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Breaking Barriers in Rett Syndrome Rehabilitation: Innovative Technologies for Cognitive and Motor Treatment, from Tele-Rehabilitation to Virtual Reality and Serious Games

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INTRODUCTION

Disability represents a complex and multidimensional condition that impacts not only individuals directly affected but also their families and society (Mackelprang, Salsgiver & Parrey, 2021). Among the most severe forms of disability are multiple disabilities, which combine intellectual, motor, sensory, and communicative impairments, significantly affecting quality of life and personal autonomy (Hale, Naglieri, Kaufman & Kavale, 2004). Within this context, Rett Syndrome (RTT) stands out as a paradigmatic example, characterized by a unique combination of neurological, cognitive, and motor deficits that require an integrated and highly personalized rehabilitative approach.

First identified in 1966 by neurologist Andreas Rett, RTT manifests as a progressive regression of previously acquired abilities, including language and motor skills, accompanied by characteristic symptoms such as stereotypical hand movements, respiratory irregularities, and communication challenges (Hagberg, Aicardi, Dias & Ramos,1983). With a prevalence of approximately 1 in 10,000–15,000 live female births, RTT is one of the leading genetic causes of intellectual disability in females (Leonard, Bower & English, 1998).

The literature highlights the critical role of rehabilitation in counteracting the progression and chronic nature of multiple disabilities (Kohzuki, 2024), including Rett Syndrome. Rehabilitation, when personalized and consistently applied, has been shown to delay functional decline, mitigate the severity of symptoms, and improve the overall quality of life of patients (Lotan, 2006; Fabio, Capri, Lotan et al., 2018). By engaging patients in targeted cognitive, motor, and communicative activities, rehabilitation enhances residual abilities and promotes neuroplasticity, allowing individuals to maintain or regain functional independence wherever possible (Rozensztrauch, Sebzda & Śmigiel, 2021).

Despite the proven benefits of rehabilitation, the literature also emphasizes the significant limitations that characterize rehabilitative pathways for RTT and multiple disabilities in general (Lim, Greenspoon, Hunt, & McAdam, 2020). Ensuring active and consistent patient participation remains challenging due to the severe motor impairments, cognitive deficits, and limited attention spans typical of RTT (Pidcock, Salorio, Bibat et al., 2016). Furthermore, the highly heterogeneous nature of the syndrome requires individualized rehabilitation plans, which can be both resource-

intensive and difficult to sustain over time (Fabio, Caprì & Martino, 2019). The success of rehabilitation often depends on the involvement and training of caregivers, who face considerable physical, emotional, and logistical burdens (Pidcock et al., 2016). Moreover, effective rehabilitation requires high-frequency and long-term interventions, a goal that is often unattainable due to financial constraints, infrastructural deficiencies, and limited access to specialized centres (Lotan, 2006; Romano, Di Rosa, Tisano, Fabio & Lotan, 2022). Finally, logistical barriers such as difficulties in traveling to rehabilitation facilities further compound these challenges, particularly for individuals who experience psychophysical fatigue and economic limitations (Lotan, Ippolito, Favetta & Romano, 2021).

Thus, while rehabilitation remains essential to delay the chronic progression of symptoms and preserve functional abilities, traditional methods often fail to deliver sustainable results. This leaves families and caregivers with limited options, emphasizing the need for innovative approaches capable of overcoming these long-standing barriers.

In this context, technology emerges as a transformative tool with the potential to revolutionize rehabilitation for RTT (Lotan, 2007). Assistive technologies, such as tele-rehabilitation platforms, virtual reality (VR), and serious games, provide opportunities to overcome many of the challenges associated with RTT rehabilitation (Craddock, 2003). The literature highlights that in pathological conditions like RTT, the integration of tools such as eye-tracking devices, interactive software, and immersive environments enables technologies to enhance patients' motivation, engagement, and participation (Lewis, Cooper, Seelman et.al, 2012). These technological solutions facilitate highly personalized interventions tailored to individual needs while also reducing logistical burdens. Furthermore, they allow for frequent, flexible, and remotely manageable rehabilitation sessions (Newell, 2011), which is particularly important for individuals with RTT (Lotan, 2006).

However, despite their proven efficacy in other neurodevelopmental and motor disorders, advanced rehabilitation technologies remain significantly underutilized in RTT, representing a critical gap in research and practice (Stasolla, Perilli, Di Leone et al., 2015; Townend, Kaufmann, Marschik, Fabio et al., 2017; Amoako & Hare, 2020). The limited application of these tools

underscores the urgent need for innovative solutions capable of enhancing rehabilitation outcomes while addressing the limitations of traditional methods.

This thesis seeks to bridge the gap between technological potential and its practical application in RTT rehabilitation. The studies of this thesis focus on adapting and developing advanced technologies to support cognitive and motor stimulation, demonstrating their feasibility and effectiveness in enhancing patient outcomes. By leveraging tools such as tele-rehabilitation platforms, immersive VR environments, and serious games, this thesis highlights the innovative power of technology to improve rehabilitation pathways for individuals with RTT and their caregivers.

The work is structured into four chapters. The first chapter provides a comprehensive overview of Rett Syndrome, describing its clinical features, progression, and the profound impact it has on the lives of patients and families. The second chapter reviews the existing literature on rehabilitation for RTT, highlighting both its capacity to delay functional decline and the inherent limitations of traditional approaches. The third chapter introduces the role of assistive technology in rehabilitation, showcasing its potential to address longstanding challenges and improve outcomes. Finally, the fourth chapter details the 5 experimental studies conducted, including the development of tele-rehabilitation systems, immersive VR environments, and serious games tailored to the specific needs of RTT patients.

In relation to this last chapter, the research on advanced technologies for RTT rehabilitation follows a progressive trajectory, evolving from tele-rehabilitation platforms to immersive solutions utilizing virtual reality (VR) and Serious Games. This progression underscores a comprehensive and innovative approach to tackling the complex challenges faced by individuals with Rett Syndrome.

The first research work concerns the development of an advanced tele-rehabilitation software specifically designed for RTT. This marked a significant initial step in overcoming logistical barriers, allowing for frequent and personalized interventions. The platform provided an effective model for remote rehabilitative care and established the foundation for integrating more advanced technologies.

Once the necessary tools, platform, and systems were identified to enable consistent, high-frequency, and personalized remote rehabilitation, the focus shifted to the development of interactive software targeting two key rehabilitation domains: motor and cognitive.

In the motor domain, immersive VR environments were introduced for gait training and upper limb movement. The initial objective was to test whether RTT patients could understand and accept these VR environments. This groundwork proved essential for the creation of targeted rehabilitation tools. After demonstrating feasibility, the research advanced to the development of a virtual game specifically designed to train upper limb motor skills, providing a more engaging and tailored therapeutic experience.

In the cognitive domain, the focus was on Serious Games. An initial project evaluated the behavioral and attentional parameters of patients to demonstrate how these games could enhance motivation and, consequently, the effectiveness of learning. One such game, the “Fiaba Interattiva”, which uses eye-tracking technology to engage patients in personalized narratives, showed improvements in memory, attention, and happiness indices. Building on these results, the research progressed to the development of the “Lettoscrittura” project, a Serious Game aimed at fostering cognitive learning and linguistic skills through immersive and accessible interactions.

The studies presented in this thesis underscore the significant potential of tele-rehabilitation platforms, immersive VR systems, and serious games in RTT rehabilitation. These tools not only improve participation, motivation, and therapeutic outcomes but also offer practical solutions to long-standing barriers such as accessibility, caregiver burden, and limited patient engagement. By fostering consistent, personalized, and engaging rehabilitation experiences, this work highlights how technological advancements can improve the effectiveness of rehabilitation. prevent the progression to chronic conditions and significantly enhance the quality of life for individuals with RTT and their caregivers.

CHAPTER ONE: RETT SYNDROME

The objective of this chapter is to provide a comprehensive overview of Rett Syndrome (RTT) within the broader framework of multiple disabilities, emphasizing its unique and complex clinical, diagnostic, and therapeutic aspects. By defining multiple disabilities as concurrent impairments that significantly affect cognitive, physical, sensory, and communicative abilities, the chapter contextualizes RTT as a paradigmatic example of such conditions. Through an exploration of its historical evolution, clinical presentation, developmental stages, and atypical forms, the chapter aims to shed light on the syndrome's multifaceted nature.

A key focus is placed on the diagnostic advancements and changes from the DSM-IV to the DSM-V, alongside insights into the genetic etiology of RTT, particularly the role of the MECP2 gene and its associated mutations.

1.1 Multiple Disability and Rett Syndrome

When discussing multiple disabilities, this term refers to "concomitant impairments (intellectual, motor, sensory, and/or communicative disorders) that cause negative functional consequences" (Hale, Naglieri, Kaufman & Kavale, 2004).

Multiple disabilities represent a combination of impairments that affect various aspects of an individual’s life, including cognitive, physical, and communicative abilities. As Smith and Tyler describe, "children with multiple disabilities exhibit a combination of limitations in multiple areas, making their active and meaningful participation in educational and social environments challenging" (Smith, 2010).

These disabilities require specific interventions and a multidisciplinary approach. Hallahan et al. emphasize that "students with multiple disabilities benefit from specialized education tailored to their complex needs, particularly through the support of assistive technology and individualized teaching methods" (Kauffman, Hallahan, Pullen et al., 2018).

Furthermore, according to the World Health Organization, "individuals with multiple disabilities face greater barriers in daily life compared to those living with a single disability, and therefore require integrated and personalized support services" (WHO, 2011).

Within the framework of multiple disabilities, specific conditions warrant particular attention due to

their complexity. One such condition is Rett Syndrome, a rare genetic disorder that predominantly affects girls.

Rett Syndrome (RTT) is a progressive developmental neurological disorder that primarily affects girls. It was first identified in 1966 by neurologist Andreas Rett, after whom it is named. Since then, approximately 70 years have passed, during which this condition has been studied from both clinical and genetic perspectives (Fabio, Colombo, Russo et al., 2014).

1.2 Historical Overview

The discovery and recognition of Rett Syndrome can be divided into several key stages that have marked progress in understanding this complex pathology.

The first stage dates back to 1966, thanks to the pioneering work of Austrian neurologist Andreas Rett, who observed certain common characteristics in 22 young patients. His attention was drawn by stereotypical hand movements in two girls sitting next to each other in his clinic's waiting room. Subsequently, Rett identified further distinctive elements of the pathology, such as dementia, autistic behaviors, facial inexpressiveness, ataxic gait, cortical atrophy, and hyperammonemia (Rett, 1966; Leonard, Downs, Benke et al., 2022). Despite the significance of his observations, Rett's articles published during those years did not receive much attention. It was not until 1982 that Swedish neurologist Bengt Hagberg and his team brought the syndrome to light, describing 35 cases with similar symptomatology. This contribution marked the second stage in the syndrome's history, leading to the international recognition of Rett's discovery and ushering in an era of studies on the nosographic identity of the pathology (Hagberg, Aicardi, Dias & Ramos, 1983; Leonard, Cobb & Downs, 2017).

The 1980s represented a third crucial stage, especially for defining diagnostic criteria. In 1984, an international conference promoted in Vienna by Rett himself established the first inclusion and exclusion criteria, later updated in 1988 by the Rett Syndrome Diagnostic Criteria Working Group (RSDCWG) (Hagberg, Goutières, Hanefeld et al., 1985). Simultaneously, the newly formed International Rett Syndrome Association, which brought together approximately 70 patients with RTT and their families, sponsored a significant workshop in Baltimore, attended by 85 professionals. This event initiated close collaboration between parents and researchers, advancing

knowledge of the syndrome, helping to understand its ethology, and differentiating it from autism (Olsson & Rett, 1985; Percy, Zoghbi & Glaze, 1987).

The fourth stage is represented by the inclusion of Rett Syndrome among the Pervasive Developmental Disorders (PDD) in the DSM-IV (1994). In this diagnostic category, RTT was placed alongside disorders such as autism, Asperger's syndrome, childhood disintegrative disorder, and PDD not otherwise specified, including atypical autism. Over time, the very definition of the pathology evolved to include atypical forms alongside the classic form. This led to the introduction of the term "Rett Complex," proposed by Zappella, Gillberg, and Ehlers (1998) to encompass the entire spectrum of variants (Sandberg, Ehlers, Hagberg & Gillberg, 2000; Coleman, Fee, Bend et al., 2022).

Another milestone was reached in 1999 when Huda Zoghbi's laboratory identified a mutation in the MECP2 gene, located on the X chromosome, as the genetic cause in approximately 80% of RTT cases (Panayotis, Ehinger, Felix et al.,2023). This discovery represented a turning point in understanding the syndrome, reinforcing the importance of genetics in its etiology.

In subsequent years, new genes and clinical phenotypes were associated with RTT. Diagnostic criteria were refined, and experimental therapies were tested to improve the quality of life for patients. These developments have allowed for a better understanding of the disorder's full clinical spectrum and the provision of personalized treatments (Gold, Krishnarajy, Ellaway & Christodoulou, 2018; Zhang & Spruyt, 2022).

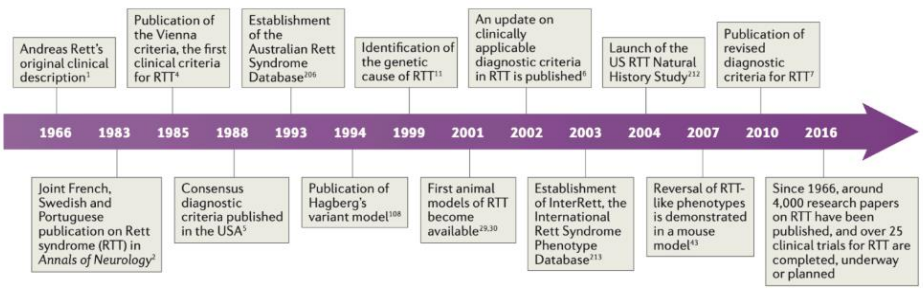


Figure 1. Key events in the discovery of Rett Syndrome (Leonard, Cobb & Downs, 2017).

1.3 Clinical Presentation, Stages, and Atypical Forms

Rett Syndrome is a neurological disorder that entails severe physical and cognitive disabilities. It is estimated to affect approximately 1 in 10,000-15,000 live-born girls (Skjeldal, von Tetzchner, Aspelund et al., 1997; Ta, Downs, Baynam et al., 2022). Although the development of girls with this syndrome may appear normal during the first six months of life, it is now accepted that subtle anomalies may be present from birth (Leonard et al., 2017).

Regarding motor development, during the first months, there is an apparently adequate progression of fine and gross motor skills, with some girls even beginning to walk (Pokorny, Schmitt, Egger et al., 2022). However, between 6 and 18 months, a slowdown or arrest in development occurs (Hagberg, 1993), progressively leading to the clinical presentation of the syndrome.

The typical manifestations have been described in detail by Hagberg in the review "Clinical Manifestations and Stages of Rett Syndrome" (2002). A hallmark feature is the stereotypical hand movements, including twisting, rubbing, and clapping during wakefulness. Among these, the most characteristic movement is the continuous hand-washing motion, resembling the gesture of washing hands, as initially observed by Rett in the first patients. Other common movements include hand-clapping, finger-twisting (hand-wringing), and bringing the hands to the mouth (hand-mouthing) (Antonietti, Castelli, Fabio & Marchetti, 2003).

