalised neurodevelopmental disorder characterised by limitations in intellectual functioning and adaptive behaviour, including social and practical skills, while autism spectrum disorder (ASD) is a developmental disability featuring core behavioural deficits in social interaction and communication and repetitive, stereotyped behaviours and interests. Rates of both ID and ASD have been on the rise in the last decades [1-3], contributing to a multidimensional social challenge of increasing concern in addition to the complex difficulties experienced by affected individuals.

Generally, individuals with ID and ASD tend to experience lower levels of wellbeing than their neurotypical counterparts, including elevated stress reactivity, responding in acutely accentuated ways to sensory, social and emotional stressors [4]. Stress manifests itself in complex physiological and psychological ways, including through cortisol-mediated hypothalamus-pituitary-adrenal (HPA) axis activity for chronic stress and salivary α-amylase (sAA)-mediated sympathetic-adrenal-medullary (SAM) axis activity, part of the sympathetic nervous system (SNS), for acute stress [5, 6]. Relationships between the autonomic nervous system (ANS) and aberrant central nervous system (CNS) development in ID and ASD are complex and poorly understood, yet evidence has emerged of altered autonomic tone and responsivity, including hyper-sympathetic arousal insufficiently attenuated by parasympathetic influences [6, 7]. A direct product of this stress pathway hyperactivity, sAA

acts as a digestive enzyme produced in a diurnal pattern by the salivary glands and pancreas, released downstream of β-adrenergic receptor activation in response to physical and psychological stress and correlated to various other physiological makers of SNS activity [6, 8]. It is quick, cost-efficient, and non-invasive to measure and as such may act as a useful and convenient marker of changes in ANS-linked stress levels [6, 9-11].

Therapies for ID & ASD – Occupational & Music Therapies

To address the challenges inherent to dysregulated stress responsivities, a number of therapies have been proven effective in reducing stress levels and increasing overall wellbeing of individuals with ID and ASD, many of which target social skills and sensory hypersensitivity [12, 13]. Occupational therapy, consisting of various activities specifically aimed at developing fine, gross motor, and daily living skills, has garnished substantial support for its effectiveness in improving play, language, social interaction, independent functioning and self-management skills [12, 14, 15]. Past research that have measured the efficacy of occupational therapy amongst persons with ID have mostly done so on populations of children, rather than adults. Nonetheless, results from these studies reveal that occupational therapy is generally an effective method of intervention for persons with ID [16]. Music therapy, consisting of listening to or performing music often as a group, has received evidence for its ability to improve social tolerance, flexibility and engagement, but also emotional responsiveness and attention span [17, 18], having been ranked among the most highly ranked forms of complementary and alternative treatments [19,20]. Previous studies that have investigated the efficacy of music therapy on adults with ASD have found that music therapy significantly alleviates autistic symptoms [21]. Taken together, the literature highlights the effectiveness of both occupational and music therapy to decrease stress and improve both skills and overall wellbeing in individuals with ID and ASD.

Study rationale

To integrate and address the above challenges, we sought to confirm the effectiveness of multisensory therapies while evaluating the much-needed validity of using sAA levels as a tool for the quick and reliable measure of stress among individuals with ID and ASD. To do so, we conducted two separate studies, the first of which monitored sAA levels in response to occupational therapy in individuals with ID, and the second of which evaluated sAA levels in response to music therapy in individuals with ASD.

General methods

sAA sampling

In both ID and ASD groups, sAA concentration levels were measured inside the therapy or control session room approximately 2 minutes prior to each session and 2 minutes after each session. sAA levels were measured using a portable sAA monitor (Nipro Co., Japan). Saliva was collected by a test strip placed under the tongue for 30 seconds and immediately measured, with the result being displayed within 60 seconds. The sAA monitor used had been thoroughly examined and assessed to be valid and reliable. All sAA levels were measured by the same research assistant who was recruited from and trained within the Affiliative Behaviour and Physiology Lab.