Another hallmark feature is acquired microcephaly, present in approximately 80% of affected girls. This condition involves slowed brain growth between the sixth and twenty-fourth month of life, associated with subsequent intellectual disability (Tarquinio, Motil, Hou et al., 2012). In terms of language, abilities typically manifest through the use of single words but rarely progress to more complex skills. However, a distinguishing characteristic of most patients is communication through an intense gaze. This "ocular language" is used as a substitute for verbal and motor communicative abilities, leading to patients being referred to as "girls with beautiful eyes," a phenotypic trait that differentiates RTT from the autistic spectrum.

Between 60% and 80% of girls with RTT develop seizures, often toward the end of the regression period or during the post-regression phase. In some cases, these seizures are difficult to control (Nissenkorn, Levy-Drummer, Bondi et al., 2015). Respiratory disorders are also frequent,

characterized by episodes of intense hyperventilation alternating with apneas lasting 30-40 seconds, primarily occurring during wakefulness.

Patients commonly experience gastrointestinal problems, including gastroesophageal reflux, swallowing air leading to abdominal distension, bloating, and feeding difficulties that may result in nutritional deficiencies and require careful monitoring to maintain body weight and overall health (Neul & Chang, 2020). Other manifestations include bruxism, variable sleep disturbances, scoliosis (which may require surgical intervention in 10% of cases), slowed foot growth with stiffness, and postures that impair walking (Menachem, Hershkovich, Ackshota et al., 2023).

Behavioral disorders, such as self-injury, anxiety, and mood alterations, have also been observed (Sansom, Krishnan, Corbett & Kerr, 1993), although these symptoms tend to decrease with age.

There is considerable variability in the severity of clinical manifestations among patients with RTT (Bebbington, Downs, Percy, et al., 2012). This variability is often associated with the type of mutation in the MECP2 gene, which serves as a significant predictor of the disorder's severity. As highlighted by Cuddapah et al. (2014), it is essential to pay attention to specific comorbidities to enable medical professionals and families to best address the needs of the patients.

The staging organization of the syndrome was initially proposed in 1985 by Hanefeld and colleagues, who divided the progression into four distinct stages (Hanefeld, 1985). This conceptualization was further developed by Hagberg and Witt Engerström in 1986 based on clinical evidence available at that time. It was subsequently revised in 1990 and 1995 by the same authors and remains the model and distinctive clinical evolution profile of the syndrome in its classic form.

The identified stages are as follows:

- Early stagnation stage: Initially, the authors outlined the onset age between 6 and 18 months, later revised to 5 months in subsequent updates. This stage is characterized by a deceleration in developmental progress but does not yet show significant abnormalities. The duration of this stage is weeks or months.
- Regression stage: This stage is estimated to begin between 18 months and 4 years of age. It is characterized by the loss of previously acquired abilities, such as communication, fine motor skills, active play, and, occasionally, a state described

by Hagberg as being "in another world." During this stage, eye contact is preserved, and respiratory problems are still mild. The duration varies, ranging from weeks to a year.

- Pseudo-stationary stage: Occurring between 2 and 10 years after passing the second stage, this period is referred to by Hagberg as the "awakening" stage. Some communication abilities may reappear, and walking abilities are seemingly preserved. The main feature of this stage is the predominance of hand apraxia, which is the inability to perform intentional gestures. This stage can last for years or decades.
- Motor deterioration stage: Typically emerging after the third stage, this phase was initially conceptualized as marked by increased weakness and scoliosis, leading to complete dependence on a wheelchair, as well as spastic and dystonic deformities that impair motor function. Subsequent revisions introduced two subgroups within this stage. Stage 4A refers to patients who could walk autonomously before this phase, while Stage 4B includes those who were never able to walk independently (Hagberg, 2002).

As previously mentioned, these stages characterize the classic form of the syndrome, while differences have been noted in atypical or variant forms. These forms appear milder clinically and differ from the classic form in terms of age of onset, severity of symptoms, or both.

Most atypical cases, within what Zappella has defined as "Rett Complex" (Zappella et al., 1998), present with milder symptoms and cannot be definitively diagnosed before school age. One of these variants is the preserved speech variant identified by Zappella and colleagues (Renieri, Mari, Mencarelli et al., 2009) where patients affected by this form regain the ability to speak using short sentences or, in some cases, more structured sentences while simultaneously improving their hand use (Zappella et al., 1998). The "forme fruste" variant, the most well-known (accounting for about 80% of atypical cases), is characterized by a later onset of symptoms (around age 4). The congenital variant, on the other hand, features an earlier onset of symptoms. Other variants described in the literature include the male variant and the early seizure variant (Sandberg et al., 2000; Gold et al., 2018; Henriksen, Breck, von Tetzchner et al., 2020).

1.4 Diagnostic Criteria According to the DSM-V and Changes from the DSM-IV

During the international conference organized by Andreas Rett in 1984 in Vienna, the first clinical criteria for diagnosing Rett Syndrome were established, later revised in 1988 by the Rett Syndrome Diagnostic Criteria Working Group (RSDCWG). These criteria were divided into three main categories: essential criteria, supportive criteria, and exclusion criteria (Henriksen et al., 2020).

With the emergence of atypical variants and the discovery of biological markers, it became necessary to revise and simplify the diagnostic criteria, which occurred in 2002, recognizing classic forms and variants as distinct entities. The process concluded in 2010 with the work of Neul and collaborators (Gold et al., 2018).

According to Neul et colleagues (2010), a diagnosis of Rett Syndrome may be considered when there is a postnatal deceleration of head growth.

The diagnosis of the typical or classic form of Rett Syndrome is based on specific criteria that must be met:

- Regression period followed by recovery or stabilization.
- The observation of all inclusion and exclusion criteria.
- The presence of supportive criteria is not mandatory but often observed.
- For the atypical form or variants, the criteria included are:
- A regression period followed by recovery or stabilization.
- The presence of at least 2 out of 4 inclusion criteria.
- The presence of at least 5 out of 11 supportive criteria.
- The diagnostic inclusion criteria are as follows:
- Partial or complete loss of acquired manual skills.
- Partial or complete loss of spoken communication abilities.
- Gait abnormalities.
- Stereotyped hand movements, such as hand-wringing, hand-clapping, hand-washing, or hand-mouthing.

It is important to note that the criteria necessary for the typical form of Rett Syndrome require the presence of a regression period and at least one of the criteria listed above. The

requirement for postnatal deceleration of head growth has been removed, as it is not present in all individuals with the typical form.

Two exclusion criteria have been identified for the diagnosis of the classic form: 1) Brain lesions (perinatal or postnatal), neurometabolic disorders, or severe infections causing neurological issues and 2) Abnormal psychomotor development within the first six months of life.

For atypical forms, a set of 11 supportive criteria has been identified. These criteria are used to better understand and classify variations that deviate from the standard presentation. They are:

- Breathing disturbances during wakefulness.
- Bruxism.
- Altered sleep patterns.
- Abnormal muscle tone.
- Peripheral vasomotor disturbances.
- Scoliosis/kyphosis.
- Growth retardation.
- Cold, small hands and feet.
- Inappropriate laughter or screaming.
- Reduced pain response.
- Intense eye communication (eye-pointing).

Some of the main clinical aspects affecting the diagnosis of Rett Syndrome include:

- Stereotypies: Stereotypies can be highly varied and complex (Hirano & Taniguchi, 2019). They often decrease and simplify with age, partly due to rehabilitative interventions. Bruxism, episodes of hyperpnea or apnea, and tremors related to posture or movement may also occur.
- Behavioral Alterations: A loss of interest in the environment and loss of language can be observed, with preserved eye contact. Over time, there is often a recovery of interest in the external world, accompanied by an intensification of visual communication.

- Sleep Disturbances: Common manifestations include nocturnal bruxism, episodes of night terrors, irregular sleep-wake cycles, and frequent nighttime awakenings.
- Epilepsy: Present in the majority of cases, with an average age of onset between 4 and 5 years. Epilepsy in RTT can be challenging to distinguish from non-epileptic paroxysmal manifestations.
- Gastrointestinal Issues: Common problems include gastroesophageal reflux, constipation, and aspiration pneumonia, often linked to swallowing difficulties.
- Respiratory Problems: Episodes of apnea or hyperventilation can occur, often associated with intense emotional situations or other clinical manifestations.
- Orthopedic Issues: Neuromuscular scoliosis is frequent, with a typical onset between 3 and 10 years. Muscle contractures and hypertonia can progressively reduce motor autonomy.
- Cardiovascular Problems: Sudden death is more common compared to the general population, often related to arrhythmias such as ventricular fibrillation or sustained ventricular tachycardia (Singh et al., 2022).
- Bone and Metabolic Problems: Reduced bone density increases the risk of fractures. Recent studies highlight the importance of calcium and vitamin D supplementation, along with the use of bisphosphonates, to improve bone health (Suzuki, Ito, Kidokoro et al., 2023).

This new conceptualization of the criteria, the history of regression, and all inclusion and exclusion criteria must be present to allow a diagnosis of typical Rett syndrome, without exceptions (Neul, et al., 2010).

1.4.1 Changes Between DSM-IV and DSM-V

Historically, girls with Rett syndrome have often been misdiagnosed with Autism Spectrum Disorder due to the similarity of symptoms and the lack of biological markers specific to the syndrome until 1999 (Young, Bebbington, Anderson et al., 2008). Since its inclusion in 1994 in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV), Rett syndrome has been classified among the Pervasive Developmental Disorders (PDDs), together with

Autism, Asperger syndrome, Childhood Disintegrative Disorder, and PDD Not Otherwise Specified, including atypical autism (Antonietti et al., 2003).

This type of classification sparked considerable debate, particularly because, in 1994, when the fourth edition of the manual was published, the genetic etiology underlying RTT was not yet known. As a result, it was difficult to clearly distinguish the typical symptoms of the syndrome from manifestations falling under the Autism Spectrum.

The publication of the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* in May 2013 was accompanied by expectations of a revolution in psychiatric diagnosis, moving away from a mere clinical observation of symptoms and signs to relying on the presence of biological laboratory markers (Hyman, 2007). This shift is evident in the modifications made to the section concerning Pervasive Developmental Disorders, which were consolidated into a single category called "Autism Spectrum Disorders" (ASD). This category now includes Autistic Disorder, Asperger's Disorder, Childhood Disintegrative Disorder, and Pervasive Developmental Disorder Not Otherwise Specified, except for Rett syndrome.

Rett syndrome is not listed in the DSM-5 because it is known to be a disorder caused by a genetic disease (Amir, Van den Veyver, Wan et al., 1999) and is therefore a genetically distinct condition from most Autism Spectrum Disorders. However, children with Rett syndrome who exhibit autistic symptoms may still receive a diagnosis of Autism Spectrum Disorder, provided that the existing medical and genetic condition is specified.

1.5 Etiological Hypotheses

During genetic research on Rett syndrome, several explanatory models have been proposed (Willard & Hendrich, 1999). Initially, the simplest explanation was that the syndrome is an X-linked dominant condition, lethal in males but not in females, who survive but manifest the pathology. This hypothesis refers to the mechanisms of genetic transmission linked to the X and Y chromosomes that determine sex: XX in females and XY in males.

In females (XX), only one copy of the two X chromosomes needs to be functional for the relevant genetic information. One of the two X chromosomes is "deactivated" in a process known as

"X inactivation," whereby, in each cell, one X chromosome is randomly "turned off" while the other remains functional. Thus, females would survive but develop the pathology.

In contrast, males (XY) lack a backup copy of the X chromosome to compensate for the defective one. Therefore, mutations on their single X chromosome would be so severe that survival would not be possible (Antonietti et al., 2003).

However, this possible explanation required further confirmation from genetic research, especially when compared with existing knowledge about other X-linked disorders of that type. At the same time, the identification (Schanen & Francke, 1998) of a male with severe neonatal encephalopathy in a family with recurrent cases of Rett syndrome reinforced the hypothesis that it was caused by a gene on the X chromosome. Consequently, a model of an X-linked dominant disorder was proposed, though it was suggested that it might not necessarily be lethal in males, who could, in rare cases like the one mentioned above, survive.

Research therefore focused on the X chromosome and comparisons between individuals from the same family, such as girls with Rett syndrome and their sisters or stepsisters. This led to the identification of the Xq28 region, where numerous mutations in the MECP2 gene were found, ultimately identifying it as the primary cause of the disease (Mok, Zhang, Sheikh et al., 2022). Although it remains unclear how mutations in this gene specifically affect neuronal development and functioning, it is well established that these mutations impair development. Specifically, MECP2 mutations are responsible for the production of the MeCP2 protein, which plays a crucial role in the development and maintenance of the nervous system (Chahrour & Zoghbi, 2007).

Recent studies estimate that MECP2 mutations are present in approximately 95% of classic Rett syndrome cases and 75% of atypical forms. Furthermore, a relationship between the type of MECP2 mutation and the overall clinical severity has been identified in RTT (Fabio et al., 2014).

While it is not known if this gene plays a specific role in the development of verbal language abilities, some relationships between the type of MECP2 mutation and language abilities have been observed in Rett syndrome (Neul & Chang, 2020). Additionally, it is still unclear how the genotype influences other specific language abilities, such as babbling or the timing of spoken language regression (Urbanowicz, Downs, Girdler et al., 2015).

Numerous studies have also sought to investigate the relationship between mutations in the MECP2 gene and the hand stereotypies typical of this syndrome, focusing on the characteristics of the stereotypies, their changes over time, their relationship with behavioral abnormalities, and comparisons with the stereotypies observed in other disorders (Ferreira & Teive, 2020).

Molecular diagnosis of Rett syndrome must therefore be preceded by genetic counseling. Traditionally, the diagnostic process involved sequencing the MECP2 gene, as the majority of patients with the classic form of Rett syndrome exhibit alterations in this gene, which was first described in the pathogenesis of Rett syndrome in 1999. Mutations in this gene are also found in patients with a clinical diagnosis of the Zappella variant. However, with the advancement of technology and the increasingly recognized phenotypic overlap, it is now preferable to use multi-gene NGS (Next-Generation Sequencing) panels.

In most cases, the mutations are point mutations, but a smaller percentage of cases involve deletions, which should be considered in the diagnostic process. The NGS panel should include at least the MECP2, CDKL5, and FOXG1 genes, as the latter two are classically associated with conditions that some of the scientific literature considers responsible for variants of Rett syndrome.

The CDKL5 gene, in fact, encodes a protein that is essential for normal brain development (Leonard et al., 2022). This gene is hypothesized to regulate MECP2 and other related genes. The discovery of the role of CDKL5 is relatively recent, with the first reports published in 2004 (Tao, Van Esch, Hagedorn-Greiwe et al., 2004; Weaving, Ellaway, Gecz & Christodoulou, 2005). A study by Fehr et al. (2013) found that at least 25% of patients with CDKL5 mutations do not meet Neul's diagnostic criteria for Rett Syndrome, suggesting that such patients should be considered as having CDKL5 disorder, distinct from RTT.

The FOXG1 gene, on the other hand, is associated with the congenital variant of Rett Syndrome. FOXG1 encodes a transcriptional repressor essential for early brain development (WU, Lu, Zhang et al., 2022). Again, it is proposed to distinguish the clinical entity of this mutation from RTT.

Research has identified other genetic mutations associated with Rett-like clinical presentations, including: MEF2C, associated with deletions in 90% of diagnosed cases, IQSEC2 and genes for GABA receptors, also involved in similar phenotypes.

Conclusions

Multiple disabilities represent a complex and multidimensional challenge requiring integrated and individualized approaches. Rett Syndrome (RTT) serves as a paradigmatic example, characterized by a complex interaction between cognitive, motor, communicative, and behavioral disabilities.

The evolution of knowledge about RTT, from its clinical discovery in the 1960s to current advances in genetics and therapy, highlights the importance of interdisciplinary research in tackling such intricate conditions.

The history of RTT has passed through fundamental milestones, from Andreas Rett's initial clinical description to the identification of the MECP2 gene mutation, which marked a breakthrough in understanding the syndrome's pathogenesis.

The clinical presentation of RTT, with its developmental stages and atypical variants, underscores the heterogeneity of the disorder and the need for personalized therapeutic approaches.

CHAPTER TWO: REHABILITATION IN RETT SYNDROME

This chapter explores the core principles of learning and cognitive enhancement (Section 2.1) and examines their connection to the effectiveness of rehabilitation strategies in addressing Rett Syndrome (Section 2.2).Through a multidisciplinary exploration, it highlights how the theoretical and biological foundations of learning, including concepts of neuroplasticity and educational mediators, can serve as the basis for innovative and personalized interventions aimed at improving cognitive, emotional, and motor abilities in patients.

The first part analyzes the psychological and biological foundations of learning, focusing on how educational techniques can be mediated to develop not only the acquisition of knowledge but also the ability to "learn how to learn". These principles are integral to this thesis, as they guided the design and implementation of the experimental studies. The second part delves into the effectiveness of rehabilitation techniques for Rett Syndrome, highlighting the critical role of personalized approaches supported by multidisciplinary teams. Additionally, this chapter explores emerging gene and pharmacological therapies, showcasing innovative methods that complement traditional rehabilitation. Collectively, these strategies underscore the significance of tailored, lifelong care in addressing the complex challenges of RTT and improving the quality of life for both patients and their families.

2.1 PRINCIPLES OF LEARNING

2.1.1 The Psychological Foundations of Cognitive Enhancement

Most educational interventions for children with disabilities aim to achieve minimal goals, but this limited investment prevents the development of the ability to "learn how to learn." What is important is not so much the ability of the individual to reproduce information, but rather how they can gradually modify the underlying cognitive processes. Cognitive enhancement is theoretically based on Vygotsky's *zone of proximal development* (ZPD) and Feuerstein's *theory of modifiability*.The *zone of proximal development* (ZPD) refers to the specific area where it is possible to observe what the child can achieve independently and what learning becomes possible with the support of a competent adult.

Figure 2. Vygotsky's Zone of Proximal Development

Vygotsky argued that the child follows the example of the adult and gradually develops the ability to perform certain tasks without help or assistance. A frequently cited definition of ZPD is:

"The distance between the actual developmental level, as determined by independent problem-solving, and the level of potential development, as determined through problem-solving under the guidance of an adult or in collaboration with more capable peers."

Vygotsky distinguished himself from other educational theorists by emphasizing that the role of learning is to provide children with experiences within their ZPD, thereby encouraging and promoting individual learning (Adler & Gielen, 2001). The lower limit of the ZPD corresponds to the level of skills achieved by the child through independent work, while the upper limit reflects what the child can achieve with the assistance of a skilled teacher.

Bruner, like Vygotsky, emphasized the importance of expert support, which provides useful guidance and instructions to help children complete tasks they are not yet capable of achieving independently. This concept, undoubtedly similar to ZPD, was elaborated by the American psychologist Jerome Bruner as the concept of *scaffolding* (Bruner, 1974). The term originates from the English word "scaffold," which literally refers to "framework" or "support" and is used in psychology and education to describe the help provided by one person to another to complete a task.

The term was first used by Wood et colleagues (1976) as a metaphor to describe the "scaffolding" action provided by parents to children during problem-solving tasks (Rodgers, D'Agostino, Berenbon et al., 2022). This form of assistance later evolved into a structured form of tutoring by adults capable of responding appropriately to the student's levels of competence (Rasheed, Kamsin & Abdullah, 2021). The authors describe this process as support that allows the

child to solve a task or achieve a goal (e.g., building a wooden block) beyond their current efforts and knowledge. The adult's assistance is not about completing the task but rather about helping the child understand the task and the correct sequence of steps towards its completion.

In Wood, Bruner, and Ross's article (1976), the scaffolding action is associated with a constant evaluation of the child's needs and levels of competence. Scaffolding must always be adapted to the student's progress until they can independently perform the task. The results of early studies highlighted the interactive and dynamic properties of this process:

a) The tutor captures the student's interest in the task;

b) Allows the student to act while regulating their actions;

c) Provides a model of the solution when the student is ready to comprehend it (Wood et al., 1976).

This process includes perceptual components (e.g., highlighting task characteristics), cognitive components (e.g., reducing degrees of freedom in actions), and emotional components (e.g., controlling frustration) (Stone, 1998).

Building on this concept, Palincsar (1991) emphasized the importance of characteristics such as: the task's objective, which enhances motivation; the active involvement of the student, maintained by alternating turns; on-the-spot evaluation; and moderated support by the teacher, who facilitates collaboration through dialogues with students.

Palincsar and Brown (1987) further refined a method called *Reciprocal Teaching* to enhance comprehension abilities in at-risk children (Muthik, Muchyidin & Persada, 2022). The central feature of reciprocal teaching is a repeated sequence in which the teacher and the student execute a series of steps to summarize, question, clarify, and predict subsequent parts of a text to aid comprehension.

In the early phases of interaction, the teacher holds primary responsibility for the task's success. This is achieved through strategies such as modeling or producing structured responses. Gradually, however, students are encouraged to take greater responsibility for applying these strategies. Through this process, students adopt a more active and strategic approach to understanding tasks, supported by the teacher's scaffolding.

In Feuerstein's *mediated learning experience* (MLE) model, exposure to environmental stimuli plays a crucial role from the earliest developmental stages, producing changes in the

organism. However, simply exposing students to a world full of colors, shapes, sounds, and movements is insufficient if their interactions with these stimuli remain superficial. Transforming experiences into learning requires key components that encourage children to classify, compare, group, label, and assign meaning to their experiences. Crucially, children learn because a mediator—a parent, family member, or caregiver—intervenes actively between the child and the environment to facilitate understanding.

Feuerstein describes this process as follows: *"Mediation means that change can be caused by another person (H), who actively intervenes between the other person (O) and the stimulus (S), playing the role of mediator."*

Through the mediated learning experience, the organism (O), directly exposed to the stimuli (S), responds to them appropriately only after the mediator (H) has selected, broken down, and modified the stimuli.

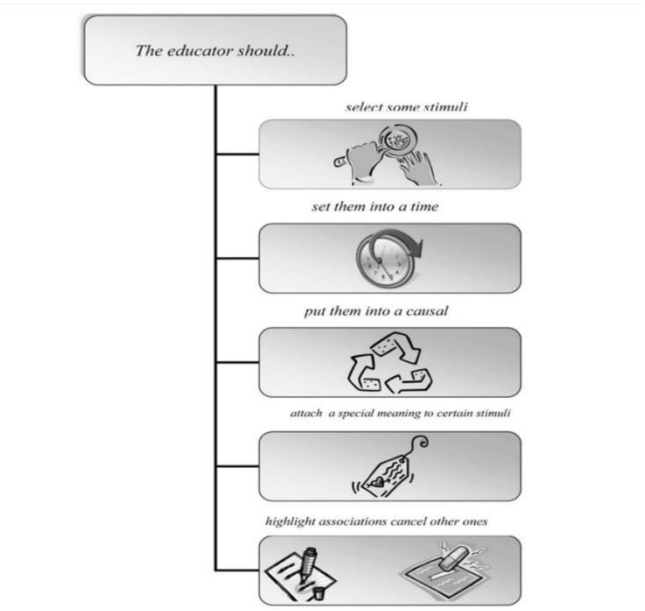


Figure 3. The Educational and Rehabilitative Relationship

The knowledge individuals acquire is then organized by the mediator, who determines relationships between stimuli. In an educational or rehabilitative relationship, educators must select stimuli, arrange them sequentially (e.g., before/after), place them within causal and spatial systems based on goals, assign meaning to specific stimuli, propose them repeatedly, highlight associations, and distinguish them from unrelated stimuli.

This type of intervention must occur on three levels: cognitive, emotional, and behavioral. At the foundation of this approach lies the importance of the *relationship* as a tool for mediation.

2.1.2 The Biological Foundations of Cognitive Enhancement

The biological foundations of cognitive enhancement refer to the concept of *neuroplasticity* (also known as brain plasticity, cortical plasticity, or cortical remapping) (Jellinger, 2022):

"The brain and its structural interconnections are modified throughout our experiences. The brain's ability to change its structure in response to experience is called neuroplasticity."

The brain consists of nerve cells or neurons (and glial cells) that are interconnected. Learning occurs by strengthening connections between neurons, adding or removing connections, or generating new cells.

During the 20th century, it was believed that the lower parts of the brain and neocortical areas became structurally immutable after childhood. This implied that learning occurred solely through changes in the strength of neural connections, while regions involved in memory formation, such as the hippocampus, retained plasticity into adulthood, allowing for the production of new neurons. However, new research has overturned this belief, suggesting that all brain areas exhibit plasticity, even beyond childhood.

Hubel and Wiesel (1972) demonstrated that *ocular dominance* (dominance of one eye) develops early in infancy and remains immutable (Baroncelli, Braschi, Spolidoro et al., 2010). However, other studies revealed that environmental changes could alter behaviors and cognition by modifying existing neuronal connections. Decades of research have shown that substantial changes also occur in subcortical areas, significantly altering neural activation patterns in response to experience.

According to the neuroplasticity theory, thought, learning, and action alter both the physical structure of the brain (*anatomy*) and its functional organization (*physiology*).

Recent advances in neuroimaging techniques have enabled better observation of the living brain, revealing structural differences resulting from diverse life experiences. Additionally, insights have been gained from studying brain cells in animals. For example, the brain structure of a mouse living in a stimulating environment with wheels, climbing structures, and social interaction differs

significantly from that of a mouse kept in isolation without any form of stimulation (Baroncelli et al., 2010). A socially subordinate animal subjected to stress will develop fewer neurons.

When neurons connect, they require energy and resources to build dendrites. This energy is supplied by an increased blood flow to the brain (Herholz & Zatorre, 2012). Using positron emission tomography (PET), Simmonds and colleagues (1993) demonstrated how this blood flow manifests in different brain regions (Evans, Burch, Frishberg et al., 2020). A particularly interesting result from these studies shows that blood flow is significantly reduced in individuals who have learned to solve a specific problem efficiently when asked to solve it again.

Once a problem has been solved repeatedly, it can be addressed effortlessly, meaning that the brain no longer requires significant material or energy, and the learning process stabilizes. This learned behavior is reinforced through repetition (Fabio, Antonietti, Castelli & Marchetti, 2009). At times, familiar problems are solved almost unconsciously. For example, we may drive from one point to another and struggle to recall the specific route we took, or we might hum a familiar melody without remembering the lyrics.

This phenomenon highlights a critical challenge: why is it so difficult to change automatic brain processes? Conscious effort is needed to alter the brain's structure. While this effort may initially seem overwhelming, repetition can gradually lead to automation. Research by Touretzky (1990) and Tesauro et al. (1995) indicates that various mammalian brain areas contribute to incremental learning. Although the cortex plays a central role, other regions, such as the basal ganglia, hippocampus, amygdala, and cerebellum, also provide vital contributions. Understanding the functions and interactions of these areas during learning is critical.

The development of incremental learning theories will improve our understanding of the learning process, with potential applications in skill development for both humans and machines. Authors emphasize that enriched environments increase the number of neural connections. These connections are formed when individuals become curious and freely explore that curiosity. During these moments, thousands of new dendritic spines sprout, resembling tree branches.

Each brain cell can generate hundreds of thousands of connectors throughout its life cycle. The brain makes these connectors available for processing sensory data and integrating them into previously acquired knowledge structures.

Enriched environments, varied experiences, and engagement in a central topic stimulate the production of these connectors, creating multiple coding options in response to experiences. For example, something as simple as changing a student's seating position in a classroom can trigger the brain to generate new dendritic spines as it incorporates a new perspective and new relationships between objects and people.

The brain adapts to environmental changes. New connectors are not necessarily permanent; they are stabilized through continuous repetition (*practice*), exploration with slight variations (*rehearsal*), or reflection on the activity (e.g., discussing it). If connections are not revisited, they can disintegrate and be lost forever. The adage "*use it or lose it*" aptly describes the importance of maintaining neural connections.

The quality and extent of connections within brain systems determine the depth of understanding and an individual's ability to execute tasks. Evidence suggests that the greater the number of connections, the better the ability to solve problems, think clearly, and comprehend events. The number of connections built depends on the individual's interest in engaging with experiences.

Encouraging dendritic growth is therefore essential. As dendrites grow, neurons form more connections with other neurons. Through repeated practice or trials, these connections stabilize, becoming permanent and usable. Enhancing the connections between brain neurons results in improved brain functionality.

These connections emerge partly due to inherited growth patterns encoded in an individual's genetic makeup but also develop in response to environmental stimuli that the brain encodes as neural impulses. This finding has clear implications for educators: since enriched environments stimulate dendritic growth, and practice stabilizes that growth, school environments must provide enriching experiences. Doing so helps students retain what they have learned and increases the likelihood of applying that knowledge to new situations.

2.1.3 The Basic Principles of Cognitive Enhancement

This section examines *"how, who, when, and where to modify"*, focusing on modifying both processes and skills, with particular attention to the automatization of basic abilities.

Before presenting methods for modifying cognitive skills and processes, it is important to clarify that *automaticity* and *skill acquisition* are closely related concepts but are not identical. Automatic processes are components of a skill, but a skill is more than the sum of its automatic components. Automaticity and skill acquisition are similar because both are learned through practice (Fabio, 2003).

In any initial stage of an activity, controlled attention processes are used to learn, resulting in slow and error-prone performance. At this stage, the entirety of working memory is engaged—meaning that all cognitive resources are dedicated to solving the new task. For example, consider a child learning to add two numbers. Initially, it is challenging for the child to hold the first number in memory, recall the second number, and then combine the two while understanding that the "+" sign means "add," "increase," or "move forward."

When asked to combine Maria's toys with Marco's toys, the child carefully thinks through the process, performs the task slowly, and eventually arrives at the correct result. During this problem-solving phase, if someone distracts the child, errors are made, and the solution is forgotten. As training progresses, performance requires less attentional control, becomes faster, and errors diminish—a transformation that can be defined as *automaticity*. Through learning, attentional strategies that once required control gradually become automatic.

Returning to the example above, as the child progresses, the "+" sign is understood more quickly, and the result is achieved effortlessly. The child can also respond to other questions during problem-solving without making errors. In this way, the child automatizes the understanding of the "+" sign.

Later, the child will need to learn more advanced tasks, such as multiplication or adding and subtracting larger quantities. If the child remains fully or partially engaged at Level A (basic addition), it would be difficult to access more complex tasks. However, because Level A has become an automated subroutine, the child can now progress to Level B tasks. Once again, the initial stages of Level B require controlled attention, leading to slow and error-prone performance. With continued training, however, automaticity is achieved again, and the child can effortlessly combine addition and subtraction.