Statistical analysis

Prior to data analysis, univariate and multivariate distributions of sAA levels and potential covariates were examined for normalcy, homogeneity of variance, outliers, influential cases and missing cases. sAA levels were normally distributed and no transformations were applied. The distance of each case to the centroid was evaluated to screen for multidimensional outliers. Other non-continuous variables (e.g. gender) were analysed with non-parametric statistics. No missing cases were found to be present in the data. A series of repeated-measures Analysis of Covariance (ANCOVA) tests were next performed on sAA levels in the two groups using age as covariate with a two-tailed p-value of .05, after which Tukey's post-hoc tests were applied to verify significance. All data analysis was performed

with R Statistical Software (2017, v 3.3.2). All values are reported as mean ± standard error of the mean (SEM).

Ethical approval

Research was undertaken with the understanding and written consent of each participant's legal caretaker in accordance with the ethical principles stated in the World Medical Association Declaration of Helsinki (version 2002).

Study 1 – Occupational therapy in ID

ID participants

Participants with ID (n = 20, mean age = 36.75 ± 11.29 years old) had received a clinical diagnosis of ID from a licensed clinician. To rule out cases of secondary ID, individuals with an ID linked to a clear neuropsychiatric syndrome (e.g. Fragile X syndrome or Down syndrome) or to obvious neurological deficits were excluded from the study. Individuals in the ID group were 50% male and 50% female. Participants in the ID group were recruited from the "La Casa di San Biagio" clinic in Camigliatello Silano, Cosenza, Italy.

Occupational therapy sessions

A random 50% of the ID participants underwent a group occupational therapy session the first week and a control session a week thereafter, while the remaining 50% of the ID participants underwent the sessions in the opposite order. The order in which participants received control and therapy sessions were counterbalanced to account for any order effects that could have occurred. For instance, participants could have gotten more accustomed to social interaction in the first condition, regardless of whether it was therapy or control session, and this could have facilitated the effectiveness of the second condition. However, in the analysis, all participants who had undergone occupational therapy were treated as one independent sample, regardless of the sequence of the therapy and control conditions which were assigned to them. Group occupational therapy consisted of 50 minutes of activities of

daily living (ADLs) (e.g. personal hygiene, feeding, bathing, grooming), instrumental activities of daily living (IADLs) (e.g. meal preparation, management of communication, management of home environment) and social participation (e.g. establishing peer-to-peer relationship). These sessions were orchestrated by a licensed occupational therapist from the "La Casa di San Biagio" clinic in groups of 3 participants. Both occupational therapy sessions and control sessions took place at 2:00 pm, consistently two hours after participants' lunch time, to control for circadian rhythm-induced fluctuations in sAA levels. Control sessions consisted of 50 minutes of regular daily activities in the "La Casa di San Biagio" clinic in groups of 3 participants.

Results

Occupational therapy decreases sAA levels in individuals with ID

A general linear model highlights a statistically significant difference ($F= 6.76$; $p < .05$) between sAA level changes in individuals with ID in response to occupational therapy versus a control session. In particular, while sAA levels rose by 50.8 U/mL, or 54.3%, from 93.6 ± 19.9 to 144.4 ± 18.9 U/mL following a control session, they remained stable, increasing only slightly by 1.4 U/mL, or 1.7%, from 84.2 ± 15.4 to 85.6 ± 13.2 U/mL, following an occupational therapy session (**Figure 1**). Age and gender were added as co-variates in Analysis of Covariance (ANCOVA), but the inclusion of these variables did not alter the main result. Indeed, these variables did not show a significant effect on the analysis and their p-values did not reach statistical significance (F =.945; p =.334; *ns*).

Study 2 – Music therapy in ASD

ASD participants

Participants in the ASD group were recruited from a patient population from the Observation and Functional Diagnosis Lab (ODFLab) at the University of Trento in Trento, Italy. ODFLab is a referral center to which individuals with a wide range of developmental disabilities are brought for assessment from across Italy. Individuals with ASD (n = 15, mean age = 11.29 ±

2.97 years old) had received a clinical diagnosis of Autism Disorder according to DSM-5 criteria between the ages of 4 and 5, which was subsequently confirmed by Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule-Generic (ADOS-G), and Childhood Autism Rating Scale (CARS). To rule out cases of secondary autism, individuals with a diagnosis of Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS) were excluded from the study, as were individuals diagnosed with any other major medical condition (e.g. seizures, Fragile X syndrome) or visual or hearing impairment. Individuals in the ASD group were 100% male.