Eventually, the child progresses to solving even more complex problems involving three mathematical operations (Level C). At this point, Level B—which already contains Level A as an automated subroutine—becomes automated, freeing cognitive resources for Level C. This process is repeated indefinitely, as symbolized by the infinity sign, indicating that there is no limit to achieving increasingly complex levels of thought. If complex problem-solving can be mastered, even more advanced tasks become accessible.

Cognitive empowerment can take place anywhere: at school, at home, during sports activities, or on the street. Providing cognitive empowerment in these settings facilitates *internalization*. Internalization can be understood as "knowing how" to apply learned skills in practical contexts.

Mastery of skills occurs through the child's active engagement within society. Another aspect of internalization involves the child adopting tools and making them their own, using them uniquely. For example, internalizing the use of a pencil allows a child to use it independently for their purposes, rather than merely replicating what others have drawn before.

It is most effective for children to work with different people in varied contexts (e.g., a teacher or educator at school, parents at home) to help them transfer what they have learned to different people and situations. It is also important to provide children with programs that respect their language and culture, fostering a sense of security that encourages active participation in the learning process, including making mistakes.

The person facilitating cognitive empowerment acts as a *cognitive coach*. Cognitive coaching is a model that requires the coach to be non-judgmental, encourage reflective practice, and guide the learner toward self-directed learning. For example, cognitive coaching focuses on the learner's thinking, perceptions, beliefs, and assumptions, and how these influence their practices.

According to Costa and Garmston (2015), a cognitive coach:

"uses tools of reflective inquiry, pauses, paraphrasing, and analysis for specificity. A cognitive coach helps others develop competencies in planning, reflection, problem-solving, and decision-making."

Cognitive coaching is a process of mutual learning between individuals. A good cognitive coach must be able to work effectively with diverse personalities, learning styles, philosophies, and developmental stages of teachers or students.

2.1.4 Basic Methods of Cognitive Enhancement

Cognitive empowerment relies on a structured approach that integrates various strategies to support a child's development. The process begins with unconditional acceptance, which lays the foundation for all subsequent methods, and progresses through the use of rules, reinforcement, and techniques that promote autonomy and automaticity.

Unconditional acceptance is a prerequisite for growth and is defined as the "ability to accept the person in their entirety" (Rogers, 2007). This does not mean endorsing every behavior but rather allowing the child to express their thoughts and emotions without judgment. Through this acceptance, the child learns to embrace their "real self," shedding the societal pressures and suppressions associated with their "unreal self." For educators and caregivers, this approach fosters a supportive environment where the child feels valued despite daily challenges.

Once this foundation is established, *rules* play a pivotal role in creating structure. By offering order to an otherwise chaotic environment, rules help children navigate their surroundings effectively. For maximum impact, rules should be few, stated affirmatively (e.g., "keep your hands still" instead of "don't move your hands"), concrete, and consistently reinforced at the appropriate time (Antonietti et al, 2003).

To motivate and reinforce adherence to these rules, *reinforcement* is employed. Reinforcements can be primary (e.g., food, sleep, water) or secondary (e.g., praise, tokens), and their effectiveness depends on the individual preferences of each child. Educators must carefully identify what each student finds rewarding, such as a favorite snack or song, to personalize the learning experience (Cooper, 2007).

For children who exhibit repetitive behaviors or difficulty focusing, *containment* can help redirect attention. For instance, gently holding the child's hands apart to interrupt stereotypies can refocus their energy on the task at hand, fostering improved concentration and engagement (Fabio et al., 2009).

As children begin to develop new skills, *shaping* becomes a key strategy. By reinforcing successive approximations of the desired behavior, shaping gradually modifies existing behaviors toward a target outcome. This method ensures steady progress, even when the final goal initially seems unattainable (Cooper, 2007).

Once the child demonstrates some level of competency, *fading* is used to reduce reliance on assistance. Initially, significant support is provided, but it is systematically decreased as the child gains confidence and mastery. This gradual withdrawal of prompts ensures that the child develops independence and does not remain dependent on external support (Cooper, Heron & Heward, 2020).

Throughout this process, *prompting* serves as a tool to encourage specific responses. Prompts can range from physical gestures to verbal cues and are applied in a hierarchy from most intrusive to least intrusive. In an errorless learning approach, prompts are systematically faded, allowing the child to achieve success while gradually moving toward self-reliance (Cooper et al., 2020).

As the child repeats tasks and gains familiarity, the goal is *automaticity*—the transition from controlled to automatic processing. Through repeated practice, tasks that once required conscious effort become effortless, freeing cognitive resources for more complex activities (Umiltà, 1995). In parallel, *abstraction* facilitates the shift from concrete experiences to symbolic understanding. For example, a child learning numerical concepts begins by counting tangible objects and later transitions to understanding abstract numerical representations (Piaget, 2016).

Finally, the principle of *economy* underscores the importance of providing minimal assistance necessary to encourage autonomy. Over-assistance can foster dependency, hindering the child's ability to develop independence. By striking a balance, educators can guide children toward self-sufficiency while maintaining a supportive role (Antonietti et al., 2003).

By combining these strategies in a cohesive and progressive manner, cognitive empowerment fosters a nurturing environment where children can develop essential skills, achieve autonomy, and build confidence to tackle increasingly complex tasks.

2.1 EFFECTIVENESS OF REHABILITATION IN RETT SYNDROME

2.2.1 Advances in Rehabilitation Strategies

Rett Syndrome (RTT), as explain in chapter one, is a complex neurodevelopmental disorder primarily affecting females, characterized by substantial variability in symptoms, progression, and severity. Common features include severe motor, cognitive, and communication impairments, along with repetitive hand movements, autonomic dysfunctions, and breathing irregularities (Lim, Greenspoon, Hunt & McAdam, 2020). This heterogeneity necessitates a personalized, multidisciplinary approach that combines medical specialists—such as pediatricians, neurologists, and gastroenterologists—with allied health professionals, including physiotherapists, speech therapists, occupational therapists, and psychologists. The involvement of caregivers is essential to provide consistent, supportive care, and complementary therapies like music therapy and sensory integration are increasingly incorporated to enhance holistic treatment (Lotan, 2006).

A scoping review by Lim et al. (2020) analyzed 62 studies on RTT rehabilitation, highlighting improvements across gross motor skills, fine motor abilities, communication, and cognitive function. Gross motor therapies, such as physiotherapy and hydrotherapy, demonstrated progress in ambulation and transitional movements, while fine motor interventions, including splinting and music therapy, effectively reduced stereotypic behaviors and improved hand functionality. Communication-focused interventions leveraging low-, medium-, and high-tech tools, such as eye-tracking technology and augmentative communication devices, showed substantial gains in choice-making, social interaction, and language use. The review emphasized the importance of combining these diverse interventions in interdisciplinary programs tailored to the needs of individuals with RTT. Beyond this key point, Lotan (2006), underscores the necessity of lifelong, individualized intervention programs that address the multifaceted challenges of RTT. Comprehensive evaluations involving healthcare professionals, caregivers, and patients form the foundation of continuous support networks aimed at enhancing quality of life and addressing physical, cognitive, and emotional needs (Rozensztrauch, Sebzda & Śmigiel, 2021). Supporting this, Yang et al. (2021) conducted a review of 91 studies examining RTT rehabilitation across the lifespan. Their findings revealed the efficacy of interventions in communication, motor

rehabilitation, and complementary therapies, with measurable improvements in mobility, cognitive engagement, and emotional well-being.

2.2.2 The Role of Non-Medical Interventions

The literature highlights that communication and learning-focused interventions are central to RTT rehabilitation, especially the use of assistive technologies (AT) in augmentative and alternative communication (AAC) and in the training of cognitive enhancement

Fabio et colleagues (2016) assessed whether cognitive training modified both behavior and brain activity using eye-tracking technology in individuals with RTT. The changes were evaluated in two phases: (a) after short-term training (30 minutes) and (b) after long-term training (5 days). This study demonstrated the efficacy of eye-tracking technology as a valid tool for RTT rehabilitation. Further studies (Fabio, Castelli, Marchetti, & Antonietti, 2013; Fabio, Giannatiempo, Oliva, & Murdaca, 2011; Vessoyan, Steckle, Easton et al., 2018) demonstrated that RTT patients who underwent intensive cognitive rehabilitation involving the assessment of non-verbal communication and language precursors showed the ability to progress beyond the pre-intentional developmental stage. These interventions allowed RTT patients to grasp the concept of subjectivity, starting with basic mental states (e.g., emotions, desires) and moving toward more complex ones, such as beliefs and false beliefs.

A recent review (Amoako & Hare, 2020) evaluated thirteen studies, which primarily included non-medical interventions such as communication strategies, music, assistive technology, augmentative and alternative communication, attention interventions, and cognitive rehabilitation. All the studies reported positive outcomes, such as improved communication skills, brainstem activity, physical fitness, quality of life, and reduced stereotypic behaviors. This review also highlighted a scarcity of research on this topic (Amoako & Hare, 2020).

Despite the generally positive trends in cognitive rehabilitation outcomes for individuals with Rett Syndrome (RTT), the long-term efficacy of specific cognitive interventions remains underexplored, with evidence limited to a few studies. For instance, a longitudinal study by Woodyatt and Ozanne (1993) investigated cognitive, communicative, and interactive development in six RTT patients over three years. The study revealed that all participants retained profound

intellectual functioning and pre-intentional communication, while also highlighting significant individual variation in cognitive and social interaction abilities.

Building on these findings, a more recent longitudinal study by Fabio, Giannatiempo, Semino, and Caprì (2021) examined the effects of cognitive rehabilitation utilizing eye-tracking technology over a two-year period. The rationale for this study was rooted in two key considerations: (a) advancements in neuroplasticity research, which suggest that highly repetitive, adaptive, novel, and targeted stimuli can enhance cognitive performance, and (b) the recognition that gaze fixation and prolonged visual engagement are critical forms of communication for RTT patients to express desires and communicate (Baptista, Mercadante, & Macedo, 2006; Schwartzman, Velloso, D'Antino, & Santos, 2015).

By leveraging eye-tracking technology, the intervention focused on improving parameters such as attention, choice behaviors, and language production. The results demonstrated that cognitive rehabilitation using this technology significantly enhanced attention span (measured in seconds), increased the frequency of choice behaviors, and facilitated greater production of vowels, phonemes, and words. These findings underscore the potential of targeted cognitive interventions, supported by advanced technologies, to improve communicative and cognitive outcomes in individuals with RTT.

Motor rehabilitation is another cornerstone of RTT care, focusing on preserving autonomy and enhancing quality of life. Fonzo, Sirico, and Corrado (2020) reviewed a variety of motor therapies, including traditional physiotherapy, hydrotherapy, environmental enrichment, and sensory-based treatments. Hydrotherapy has proven effective in reducing stereotypic movements, improving motor skills, and promoting social interaction. Music therapy has similarly enhanced intentional hand use and communication. Lotan and Hanks (2006) emphasized that individualized physical therapy programs, coupled with emotional and motivational support, are essential for maximizing functional independence. Romano et al. (2022) highlighted the success of remote-supervised intensive motor and postural activity programs. These caregiver-supported interventions not only prevented scoliosis progression—a common comorbidity in RTT—but also improved overall motor function, emphasizing the value of early, proactive management.

Related to both cognitive and motor rehabilitation, the literature highlights that music therapy (MT) has emerged as a highly effective intervention in RTT care. Chou et al. (2019) demonstrated the multifaceted benefits of MT for individuals with RTT and their families. Participants showed significant improvements in non-verbal communication, such as enhanced eye contact and vocalization, as well as reduced stereotypic behaviors during music-based activities. Families also reported reduced stress and strengthened emotional bonds with their children, underscoring the relational benefits of MT. Additional studies (Elefant, 2002; Wigram & Lawrence, 2005; Tortoriello, Frosolini, Pianigiani et al., 2023) corroborate the efficacy of MT in fostering communication, reducing behavioral challenges, and enhancing social interaction.

Given that Rett Syndrome is a multi-disability affecting various systems, sensory integration appears to play a fundamental role in rehabilitation. Pizzamiglio et al. (2008) implemented a program based on Piaget's theory of sensorimotor intelligence, combining visuomotor coordination with sensorimotor rehabilitation. Over three years, this approach significantly improved hand functionality, spatial awareness, and receptive language in a young RTT patient. Similarly, Drobnyk et al. (2019) investigated Ayres Sensory Integration (ASI) therapy, which aims to address sensory processing challenges. Their study demonstrated small but significant improvements in grasping abilities among RTT children, emphasizing the importance of integrative approaches in tackling the disorder’s motor and sensory complexities.

Despite these advancements, RTT rehabilitation faces systemic challenges. Barriers include high costs, limited access to specialized centers, insufficient staff training, and discontinuity in care pathways (Lotan, 2004; 2006). Methodological limitations, such as small sample sizes and lack of standardization, constrain the generalizability of findings (Derer, Polsgrove & Rieth, 1996). Most studies focus on children and adolescents, leaving a critical gap in understanding interventions for adults with RTT (Lim et al., 2020). Moreover, the absence of long-term studies assessing skill retention and the impact of interventions across the lifespan hinders the development of comprehensive care strategies.

In conclusion, rehabilitation for RTT demonstrates considerable potential when approached through a comprehensive, multidisciplinary framework. Integrating medical, technological, and therapeutic interventions has yielded promising results, with significant improvements observed in

communication, motor function, and overall quality of life. However, to maximize these benefits and ensure sustained impact, systemic barriers must be addressed, and continuity of care must be prioritized. By advancing research, enhancing resources, and fostering collaborative care, RTT rehabilitation can continue to evolve, offering hope and improved outcomes for patients and their families.

2.2.3 Gene and Pharmacological Therapies

Alongside rehabilitation, another crucial aspect of managing the lives of patients with Rett Syndrome is the exploration of gene and pharmacological therapies. These innovative approaches have gained significant attention in recent research, offering new avenues for treatment and hope for improved outcomes.

Gene therapy has emerged as a promising option, focusing on the administration of adeno-associated viral (AAV) vectors to deliver a functional copy of the MECP2 gene. Preclinical studies conducted on animal models, such as mice, have shown encouraging improvements in motor and respiratory functions. However, the therapy is not without challenges. One significant issue is the potential overexpression of the MeCP2 protein, which can lead to severe side effects, including liver toxicity (Panayotis et al., 2022).

Equally exciting is the potential of gene editing through advanced technologies like CRISPR/Cas9. This approach enables the correction of specific mutations in the MECP2 gene, potentially restoring the disrupted epigenetic balance that characterizes Rett Syndrome. Despite its promise, gene editing remains in the experimental phase, requiring further studies to ensure its safety and efficacy before it can be considered a viable clinical option (Panayotis et al., 2022).

Meanwhile, pharmacological approaches have also been explored to address molecular dysfunctions associated with Rett Syndrome. For example, drugs that modulate GABA and glutamate receptors—key components of brain signaling pathways—have shown positive results in improving cognitive and behavioral functions in preclinical studies (Panayotis et al., 2022).

Despite the progress, treating Rett Syndrome continues to present significant challenges. The disorder's phenotypic variability, difficulties in optimizing therapeutic doses, and potential risks of toxicity remain obstacles to overcome. As a result, experts increasingly advocate for combined

approaches that integrate genetic therapies, pharmacological treatments, and traditional rehabilitation. This multidisciplinary strategy holds the greatest promise for addressing the complexity of Rett Syndrome and enhancing the quality of life for those affected.