Music therapy sessions

A random 50% of the ASD participants underwent a group music therapy session the first week and a control session a week thereafter, while the remaining 50% of the ASD participants underwent the sessions in the opposite order. Similar to Study 1, the sequence in which participants received control and therapy sessions were counterbalanced to account for order effects. In the analysis, all participants who had gone for music therapy were treated as one sample, independent of the sequence of the therapy and control conditions. Music therapy consisted of 50 minutes of group interaction and discussion related to emotions upon listening to composed musical pieces, and group-based musical improvisation with the use of musical instruments (e.g. drums). Discussions during group therapy develops emotional recognition and fosters social cohesion, group-based improvisation enhances cooperation. These sessions were orchestrated by a licensed music therapist from the ODFLab in groups of 3 participants. Both music therapy sessions and control sessions took place at 2:00 pm, consistently two hours after participants' lunch time, to control for circadian rhythm-induced fluctuations in sAA levels. Control sessions consisted of 50 minutes of regular daily activities in the ODFLab in groups of 3 participants.

Results

Music therapy decreases sAA levels in individuals with ASD

A general linear model highlights a statistically significant difference ($F= 5.29$; $p < .05$) between sAA level changes in individuals with ASD in response to music therapy versus a control session. In particular, while sAA levels remained stable, increasing only slightly by 1.6 U/mL, or 3.8%, from 42.4 ± 5.9 U/mL to 44.0 ± 5.8 U/mL following a control session, they dropped 7.9 U/mL, or 17.4%, from 45.3 ± 5.1 U/mL to 37.4 ± 5.2 U/mL following a music therapy session (**Figure 2**). Age was added as a co-variate but the inclusion of this variable did not alter the main result. Indeed, this variable did not show a significant effect on the analysis and its p-value did not reach statistical significance ($F =.80$; $p =.528$; *ns*). In the ASD population, only male participants were recruited, and as such, gender was not added as a co-variate.

Discussion

Implications

sAA may act as a quick and reliable marker of stress levels in individuals with ID and ASD

These results support the use of sAA measures as a means of monitoring general stress, as well as, within a more confined time window, the specific short-term impact of therapy: sAA levels generally return to baseline within 10 minutes after an acute change [6], and as such may act as a short-term, cost-effective and easily accessible marker of stress and general affect. This may inform clinicians, therapists and families on the state of an individual in a quick and reliable way, contributing to the ongoing implicit and explicit understanding of the individual and the facilitation of their case management.

Occupational and music therapy alleviate stress and associated difficulties in ID and ASD with far-reaching effectiveness

Building upon previous literature, these results confirm that occupational and music therapy are effective in decreasing sAA concentrations in individuals with ID and ASD. Interestingly, while occupational therapy accentuated an already existing trend of decreasing sAA levels, music therapy was able to reverse the direction of sAA change, decreasing, instead of increasing, sAA levels. Taken together, these results show that such therapies not only further alleviate stress levels which were declining, but also decrease baseline stress levels which were previously on the rise.

In addition, beyond stress, sAA levels have been linked to illness, fatigue and aggression [6], higher cortisol reactivity and disruptive behaviours [22], and anxiety, panic disorders and depression [11]. sAA and cortisol levels are also higher in individuals with ASD with lower intellectual functioning [23], and since stress compromises cognition [24], this may feed into a cycle of anxiety and misunderstanding, all of which highlight the importance of rapid and effective intervention. In this light, these results confirm the potential of alternative therapies to alleviate not only stress but multiple other important and persistent difficulties among individuals with ID and ASD. In addition, in our studies, since participants spanned a range of ages and gender, and the therapies used were of two distinct types, the use of sAA as a tool to monitor stress and well-being appears effective irrespective of therapy type, age, and gender. As such, these results provide robust evidence for the far-reaching nature of therapeutic effectiveness.

Limitations and future directions

Despite providing supportive evidence for the stress-relieving nature of various interventional therapies, methodological and conceptual limitations remain. With regards to the data, the sample size remains modest and as such, the results remain to be reproduced. Within the ASD group, only male participants were recruited, which limited our analyses and understanding of the effect of gender on music therapy efficacy. Replications of this study with larger sample sizes, and inclusion of both male and female participants, will generate greater reliability in the findings that have been obtained. In addition, sAA levels rose 54.3%

after the control session in the ID group but remained stable after the control session in the ASD group—since time of day, location and duration of the control session were controlled for, plausible factors for this discrepancy include age, which was higher in the ID group, and gender, which was mixed in the ID group.