Conclusions

Chapter Two has explored the multifaceted approaches to rehabilitation and management in Rett Syndrome (RTT), underscoring the importance of both traditional and innovative interventions. By integrating theoretical and biological principles of cognitive enhancement, the chapter establishes a foundation for personalized strategies aimed at addressing the unique challenges posed by RTT.

The psychological and biological principles of learning, such as neuroplasticity and mediated learning, provide critical insights into designing interventions that foster cognitive, motor, and emotional development. These principles guide the implementation of rehabilitation techniques that empower individuals with RTT to overcome developmental challenges and achieve meaningful progress.

Rehabilitation strategies discussed in this chapter emphasize the importance of tailored, multidisciplinary approaches. Non-medical interventions, such as assistive technologies, augmentative and alternative communication (AAC), music therapy, and sensory integration, have demonstrated measurable improvements in attention, communication, motor function, and social interaction. The use of advanced technologies like eye-tracking further illustrates the potential of innovative tools to enhance rehabilitation outcomes, supporting both short- and long-term development.

In addition to rehabilitation, this chapter highlights the promising advancements in gene and pharmacological therapies. Gene therapy, utilizing adeno-associated viral vectors to deliver functional copies of the *MECP2* gene, and gene editing through technologies like CRISPR/Cas9 represent groundbreaking steps toward addressing the genetic basis of RTT. However, these approaches are still in experimental stages and face challenges, including potential toxicity and phenotypic variability. Pharmacological treatments targeting neurotransmitter pathways offer

further avenues for improving cognitive and behavioral outcomes but require careful dose optimization and safety validation.

Despite these advancements, the management of RTT continues to face systemic and methodological challenges. Limited access to specialized care, high costs, and the lack of standardized protocols hinder the widespread implementation of effective therapies. Furthermore, the scarcity of long-term studies, particularly for adult patients, underscores the need for comprehensive research to address the lifelong trajectory of RTT.

In conclusion, the successful management of RTT necessitates a holistic, collaborative framework that integrates medical, technological, and therapeutic interventions. By addressing systemic barriers and fostering interdisciplinary collaboration, researchers and clinicians can continue to improve the quality of life for individuals with RTT and their families. Sustained efforts in research, innovation, and resource development hold the promise of transforming RTT care, offering enhanced possibilities and hope for those affected by this complex disorder.

CHAPTER THREE: INNOVATION IN REHABILITATION WITH ASSISTIVE TECHNOLOGIES

This chapter examines the role of assistive technology (AT) in enhancing the quality of life and independence of individuals with disabilities, with a focus on rehabilitation and its application for Rett Syndrome (RTT). It highlights how AT, including Eye-Tracking Technology (ETT), Virtual Reality (VR), Internet of Things (IoT)-enabled systems, and telerehabilitation, can improve motor, cognitive, and social functions. These technologies empower individuals to overcome barriers, foster social inclusion, and enable active participation in education and community life.

3. ASSISTIVE TECHNOLOGY (AT) AND REHABILITATION IN DISABILITIES

3.1 Overview of Assistive Technologies (AT)

As indicated by the WHO, around 15% of the global population experiences some form of disability, and 2-4% of them face significant difficulties in functioning (Newell, 2011; WHO, 2013). People who have difficulty carrying out daily functional activities such as walking, speaking, hearing, or seeing are considered individuals with non-severe disabilities (McNeil, 1997). In contrast, those unable to perform even one of their daily living activities are classified as individuals with severe disabilities. Within this group are children with multiple disabilities, including those with Rett Syndrome (RS), who often encounter significant barriers to accessing and participating in essential activities such as self-care, play, leisure, and education (Mount & Cavet, 1995). Rehabilitation technologies play a crucial role in helping individuals with disabilities achieve greater independence in performing daily activities through the use of assistive technologies (Newell, 2011).

Rehabilitation is defined as reducing deficiencies in function, involvement, activity, and quality of life by integrating sensory management, instruction, perceptual training, and counseling. The primary goal of rehabilitation for individuals with disabilities is to empower them to live independently and support their ability to be productive (Ge, Chen, Tang et al.,2021).

The concept of disability and rehabilitation finds a point of convergence in technology. According to the WHO, Assistive Technology (AT) refers to any assistive items, services, or related

systems designed to preserve or restore an individual's functioning, improve their independence, and thereby enhance their well-being (Cowan & Turner-Smith, 1999; Craddock, 2005).

AT include tools, devices, systems, software, or services that help individuals with disabilities or those facing challenges with visual, auditory, physical, or mental tasks to improve their functional capabilities and compensate for a loss of autonomy in both medical and social contexts (Newell, 2003). AT also aims to enhance participation, especially for children, in educational settings by providing tools that support interaction and independence. It spans a wide range of solutions, from low-tech devices such as adapted utensils with built-up handles to high-tech solutions like microswitches, electronic communication aids, powered mobility devices, and environmental controls (Parette, 1997).

The term "adaptive technology" is often used interchangeably with assistive technology; however, adaptive technology is a subset of assistive technology (Tuntland, Kjeken, Nordheim, Falzon et al., 2009). Assistive technology broadly refers to any product, tool, equipment, or system—whether commercially available, adapted, or customized—that helps retain, improve, or enhance the functional abilities of individuals with disabilities (Lewis, Cooper, Seelman et al., 2012). Adaptive technology, specifically, includes products designed exclusively for disabled individuals and rarely used by people without disabilities.

Thus, AT is a comprehensive term encompassing rehabilitation equipment, adaptive and assistive devices, and the processes involved in selecting, acquiring, and utilizing these tools (Oliver, 2018). Assistive technology helps elderly individuals, people with disabilities, or those with medical conditions or injuries by making their lives safer and easier. It offers them opportunities for independent living without the need for assistance, facilitates their participation in education, the labor market, and civic life, and enables them to live productive, dignified lives while engaging actively in society. AT also fosters interaction with non-disabled individuals. Without AT, people with disabilities are often excluded, isolated, and trapped in poverty, which amplifies the negative effects of disability and illness on these individuals, their families, and society.

Assistive technology is an essential resource, intervention, and support mechanism for rehabilitation, enabling individuals to perform activities of daily living and participate in their communities (Lewis et al., 2012).

3.2 AT and rehabilitative applications: strengths and weaknesses

Defined by the World Health Organization (WHO) as tools, devices, or systems aimed at conserving or restoring an individual's physical, sensory, or cognitive abilities, AT has evolved into a cornerstone of modern rehabilitation strategies. By leveraging technological advancements, AT empowers individuals to overcome barriers in mobility, communication, and daily living tasks, thus promoting greater autonomy and social inclusion.

ATs can be found and applied in many places within homes, workplaces, schools, and others. In literature, assistive technologies are classified based on application areas such as survival, environmental interaction, communication, mobility, positioning, and physical education (Boelen, DEN BOUT, De Keijser & Hoijtink, 2003; McCreadie & Tinker, 2005; Simpson, McBride, Spencer, Lodermilk & Lynch, 2009; Poels, De Kort & IJsselsteijn, 2012).

Assistive technologies are classified into (Aqel, Issa, Elsharif, Ghaben, Alajerami, et al., 2019):

- **ATs for Survival**: Devices and tools needed to sustain life, including bathing aids, feeding, dressing, and sleeping. Some of these technologies include aids for daily living such as special utensils and cutlery (e.g., jar opener, potato peeler), special door handles, tap turner, bath seat, bath or toilet hoist, ripple mattress, special chairs and tables, button hook, and put on/off clothing.
- **ATs for Mobility**: Tools and devices to assist people with mobility challenges by allowing free navigation in indoor and outdoor environments. These ATs give individuals the opportunity to navigate their homes and workplaces and to independently walk, climb stairs, and transfer from wheelchairs to seats. Examples of these technologies include wheelchairs, exoskeletons, and walking canes.
- **ATs for Communication**: Gadgets and devices important for social interaction, visual and auditory reception, and oral/composed articulation. Examples include tape recorders, speech synthesizers, low-vision aids, magnifying glasses, hearing aids, telephone amplifiers, Braille displays and writers, flashing lights instead of doorbells, and flashing alarm clocks.

- **ATs for Physical Education**: Tools designed to empower persons with physical inabilities to participate in physical exercises. There are numerous ATs designed for this purpose, including adapted wheelchairs and adapted tools empowering disabled persons to engage in swimming, biking, basketball, and other recreational activities.
- **ATs for Environmental Interaction**: Devices and tools necessary for people with disabilities to interact with their surroundings, such as remote-control devices, electronic doors, foot mice for patients with hand-function challenges, adapted light switches, door answering/opening systems, stair ramps, handrails, and bath grab-rails.
- **ATs for Positioning**: These ATs meet the needs of individuals with physical incapacities who cannot support themselves in positions such as standing, sitting, and lying down. Such ATs include adaptive seating, height-adjustable chairs and tables, standing frames, bean bag chairs, and wedges.

Studies on the use of assistive technologies (AT) have highlighted benefits that extend beyond merely enabling users to perform tasks they would otherwise find impossible. A key advantage, especially relevant for children with multiple disabilities, is the ability to gain control over their environment, fostering exploratory play and independence in daily activities (Hutinger, Johanson & Stoneburner, 1996; Cowan and Turner-Smith, 1999; Sullivan and Lewis, 2000; Senjam, Foster & Bascaran, 2021). Comprehensive studies on AT applications in schools (Derer et al., 1996; Hutinger et al., 1996) identified the development of autonomy and self-determination as some of the most frequently cited benefits by parents and teachers. A notable benefit contributing to self-determination is the ability to make choices and direct personal care through augmented or alternative communication (Todis and Walker, 1993; Hutinger et al., 1996). For individuals with speech and language impairments, devices such as eye-tracking systems and speech-generating devices offer a pathway to express thoughts and interact with others. Eye-tracking technologies, in particular, have revolutionized communication for individuals with Rett syndrome or amyotrophic lateral sclerosis (ALS), enabling them to navigate digital interfaces and select words or symbols through gaze alone. This fosters not only functional communication but also emotional expression and social connectivity (Lopresti, Mihailidis & Kirsch, 2004).

Additional reported benefits about the use of AT, include improved social interactions, as noted by several studies (Mistrett, Constantino & Pomerantz, 1994), as well as increased motivation and self-esteem (Senjam, et al., 2021; Swinth and Case-Smith, 1993). Skill acquisition and enhancement, such as handwriting, motor skills, reading, visual attention and perception, and math skills, have also been documented (Reed & Kanny, 1994). Furthermore, cognitive benefits have been associated with AT use, including an improved understanding of cause-and-effect relationships, extended attention span, and better problem-solving abilities ((Reed & Kanny, 1994; Todis and Walker, 1993; Hutinger et al., 1996).

The article by LoPresti, Mihailidis, and Kirsch (2004) reviews the state of assistive technology for cognition (ATC), highlighting its critical role in supporting individuals with cognitive disabilities. The authors discuss ATC's potential to aid in tasks requiring complex attention, executive reasoning, prospective memory, and behavior monitoring, emphasizing its ability to enhance traditional rehabilitation methods and introduce innovative approaches.

ATC interventions serve diverse populations, including individuals with traumatic brain injury, stroke, dementia, autism, and learning disabilities. Depending on the individual's needs, ATC can either capitalize on residual abilities to achieve tasks or provide external supports for essential activities of daily living (Mihailidis, Fernie & Barbenel, 2001; Evans, Ricciuti, Hope et al., 2010)

Examples include devices that assist with memory, such as alphanumeric paging systems, which have demonstrated significant improvements in task completion and independence (Wilson et al., 1998). Additionally, personal digital assistants (PDAs) and wearable technology can provide location-based cues, reminders, and task guidance, improving user autonomy (Friedman, 1993).

A significant strength of ATC lies in its customizability, allowing interventions to be tailored to individual strengths, preferences, and needs. For instance, the COACH system utilizes artificial intelligence to assist dementia patients with handwashing, adapting to their specific task patterns (Mihailidis et al., 2001).

Similarly, task guidance systems like COGORTH have shown effectiveness in enabling users to complete multi-step activities through structured prompts (Kirsch & Weixel, 1988). While ATC offers considerable benefits, challenges remain. The authors highlight the complexities of matching individuals with appropriate technologies, as well as the need for better integration of

user-centered design and participatory approaches (LoPresti et al., 2004). Overall, LoPresti (2004) conclude that ATC is an essential and evolving component of cognitive rehabilitation, with significant potential to enhance the independence and quality of life for individuals with cognitive impairments.

To reinforce these findings, teachers also reported improvements in general academic behaviors using AT, such as work habits and productivity (Derer et al., 1996). Observing these overall benefits, parents and teachers have recognized AT's ability to provide children with new opportunities, reveal their potential, and equip them with tools to realize that potential (Derer et al., 1996; Hutinger et al., 1996). This increased awareness of children's abilities has, in turn, led to heightened expectations from parents and teachers (Anderson & Horney, 2007; Hutinger et al., 1996).

In addition to its academic impact, the psychological benefits of AT are equally significant (Aqel et al., 2019). By reducing reliance on caregivers, AT boosts self-confidence and minimizes the stigma associated with disability. For caregivers, these technologies alleviate the physical and emotional burden of care, allowing for a more supportive and balanced dynamic between them and the individual (Aqel et al., 2019).

Building on this, AT plays a transformative role in addressing the challenges faced by individuals with motor disabilities. From mobility aids such as wheelchairs and exoskeletons to communication tools like speech-generating devices, these technologies bridge gaps in physical and cognitive impairments. For example, robotic exoskeletons have been shown to facilitate gait training and improve motor functions, particularly in individuals recovering from spinal cord injuries or neurological disorders. For example, robotic exoskeletons have been shown to facilitate gait training and improve motor functions, particularly in individuals recovering from spinal cord injuries or neurological disorders. Rehabilitation devices are essential tools in physical rehabilitation, physiotherapy, and exercise programs designed to address physical disabilities and motor-function deficiencies in various body parts, including the shoulder, elbow, wrist, hand, hip, knee, and ankle joints. These devices are often developed to assist or substitute physiotherapists, especially in intensive post-injury treatments that demand extensive time and effort. Challenges such as low physiotherapist-to-patient ratios and fatigue among therapists highlight the importance

of rehabilitation devices as reliable, precise alternatives for repetitive exercises. Rehabilitation devices are equipped with sensory components, making them adaptable to patient conditions.

However, despite these benefits, challenges in the effective application of AT for students with multiple disabilities remain documented. Studies reveal infrequent usage of AT devices outside the school setting and limited application in classrooms due to inadequate staff training, lack of ongoing support, and logistical challenges (Hutinger et al., 1994; Derer et al., 1996).

The absence of coordinated planning and collaboration between school and family further limits AT integration in educational programs (Carey & Sale, 1994; Hutinger et al., 1996). Additionally, funding constraints and maintenance costs complicate access to necessary equipment, with schools often lacking sufficient resources to meet these demands (Derer et al., 1996). Issues with equipment portability, maintenance complexity, and device sharing among classrooms reduce personalized AT usage (Scherer & McKee, 1990; Carey & Sale, 1994).

Evidence shows that AT can yield substantial benefits for children with multiple disabilities (Hutinger et al., 1996; Sullivan & Lewis, 2000), yet it remains underutilized and often suboptimally implemented in educational environments (Derer et al., 1996).

3.3 AT and the software applications for rehabilitation

Technological advancements have revolutionized rehabilitation practices, integrating innovative tools to enhance the effectiveness and accessibility of therapeutic interventions.