Certain empirically derived conceptual limitations with regards to the role of sAA also remain. In a behavioural genetic study, sAA has been shown to display a certain degree of state variance in such a way that it may increase or decrease in response to stressful stimuli [25]. In line with these observations, individuals with ASD have been shown to be both hypo-responsive to stress, such as following a social evaluative threat, and hyper-responsive to stress, such as following unpleasant stimuli [26]—they also display significant intra-individual variability [4], with one report suggesting that sAA was downregulated in individuals with ASD [27]. While they generally demonstrate a therapy-induced decrease in stress levels, these results are to be interpreted with caution in the greater context of ASD stress research, both in terms of how they affect basal stress levels and in terms of how they influence stress responsiveness. Finally, at a physiological level, while directly reflective of SNS activity, sAA is also influenced by parasympathetic nervous system (PNS) activity—while SNS activity increases salivary protein secretion, PNS activity increases salivary flow rate, both resulting in higher overall levels of sAA [28], thus confounding the arguably over-simplified link between sAA and stress-responsive SNS activity.

This said, these results lay the foundation for a number of interesting future questions. In light of the aforementioned, it will be important to keep disentangling the precise physiological and psychological mechanisms of action of occupational and music therapy, especially in light of the role of sAA, beyond increasing self-awareness, sensory integration, and social interaction. Specifically, systematic analyses of the individual components of these therapies, and their combinatorial effects on various parameters of the patient's psychological and physiological health would be required. In addition, links to sensory integration differences in ASD, including the differential influence on synesthetic populations,

as well as links between levels of the digestive enzyme sAA and prevalent metabolic disorders in ASD, may provide fecund avenues for further exploration.

Conclusions

These results support the benefits of alternative therapies for individuals with ID and ASD, defending their widespread adoption and implementation. In conjunction, they support the monitoring of sAA-reflected stress levels as a quick, reliable tool for better understanding and managing the implicit states of individuals affected by ID and ASD.

Figure 1. Changes in sAA concentration (U/mL) in response to occupational therapy sessions in individuals with ID. The difference between pre- and post-session sAA concentrations is expressed as a fraction (%) of pre-session sAA levels. Control sessions increase sAA levels by 54.3%, while occupational therapy sessions increase sAA levels by 1.7%, resulting in a 31-fold difference in the effect on sAA levels of control versus occupational therapy sessions (* p-value < 0.05, ** p-value < 0.01).

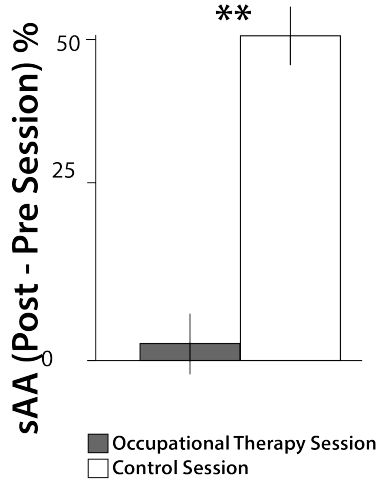
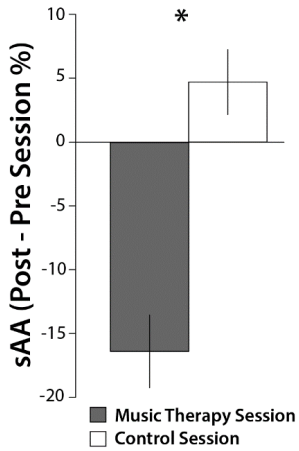


Figure 2. Changes in sAA concentration (U/mL) in response to music therapy sessions in individuals with ASD. The difference between pre- and post-session sAA concentrations is expressed as a fraction (%) of pre-session sAA levels. Control sessions increase sAA levels by 3.8%, while music therapy sessions decrease sAA levels by 17.4%, resulting in a nearly 5-fold difference in the effect on sAA levels of control versus music therapy sessions (* p-value < 0.05, ** p-value < 0.01).



Conflicts of Interest

The authors declare no conflicts of interest.

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