Among these, deep learning and neural networks, augmented reality (AR), virtual reality (VR), and the Internet of Things (IoT) have emerged as transformative solutions in the field. Each of these technologies contributes uniquely to improving patient outcomes and the quality of care provided by healthcare professionals.

- Deep learning and neural networks have shown significant potential in aiding physiotherapists with faster, more accurate diagnoses and evaluating rehabilitation exercises by analyzing patient data and medical images like MRIs, CT scans, and X-rays. For example, Dorofeev and Wenger, (2019) developed an AI model based on neural networks to optimize spinal rehabilitation by analyzing medical data from MRT, CT, EEG, and EMNG tests. This model helped identify and correct

movement defects and predict diseases such as osteochondrosis and spondylosis, facilitating quicker rehabilitation interventions. Liao et al. (2020) used deep learning frameworks, including convolutional (CNN), recurrent (RNN), and hierarchical (HNN) neural networks, to assess the quality of rehabilitation exercises by analyzing captured motion data. Similarly, Vakanski et al. (2016) utilized neural networks and Microsoft Kinect to evaluate patient movements during rehabilitation exercises, comparing them to pre-recorded physiotherapist-designed programs. Han et al. (2017) applied a deep neural network (DNN) for posture correction in patients with conditions like stroke and Parkinson's disease, using data from Kinect sensors to optimize rehabilitation.

- Augmented Reality (AR) enhances rehabilitation by integrating virtual objects into training, making exercises more engaging and effective. Bai et al. (2014) demonstrated the use of AR systems to foster pretend play and social interaction in children with autism, positively impacting their understanding of their environment. Lee and Park (2014) designed an AR-based postural training system to improve gait and balance in stroke patients, using GAITRite testing to assess effectiveness. Assis et colleagues (2019) developed the NeuroR system for upper-limb rehabilitation post-stroke, which allowed both patients and caregivers to practice motor rehabilitation exercises through virtual simulations. Lin and Chang (2015) created an AR system using Scratch 2.0 and web cameras to improve the physical activity of children with developmental disabilities. These studies demonstrate AR's potential to increase patient interaction, improve physical performance, and promote social integration during rehabilitation.
- Virtual Reality (VR) combines therapy and entertainment, offering immersive environments for rehabilitation. Shi and Peng (2018) developed a game-based application for upper-limb rehabilitation using Kinect motion sensors, providing interactive and enjoyable exercises suitable for home use. Reyes and Arteaga (2018) created a 3D Maze system to improve the shoulder, arm, and hand movement of patients, utilizing low-cost and adaptive VR environments (Aqel, Issa,

Elsharif, et al., 2019; Maggio, De Luca, Molonia et al., 2019) evaluated VR's role in rehabilitating patients with multiple sclerosis, noting significant improvements in motor skills, balance, and cognitive functions while increasing patient motivation. VR technologies thus provide engaging and effective rehabilitation solutions that cater to physical and cognitive disabilities.

- The Internet of Things (IoT) facilitates remote rehabilitation by integrating devices and software for monitoring and data analysis. Dobkin (2017) designed an Android app to collect patient data and send it to a medical cloud for analysis, enabling physiotherapists to monitor and adjust rehabilitation plans remotely. Ferrag et colleagues (2017) developed a wireless rehabilitation system using motion sensors and cloud-based data storage, allowing doctors to track patient progress. Alexandre and Postolache (2018) created an IoT-enabled VR system using smart gloves for upper-limb rehabilitation, enabling therapists to monitor patient performance via data collected from sensors. Bilic et al. (2017) introduced a wearable IoT system for monitoring elbow rehabilitation using accelerometers and gyroscopes to characterize movement. These studies highlight IoT's role in enabling cost-effective tele-rehabilitation, providing specialists with tools to monitor, assess, and support patients remotely.

Deep learning, AR, VR, and IoT technologies play vital roles in advancing rehabilitation by offering innovative, cost-effective, and interactive solutions. These technologies not only enhance diagnostic and therapeutic capabilities but also improve patient motivation, engagement, and overall outcomes in diverse rehabilitation settings.

3.4 AT in Rett Syndrome

As described by Neul et al. (2010), RTT presents severe developmental limitations that lead to isolation, passivity, and diminished social engagement, significantly affecting quality of life (Felce & Perry, 1995). Assistive Technology (AT) can play a crucial role in motor and cognitive rehabilitation for individuals with Rett Syndrome (RTT).

To address these challenges, AT and cognitive-behavioral interventions have emerged as effective

strategies for rehabilitation. The Australian RTT study (Leonard, Gold, Samaco et al., 2022) has gathered information regarding the use of assistive technology among individuals with RTT. Their findings suggest that the majority of individuals with RTT are using such devices. As detailed in this review, the most commonly used AT for people with RTT are:

(a) mobility aids,

(b) AT for physiological needs,

(c) transport aids,

(d) foot supports,

(e) communication aids,

(f) arm supports, and

(g) lifting aids (see Figure 1).

Figure 1. Equipment use in RTT. AT most commonly used in families with a dependent with RTT as shown by Leonard's monitoring in 2002.

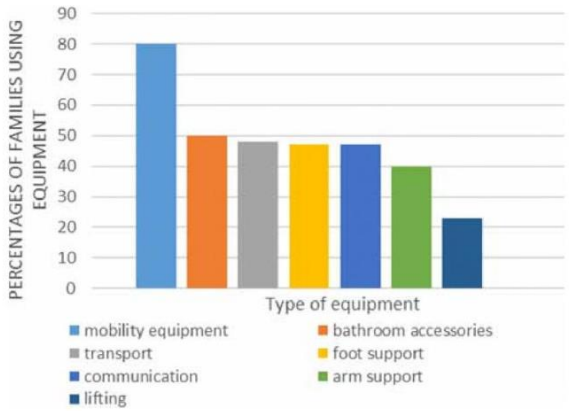


Figure 4. Equipment use in RTT. AT most commonly used in families with a dependent with RTT as shown by Leonard's monitoring in 2002.

Despite the limited application of AT in RTT rehabilitation, research demonstrates positive outcomes, emphasizing its potential for enhancing motor and cognitive functions (Sigafoos, Green, Payne et al., 2009; Didden, Korzilius, Smeets et al., 2010).

Eye-tracking technology (ETT) has proven instrumental in RTT rehabilitation. ETT enables the assessment of visual and cognitive abilities, facilitates non-verbal communication, and monitors progress over time. For example, Wandin and colleagues (2022) demonstrated ETT's effectiveness in evaluating visual abilities, while Byiers and Symons (2013) highlighted its utility in assessing

cognitive functions. ETT also allows individuals with RTT to communicate by selecting objects or symbols through gaze movements (Vessoyan et al., 2018). Fabio et al. (2021) emphasized its potential in enhancing attention span and decision-making behaviors, demonstrating longitudinal improvements through cognitive rehabilitation. Additionally, cognitive stimulation with ETT has shown promising results. Migliorelli et al. (2022) observed changes in brain activity using Tobii Dynavox PCEye Explore, where tasks facilitated by the Look to Learn software resulted in decreased delta activity and increased beta rhythms, suggesting enhanced brain complexity. Similarly, Ahonniska-Assa et al. (2018) used ETT in language comprehension tasks, revealing notable improvements in receptive vocabulary among younger participants.

Innovative applications of ETT, such as the Speak with Your Gaze (SWYG) program developed by Iannizzotto et al. (2020), have further expanded its utility. This program enables remote communication by detecting gaze direction during teleconferencing, which proved valuable during the COVID-19 pandemic, enhancing attention and reducing stereotypical behaviors. Interactive multimedia tasks have improved cognitive and social engagement in RTT patients (Dovigo et al., 2021). Parental feedback underscores the transformative impact of ETT-enabled speech-generating devices (SGDs) in RTT rehabilitation. Vessoyan et al. (2018) reported high satisfaction among caregivers, emphasizing reduced burden and improved communication, supported by ongoing assistance from Augmentative and Alternative Communication (AAC) therapists.

AT, particularly ETT, represents a powerful tool for addressing the motor and cognitive challenges of RTT. By enabling communication, improving cognitive functions, and fostering social interaction, AT significantly enhances the quality of life for RTT individuals and their caregivers.

In addition to the use of ETT, technological aids such as sensors and personal computers offer promising avenues for improving self-determination in individuals with Rett Syndrome (RTT). This approach enhances opportunities for occupation, constructive engagement, and choice-making, which are often limited in RTT patients. In five studies (Lancioni et al., 2014, 2018; Stasolla et al., 2018, 2015; Stasolla & Caffò, 2013), technologies were employed in RTT rehabilitation, using sensors connected to computers to allow participants to activate reinforcers such as videos, music, or images through target movements. These studies consistently demonstrated an increase in the

frequency of correct responses when access to technology-enhanced rewards was visible, highlighting the potential of assistive technologies in fostering active participation.

In line with these findings, a recent study by Caprì and colleagues (2024) examined the effectiveness of a low-tech device called Click4all in promoting social interaction among RTT patients and their classmates in a school setting. The study involved 27 RTT patients divided into experimental and control groups. The experimental group used Click4all during cognitive and motor training activities, while the control group received traditional treatments. Results showed significant improvements in social interactions—such as eye contact, smiles, and physical engagement—within the experimental group over two years. Additionally, classmates in the experimental group exhibited greater awareness of RTT patients' preferences and needs, improving interpersonal relationships. Although no statistically significant differences were observed in cognitive and motor skills between the groups, participants in the experimental group displayed slight improvements, suggesting the therapeutic potential of the device. The study emphasized Click4all's effectiveness as a customizable tool for reducing isolation and enhancing social inclusion in RTT patients.

Regarding rehabilitation software and the Internet of Things (IoT), telerehabilitation has emerged as a novel approach in RTT care. Lotan and colleagues (2021) examined a Skype-supervised, individualized, home-based rehabilitation program for RTT patients, where parents acted as primary facilitators of daily physical activities. This 12-week program, with bi-weekly remote guidance, yielded positive functional outcomes and high parental satisfaction. Parents reported improved motor abilities in their children and increased confidence in supporting these exercises independently post-program. Similarly, Romano (2022) investigated a remotely supervised, family-centered motor rehabilitation program involving 13 participants over three months. The program achieved 78.7% of its rehabilitative goals, with notable improvements in gross motor functions. Parental satisfaction was again high, underscoring the feasibility and effectiveness of remote, family-centered care, particularly during the COVID-19 pandemic. These studies illustrate the adaptability of telerehabilitation in RTT, empowering families and ensuring consistent, accessible care in familiar environments.

Virtual Reality (VR) has also been explored in RTT rehabilitation. Mraz, Eisenberg Diener et al. (2016) conducted a study involving six RTT patients who participated in a VR-based upper-limb rehabilitation program using Microsoft Kinect and FAAST (Flexible Action and Articulated Skeleton Toolkit) software. The 12-week intervention involved three 60-minute sessions per week, focusing on forward-reaching tasks to improve elbow extension and shoulder flexion/extension. YouTube videos chosen by participants were integrated to maintain motivation, requiring participants to perform specific movements to play or pause the videos. The program progressively increased in complexity, alternating between right and left arms. Observations revealed that the intervention was most effective when activities were engaging, established clear cause-and-effect relationships, and matched participants' cognitive abilities. Outcomes included improved upper-extremity functionality, increased reach frequency, and reduced stereotypical hand movements.

Conclusions

The integration of assistive technologies (AT) in rehabilitation for Rett Syndrome (RTT) demonstrates promising potential, as evidenced by the positive outcomes reported in limited RTT-specific studies and the broader literature on disability interventions. The application of AT has consistently shown its ability to enhance self-determination, social interaction, and motor and cognitive skills in RTT patients. Technologies like Eye-Tracking Technology (ETT), low-tech tools such as Click4all, Virtual Reality (VR), and Internet of Things (IoT)-enabled telerehabilitation exemplify the adaptability and versatility of AT in addressing the complex needs of this population.

The studies reviewed indicate that AT can foster significant improvements in various domains. ETT enables non-verbal communication, facilitates cognitive stimulation, and improves attention and decision-making, offering a means of overcoming communicative and cognitive barriers. Low-tech devices like Click4all promote social inclusion in educational settings, reducing isolation and enhancing interpersonal relationships. VR-based interventions encourage engagement and physical activity through immersive and interactive environments, while IoT-powered telerehabilitation programs extend access to therapy, empower families, and ensure continuity of care.

Although the application of AT in RTT remains limited, the positive outcomes reported align with findings from broader research on disability-related rehabilitation. Studies in other disability contexts underscore the role of AT in improving functional independence, fostering social inclusion, and enhancing quality of life. These findings establish a strong foundation for further exploration and innovation in RTT-focused AT applications.

CHAPTER FOUR: EXPERIMENTAL STUDIES

The preceding chapters provide the theoretical and methodological framework upon which this doctoral thesis is built. These foundations have directed the development of the experimental projects presented in this chapter.

This chapter presents five experimental studies exploring the application of innovative technologies in rehabilitation and learning, with a particular focus on patients with Rett Syndrome.

The first study focuses on the design and implementation of a specific telerehabilitation software aimed at enabling professionals to support and train caregivers while simultaneously providing personalized, consistent, and high-frequency rehabilitation interventions. The objective was to evaluate the effectiveness of this platform as a remote tool for improving the motor and cognitive skills of patients.

Once this infrastructure was established, the work transitioned toward the adoption of interactive and immersive technologies to enhance and expand rehabilitation pathways. Within this framework, the second study investigates the impact of immersive virtual reality combined with treadmill usage, evaluating its potential to improve lower limb motor function and increase engagement in rehabilitation programs.

The third and fourth studies are closely related to the realm of virtual reality and focus on the development of therapeutic games for upper limb rehabilitation and cognitive improvement.

The fifth study, finally, focuses on cognitive rehabilitation through the use of Serious Games as educational tools. Specifically, it analyzes the effectiveness of an “Fiaba Interattiva” in improving attention spans, enhancing memory, and facilitating concept acquisition. This approach was further developed through an ongoing study focused on the creation of an interactive game, “Lettoscrittura,” designed to support cognitive and communication rehabilitation by combining playful and engaging learning with targeted exercises.

These studies outline a research trajectory focused on enhancing the quality of life for patients with Rett Syndrome through the utilization of advanced technological solutions.

4.1 Experimental Study 1: Comparison Between Advanced and Basic Telerehabilitation Technologies for Patients with Rett Syndrome: A Pilot Study on Behavioral Parameters

Tele-rehabilitation (TR) is the accessibility to therapeutic and rehabilitative services through the support of various technologies, such as video, websites, computer programs, and video conferencing platforms (Schwamm, Holloway, Amarenco et al., 2009). TR also refers to the use of information and communication technologies (ICT) to deliver remote rehabilitation services to people at home or in other remote locations (Brennan, Mawson & Brownsell, 2009). These services include therapeutic interventions, remote monitoring of progress, and training and consulting for personnel involved in the rehabilitation process.

The rapid evolution of technology has allowed healthcare professionals to provide care services in new and different ways. Consequently, TR interventions employ a wide variety of technologies depending on the needs of the patient and the involved personnel: from basic common tools (e.g., phone, video conferencing) to more advanced devices (e.g., wearable sensors, 3D cameras, eye-tracking systems, and virtual reality) (Marzano, Ochoa-Siguencia & Pellegrino, 2017). Essentially, TR applications can be divided into two main areas:

- Basic applications, which include evaluation and treatment services using phones or computers via video conferencing.
- Advanced applications, which include real-time, bidirectional interactions with live audio and video, synchronous evaluations, and virtual reality (Marzano et al., 2017).

The type of technology used in TR interventions can influence the effectiveness of TR both negatively and positively. For instance, basic tele-rehabilitation technology using video conferencing systems has been considered difficult to use for individuals generally unfamiliar with this type of technology (Pramuka & Van Roosmalen, 2009). Conversely, some studies have shown that advanced technologies combined with TR applications can achieve better outcomes (Kairy, Lehoux, Vincent et al., 2009).

TR has also been utilized in several clinical fields (Woolf, Caute, Haigh et al., 2016; Peretti et al., 2017). Regarding the field of neurodevelopmental disorders (NDD), a recent systematic

review identified three categories of technologies used in TR interventions for children with NDD: integrated systems, game-based technologies, and video-based technologies (Caprì et al., 2021). This review demonstrated that TR proves to be an effective tool for improving adaptive skills in children with NDD. The literature also indicates that TR can be a promising intervention for children with neurological diseases (Corti, Oldrati, Oprandi et al., 2019)cas well as for the treatment of motor (Agostini, Moja, Banzi et al., 2015; Giordano, Bonometti, Vanoglio et al., 2016), cognitive (Coleman et al., 2022), and language disorders (Theodoros, Russell & Latifi, 2008; Van De Sandt-Koenderman, 2011; Cardullo, Gamberini, Milan & Mapelli, 2015). Another recent review (Valentine, Hall, Young et al., 2021) emphasized the effectiveness of providing TR interventions to parents and children with NDD, highlighting the importance of understanding which technologies are most suitable for the different individuals involved. Furthermore, another study reported that among the benefits of advanced TR is a reduction in parental burden, the guarantee of continuous and immediate feedback to the child, and greater control over acquired skills (Maresca, Maggio, De Luca et al., 2020). It has also been found that the use of advanced TR can produce improvements in cognitive, motor, and language skills within the same treatment session (Maresca et al., 2020), a crucial factor given that children with NDD may exhibit multiple disabilities.

The combined findings from these systematic reviews on advanced TR have highlighted the accessibility, effectiveness, and suitability of using such technologies to improve cognitive, emotional, motor, and adaptive abilities in children and adolescents with NDD (Stasolla, 2021). These promising results provide a solid theoretical background for using these technologies as unprecedented opportunities to support the implementation of TR services and develop best practices for patients with NDD. However, it is important to note that the application of advanced and basic TR technologies to patients with cerebral palsy and rare genetic diseases (e.g., Rett Syndrome) has not been sufficiently studied (Stasolla, 2021). This may be related to the fact that RTT is a rare genetic disease and a heterogeneous syndrome with varying levels of severity, including cognitive, linguistic, motor, neurological, and behavioral deficits (Castelli et al., 2013; Fabio et al., 2018). Additionally, RTT patients exhibit attention deficits, so the educator or therapist must simultaneously implement an intervention to stimulate the patients' attention and reduce stereotypies (Fabio et al., 2018; Fabio et al., 2021). Given the complexity of RTT, treatments

require a multidisciplinary team of specialists from various areas: medical, psychological, social, educational, and occupational. They must provide support across all areas of daily life. For these reasons, it can be difficult to develop a comprehensive intervention using TR, as it would need to address multiple skill areas.

According to previously described studies on treatments for neurodevelopmental disorders (Caprì et al., 2021; Valentine et al., 2021; Stasolla et al., 2021), the use of TR also seems suitable for RTT patients.

The main objective of the present study is to determine whether the use of advanced tele-rehabilitation technology (ATR) in RTT patients leads to greater (or equal) improvements in motor and cognitive functions compared to basic tele-rehabilitation (BTR). The rationale is that the ATR system includes eye-tracking support, enabling the therapist and caregiver to monitor patient choices with high precision and rely on video recordings and real-time 3D reconstructions produced using computer vision and artificial intelligence (AI) techniques. The therapist and caregiver can also see the patient's reconstructed skeleton overlaid on the patient's image in the video. This combination of tools thus aims to ensure greater engagement from therapists, caregivers, and patients.

It was hypothesized that patients undergoing ATR would show greater improvement in almost all motor and cognitive scoring activities administered. Additionally, the following outcomes were expected:

- An increase in the attention levels of the ATR group and a simultaneous decrease in stereotypies due to therapist interventions conducted remotely.
- Improvements in the areas of basic prerequisite behaviors, neuropsychological skills, basic cognitive abilities, advanced cognitive abilities, communication, emotional abilities, fine and gross motor skills, and life autonomy.
- These general improvements would also impact global scales measuring disease severity and functional ability levels.

4.1.1 Materials and Methods

4.1.1.1 Participants

A total of 20 young girls and women with a diagnosis of RTT, ranging from age 4 to 31 years old (Median: 12.50; IQR: 9.50–17.25) were recruited from the Italian Rett Association (AIRETT). All the participants were born into non-consanguineous marriages. Regular immunizations had been carried out. At birth, the weight and height were all normal. The RTT patients were classified as clinical stage III (characterized by prominent hand apraxia/dyspraxia, apparently preserved ambulation ability, and some communicative ability, mainly eye contact) or stage IV (late motor deterioration, with progressive loss of ambulation ability), according to the criteria for classic RTT by Hagberg et al. (1985). All the participants showed pervasive hand stereotypies. All attended schools or socio-educational centers. A general assessment was carried out by a psychologist using the Vineland Adaptive Behavior Scale (VABS; Sparrow & Cicchetti,1985) and the Rett Assessment Rating Scale (RARS; Fabio, Martinazzoli, & Antonietti, 2005). Table 1 shows the characteristics of the groups.

Table 1

Characteristics of participants.

Partecipanti	Nome	Stadio Clinico	Età	Mutazione Mecp2	Livello di gravità (RARS)	Livello di funzionamento (VABS)
ATR						
1	L.G	IV	25	T158M	75,5	75
2	L.A	IV	25	T158M	75,5	75
3	D.D	IV	31	R306C	75	90
4	C.A	III	5	T158M	58	84
5	A.C	III	5	----	71	71
6	C.L	III	4	P152R	69,5	109
7	F.D	IV	18	T158M	64	136
8	S.M	III	14	T158M	62	91
9	D.F	IV	25	R255X	64	111
10	C.M	III	7	C965C	65,5	104

11	S.D	IV	15	P133C	72	151
12	B.C	III	5	R255X	81,5	85
13	S.A	III	10	C965C	70	108
14	B.G	III	24	P152R	63,5	74
15	G.L	IV	10	R255X	64,5	84
16	S.L	IV	9	T158M	67,5	78
18	B.A	III	10	P152R	75	98
19	P.V	III	8	----	66,5	69
20	L.M	III	9	C965C	58	80

The Mecp2 mutation was seen in most of the participants; specific mutations of the Mecp2 gene were: 40% showed T158M, 20% showed P322L, 15% showed R255X, and 15% showed P152R; for 10%, it was not possible to specify the type of Mecp2 gene mutation. With reference to the eligibility criteria of the participants from the Rett population, inclu- sion criteria of the participants with Rett syndrome, are that they had to be able to stay seated to independently watch the presented stimuli or with some support. The exclusion criteria refer to patients with genetic mutations FOXG1 and CDKL5.

The participants were matched for age, severity level of the disease, and functional ability level and randomly assigned to the ATR and BTR rehabilitation groups.

We asked the reference neuropsychiatry of each patient to give a medical judgment of severity based on the typical characteristics of the syndrome (epilepsy, mood swings, convulsions, aerophagia, scoliosis). The severity level ranged from 5 (mild severity) to 20 (severe severity). The mean severity index about the typical characteristics of the syndrome was 9.

4.1.1.2 Study Design

This study employed a pre-test, post-test 1, and post-test 2 design with two groups: the control group and the experimental group (Figure 5).

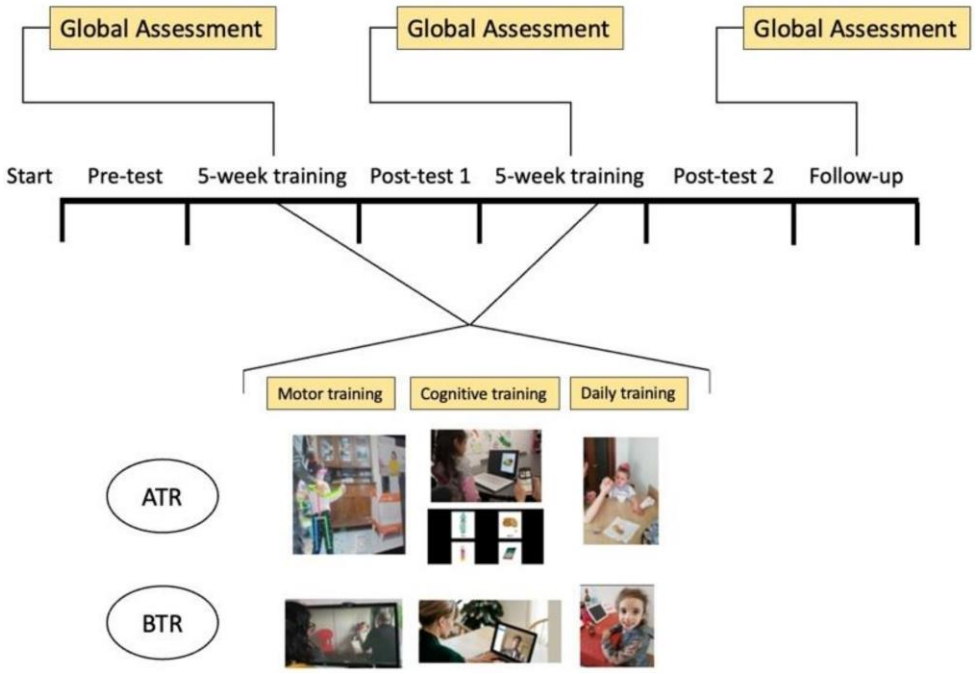


Figure 5. The study design with all phases. In ATR, the monitoring of the skeleton and eye-tracking are shown, while in the BTR group, only the video-call is shown.

The former received evaluation and treatment with the use of BTR (a simple online Skype platform), whereas the latter received evaluation and treatment with the use of an ATR system that was equipped with enhanced tools to acquire eye-gaze data and a reconstructed patient's skeleton that was superimposed on the patient image in the video. In the pre-test phase, all the participants underwent a cognitive assessment to evaluate attention, the intensity of stereotypies, the intensity of trainer aids, and global functioning before the treatment (cognitive empowerment): a total of ten of the patients were assessed with the traditional online system and ten with the ATR system. This same assessment was repeated once after treatment (post-test 1) after 5 weeks and once at 10 weeks after the conclusion of treatment (post-test 2). The scores that were obtained in the pre-test phase were compared with those that were observed in the post-test 1 assessment phase and post-test 2, to evaluate the effects of the intervention in the control group and the experimental group. To avoid subjective bias in the present study, blinded assessment was applied. One independent blinded investigator registered all the study outcomes in the three phases (Cohen's kappa was always higher than 0.86).

4.1.1.3 Assessment and Measures

As stated above, all A phases of this study consisted of a cognitive assessment in which attention span, intensity of stereotypies, Global Functioning Rett Scale (Hagberg et al., 1985), Vineland Adaptive Behavior Scale (Sparrow et al., 2013), and Rett Assessment Rating Scale (Fabio, Martinazzoli, & Antonietti, 2005) were measured for participants in both groups. In the controls, BTR was used; in the experimental group, ATR was used.

- Attention

The attention measurements started when the patient looked at the object of focus as requested by the educator and continued until the patient looked away from the object or stared into space. The participant was asked to look at a picture of an object (food, familiar objects, or animals) that was presented on a computer screen without eye-tracking for the control group and with eye-tracking with the experimental group. The total time that was spent by the participant looking at the stimuli or correctly at the face of the therapist was considered the parameter (in seconds).

- Type and Duration of Stereotypies

These data were recorded in a free and unstructured examination during a preliminary observation session lasting 5 min which was video recorded with a video camera. The therapists coded the data regarding the presence of stereotypies and counted the numbers of stereotypies during a 5 min period and registered the type of stereotypies.

For the Global Evaluation:

- The Vineland Adaptive Behavior Scales-Interview second edition (VABS) and the Rett Assessment Rating Scales (RARS) were used. VABS is subdivided into four domains: communication, daily living, socialization, and motor skills. The interviewer asks general questions about the patient's functioning in each domain and uses the responses to rate the participant on each critical behavior item (2: always present, 1: sometimes present, 0: seldom or never present). Typical interviews require approximately one hour. A total score is computed by summing the individual ratings for each scale, named the Vineland composite scores. The reliability of VABS was established as follows: split-half, 0.73–0.93 for the communication domain, 0.83–0.92 for daily living skills, 0.78–0.94 for socialization, 0.70–0.95 for motor skills, 0.84–0.98 for adaptive

behavior composite, and 0.77–0.88 for maladaptive behavior (survey form) (0.80 and 0.90 for the Survey Form). The interrupter reliability coefficients for the survey and expanded forms ranged from 0.62 to 0.75. The standard error of measurement ranged from 3.4 to 8.2 over the four domains, and from 2.2 to 4.9 for the Adaptive Behavior Composite, on the survey form.

- RARS is a standardized scale that is used to evaluate patients with RTT. It is organized into seven domains: cognitive, sensorial, motor, emotional, autonomy, typical characteris- tics of the disease, and behavior. Typical characteristics of the disease and behavior domains measure the following characteristics: mood swings, convulsions, dyspnea, hyper-activity, anxiety, aggressivity, bruxism, oculogyric crises, epilepsy, aerophagia, muscle tension, and food preferences. A total of 31 items was generated as representative of the profile of RTT. Each item is provided with a brief glossary explaining its meaning in a few words. Each item is rated on a 4-point scale, where 1 = within normal limits, 2 = infrequent or low abnormality, 3 = frequent or medium-high abnormality, and 4 = strong abnormality. Intermediate ratings are possible; for example, an answer between 2 and 3 points is rated as 2.5. For each item, the evaluator circles the number corresponding to the best description of the patient. After a patient has been rated on all 31 items, a total score is computed by summing the individual ratings. This total score allows the evaluator to identify the level of severity of RTT, conceptualized as a continuum ranging from mild symptoms to severe deficits (Mild = 0–55; Moderate = 56–81; Severe = > 81). RARS was established by a standardization procedure involving a sample of 220 patients with RTT, proving that the instrument is statistically valid and reliable. More precisely, normal distribution analyses of the scores were computed and the mean scores of the scale were similar to the median and the mode. The skewness and kurtosis values, calculated for the distribution of the total score, were 0.110 and 0.352, respectively. The distribution was found to be normal. Cronbach's alpha is used to determine the internal consistency for the whole scale and sub-scales. The total alpha was 0.912, and the internal consistency of the sub-scales is high (from 0.811 to 0.934).

GAIRS (Fabio, Semino & Giannatiempo, 2022). is a global assessment and intervention rating scales checklist for Rett syndrome coming from the items of assessment in multi-disability disorders (Shearer, Billingsley, Frohman et al., 1972; Kozloff, 1979;; Fabio, Martinazzoli, & Antonietti, 2005; Sparrow, 2011; Castelli et al., 2013; Downs, Stahlhut, Wong et al., 2016; Rodocanachi, Isaias, Cozzi et al., 2019; Cervi, Saletti, Turner et al.,2020) that was adapted for Rett syndrome. Through a global analysis, it gives an overview of the different areas and is intended for use in the functional analysis of the overall abilities of the patient. The GAIRS checklist is composed of 10 macro-areas: basic or pre-requisite behavior, neuropsychological abilities, basic cognitive concepts, advanced cognitive concepts, communication abilities, emotional-affective abilities, hand motor skills, graphomotor skills, global motor abilities, and the level of autonomy in daily life. The 10 areas are described in Table 2. For each area, different sequential skills, hierarchically structured, are evaluated. A total of 85 skills are evaluated. Each skill has a numerical score ranging from 1 to 5, where 1 is the minimum level of capacity and 5 is the maximum level of capacity to perform a specific activity. Below, we present some examples. In the area of basic behavior, the first skill that is evaluated is spontaneous eye contact. The score of this skill is: 1 if the child is unable to establish spontaneous eye contact, 2 if the child can establish spontaneous eye contact 2/3 times out of 10, 3 if the child can establish spontaneous eye contact 4/6 times out of 10, 4 if the child can establish spontaneous eye contact 7/8 times out of 10, and 5 if the child always establishes spontaneous eye contact. Instead, the sixth skill that is investigated in the hand motor area is grasping ability and the score is: 1 if the child cannot grasp an object on the table, 2 if the child can grasp a 5 cm object with palmar cubitus grip, 3 if the child can grasp a 5 cm object with palmar grip, 4 if the child can grasp a 1 cm object with pluri-digital grip, and 5 if the child can grasp a 1 cm object with plier's grip (thumb-index).

Table 2

Description of GAIRS checklist areas.

1. Basic Behaviors Area:
Evaluates the prerequisite behaviors for learning and communication, they are: spontaneous eye contact, eye contact on request, looking at objects, tracking objects and faces, functional gestures, cooperation with simple spoken requests (reply to their name, look for mother), sitting long enough to complete a task, object permanence, be able to wait for their turn before starting an activity, be able to communicate basic needs (need to eat, drink, sleep, play, walk, go to the bathroom, and feel good or bad).

2. Neuropsychological Area:
Evaluates brain-based skills which are needed in acquisition of knowledge, manipulation of information, and reasoning. They have more to do with the mechanisms of how people learn, remember, problem-solve, and pay attention, rather than with actual knowledge. This area includes selective attention, types and intensity of stereotypes, lateralization, temporal orientation, spatial orientation, memory span, logical sequences, categorization (animals, dress, foods, drinks, objects, places, actions).

3. Basic Cognitive Area:
Evaluates the basic cognitive concepts that allow the understanding of reality (spatial concepts, topological concepts, etc.). This area includes object recognition, color discrimination, geometric form discrimination
measure concepts, spatial concepts, human body discriminations, time concepts, cause-effect relationship.

4. Advanced Cognitive Area:
Evaluates the concepts of school learning that include the sub-areas of writing and mathematics. This area includes global words recognition, syllables recognition, recompleting words through syllables, alphabetic symbols recognition, recompleting words with alphabetic symbols, recognition of words representing actions, using words to communicate, math pre-requisite concepts, recognition of numbers, biunivocal relation between number and quantity.

5. Communication Area:
Evaluates the development of language by measuring responses to environmental sounds and speech, as well as the production of sounds and words. The skills of communication, comprehension and expression that allow the person to interact with others. This area includes expressing a basic need at a corporal level, recognizing, and expressing a basic need through pictures, understanding the biunivocal relation between the corpora, recognizing and expressing a basic need through word, understanding the biunivocal relation of a basic need between a picture and the word that expresses it, verbal comprehension, verbal production.

6. Emotional Area:
Evaluates the person's abilities and ways of experiencing, expressing, and understanding their own emotions and those of others are analyzed. This area includes identify emotions and express emotions.

7. Hand motor Area:
Evaluates the ability to make movements using the small muscles in our hands and wrists. Kids rely on these skills to do key tasks in school and in everyday life. Fine motor skills are complex, however. They involve the coordinated efforts of the brain and muscles, and they are built on the gross motor skills that allow us to make bigger movements. This area includes musculoskeletal alterations, hand-eye coordination during motor tasks lateralization, reaching movement, touching ability, grasping ability, releasing movement, repositioning movement, bimanual coordination, ability to push and pull an object.

8. Graphomotor Area:
Evaluates the fine motor skills incorporating, among others, graphomotor skills (GS) which, in turn, involve strength and control of the finger muscles, and incorporates important daily skills such as writing and drawing, necessary for the academic achievement of all students. This area includes grasping of pencil, drawing patterns and use of school tools.

9. Global Motor Area:
Evaluates the gross-motor skills which are important for an upright posture, walking, running, and climbing. It allows for the observation of physical weakness or disability or defects of movement. This area includes: standing, sitting, parachute reactions, rolling supine – on one side, rolling supine – prone, supine – to seated on the floor, seated on the floor – to standing, seated on a chair – to standing, standing – to seated on the floor, standing – to seated on a chair, walking, body spatial orientation in standing, stepping, running, climbing upstairs, descending stairs, jumping, picking up an object from the ground (small ball), playing with a ball and walking on a slope.

10. Autonomy in Daily Life Area:
Measures early adaptive and self-help behavior typically seen at home, as well as social behavior that develops through early adult-child interactions; therefore, this area analyses the level of autonomy in the praxis of daily life This area includes daily autonomy such as, eating, drinking, coughing or difficulty breathing during meal, type of food's consistence, washing, autonomy in the bathroom and dressing, and other skills such as, playing and socialization skills and advanced autonomy activities.

4.1.1.4 The Local Workstation

The hardware of the local workstation was composed of a laptop with an installed Skype Platform for the BTR; for the ATR, a laptop that was equipped with an eye-tracker, a webcam, a 3D camera, and a headset with a BTR system installed was used, as described below.

4.1.1.5 Technological Architecture

The ATR system (Figure 6) is composed of two types of workstations, namely a local workstation and a remote workstation.

The local workstation is intended to be used at the patient side, while the remote one is located at a specialized care center. Through the local workstation, the patient and caregiver connect with specialists for a cognitive or physical rehabilitation session. It is worth noting that, for Rett Syndrome subjects, the patient is never alone but always accompanied by a caregiver (with different skill levels, ranging from parents to therapists). The software platform is a web application, that leverages on the CISCO Webex API for network connection and video streaming. The following sections discuss the main components of the platform.

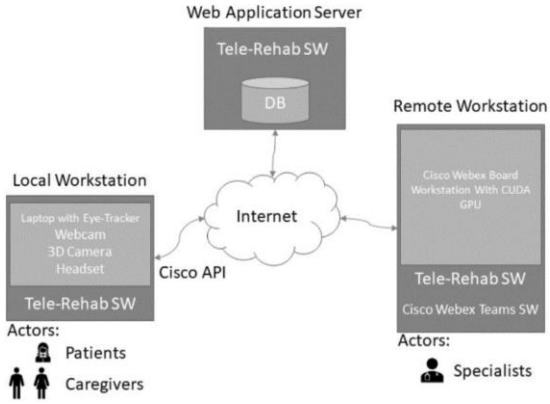


Figure 6. Technological architecture of the software with a web application server that was connected to both a remote workstation and a local workstation.

4.1.1.6 The Telerehabilitation Software

The special requirements of patients that are affected by Rett Syndrome, and other MDs with similar characteristics, demand very specific approaches and tools. As a consequence, we designed and implemented an ad hoc architecture to manage the TR sessions and to store and analyze the acquired data that is tailored to the needs of the Rett subjects, exploiting all the knowledge that was acquired with AIRETT after years of experience in using new technologies for both cognitive and physical rehabilitation. The main characteristic of the software is that it does not interfere with the ordinary tools that the patient uses in their everyday rehabilitation activities; in addition, a caregiver can be supervised and trained by a remote specialist during the rehabilitation sessions.

The software (Associazione Italiana Rett O.N.L.U.S., Siena, Italy) is a web application with a standard set of features (such as user authentication, videoconferencing, recordings, annotations, general patients' data) and domain-specific features. Such features exploit data acquisition and artificial intelligence techniques to add advanced tools giving therapists and caregivers a more sophisticated way to interact with the patient during the sessions and to ease further data analysis about the sessions.

More specifically, the ATR system is equipped with support for eye-tracking, so that the therapist can monitor how the patient interacts with the system during a cognitive session. In the context of motor rehabilitation, during the sessions, the movements of the patient are not the only

video recorded, but there is also a 3D reconstruction that is mapped in real-time, by applying computer vision and artificial intelligence (AI) techniques. The therapist can then see the reconstructed patient's skeleton superimposed on the patient's image in the video stream and can even annotate if the data that are acquired during the session can be considered valid or not. The skeleton data are then used to better observe the overall patient's pose and the angles between the bones near articulations, giving an objective measure of improvements in the patient's movements. Moreover, we plan to apply machine learning and advanced analysis techniques to the acquired data to provide decision-making support to the specialist when the amount of data is adequate for this purpose (Nucita et al., 2013).

4.1.1.7 Procedure

This study lasted from the 1 February to mid-June 2021. The performances were recorded according to the following steps: pre-test phase; cognitive and motor empower- ment; post-test phase 1 (after 5 weeks); cognitive and motor empowerment; and post test phase 2 (after 10 weeks). In the pre-test phase the AIRETT center professionals contacted the family by phone and with a brief interview that ascertained their availability for GAIRS administration sessions and all the procedures of TR. Then, the parents were invited to a session in which they completed the RARS scale that allows identifying the severity of the patients with Rett syndrome, and the Vineland questions to identify behavioral features.

After these sessions, the GAIRS Checklist was administered to the patients by the AIRETT team, composed of a physician, speech therapist, and psychologist, during the evaluation sessions at the Rett Centre. All professionals had certified, special training in Rett syndrome. Some skill scores that cannot be assigned directly during the evaluation, such as the ability to go to the bathroom, were evaluated through video or interviews with parents. Every skill was requested ten times, but if the participant gave the first 3 correct answers, the skill was considered acquired; in the same way, if the participant gave the first 3 wrong answers, the skill was considered not acquired. The total administration time was around 4 hours (range from 3 to 7) but for the most seriously affected patients, it was necessary to divide checklist administration into multiple sessions (2 or 3).

Successively, the therapist contacted the families of the control group through BTR for the GAIRS administration session and evaluation of attention and type and intensity of stereotypies, while they contacted the families of the experimental group to collect the same data through the ATR system.

All the caregivers and the therapist received specific training in the use of BTR and ATR systems from the specialist of the AIRETT team. During all the cognitive empowerment phases, one specialist of the AIRETT Center contacted the families of the control group and the families of the experimental group to conduct a session of cognitive and motor training. Families of both groups conducted the session three times a week with the trainer who collected data on the performance of the girls and women. Each session lasted for one hour

or an hour and a half and there were 30 sessions overall that were collected.

A session of cognitive training consisted of carrying out some cognitive discrimination tasks. The choice of the tasks depended on the level of the participant that was measured through the GAIRS Checklist. Cognitive stimuli were concrete objects or coloured pictures of objects (food, animals, toys, and familiar objects) that were presented with a distracting image in a randomized right-left order in a PowerPoint presentation. The participant was asked to look at the target, for example, an apple. Each task involved three repetitions of the presenting stimuli. The criterion allowing one to proceed to the next step was always the same: three correct answers (with eye contact) that were obtained in each of the three sessions of treatment.

A session of motor training consisted of some global and/or fine motor exercises. Each task was chosen by the level of the participant that was measured through the GAIRS Checklist. Exercises could be a passive movement to empower the range of motion of the main articulation or motor skills, such as touch a target or walk in an open space. In motor evaluation, the score depends (a) on the number of times the participants performed the exercise correctly, for example in hand-eye coordination during motor tasks where the score went from 1, the participant never looks at her hand during the 5 motor tasks, to 5, always looks at her hands during the motor tasks; (b) on the type of performance, such as in the standing skill where the score went from 1, the participant is unable to hold the posture, to 5, holds posture without assistance for more than 3 min. During a TR session, the software allows the local caregiver to video call a remote specialist. As shown in Figure

2, once the video call starts, the software acquires video streams from the laptop screen, the laptop webcam, an external webcam, and data from the eye-tracker. The local caregiver starts a cognitive rehabilitation session with rehabilitation software that was specifically tailored for Rett patients. During the session, the patient is always in audio contact with the remote specialist, who follows what is going on through the shared screen and the videos. The specialist can also see patient interactions, i.e., the patient's gaze on the screen. Hence, thanks to this setting and the acquired data, the specialist can monitor how the patient and the caregiver interact (from front and side views), and how the patient interacts with the rehabilitation software (through the eye-tracker pointer on screen). The parents were suggested to repeat each new learning related to motor and cognitive training in the life situations (daily training).

After the session ends, the video call is terminated, and the acquired data are stored locally and shared between the caregivers and therapists through a storage cloud system.

Importantly, the remote specialist can start and stop recording videos during sessions and annotate text on their own, simply by interacting with the system and without distracting the local caregiver and/or patient.

A motor TR session can be started by video calling a remote specialist, as for cognitive rehabilitation. In this case, however, there is no interaction between the patient and the local workstation. The local workstation is only used to acquire data and to let the caregiver and the specialist communicate. In the context of motor rehabilitation, the novel contribution of our approach is that during the session the movements of the patient were not only video recorded, but also reconstructed and 3D-mapped in real-time. In the post-test phase, all the participants were re-evaluated after 5 weeks and new cognitive and motor aims were established. Finally, they were re-evaluated after more than 5 weeks (post-test 2).

4.1.2 Statistical Analysis

The data of each subscale of the GAIRS were obtained following standardized instructions (Fabio et al., 2021), and the mean of the items for each patient was calculated for the subscales, ranging from 1 to 5 (with higher scores indicating that the patients reached the mastery performance for that subscale). The total score of GAIRS was computed by the mean of all areas.

Data analysis was performed using the IBM SPSS Statistics, Version 24 (IBM, Armonk, NY, USA). A mixed model ANCOVA for repeated measures was applied with a repeated factor time (T0–pre-intervention baseline, T1–5 weeks, post-test 1, T2–10 weeks, post-test 2), a factor group (experimental ATR, control BTR), and RARS (severity of disease at T0) as a covariate. Each of the measured parameters was the dependent variable. A Bonferroni correction was applied for multiple comparisons. The alpha level was set to $p < 0.05$ for all statistical tests. In the case of significant effects, the effect size of the test was reported. The effect sizes were computed and categorized according to eta squared η^2 (Fisher, 1973).

4.1.3 Results

The results are first discussed with reference to the level of attention and intensity of stereotypies and trainer interventions; secondly, they are analysed with reference to each subscale of GAIRS and to the total GAIRS; finally, the impact on general scales of RARS and VABS is analysed.

Table 3 summarizes means and SD (standard deviation) of attention, intensity of stereotypies, and trainer interventions at the three assessment times, and ANCOVA group- by-phase interaction results after checking for the effects of the covariate RARS. We used a mixed model ANCOVA for repeated measures with a repeated factor phase (T0–pre- intervention baseline, T1–5 weeks, post-test 1, T2–10 weeks, post-test 2), a factor group (experimental, control), and RARS as a covariate.

Table 3
The means and standard deviations of attention, intensity of stereotypies and trainers' interventions.

Measures	Pre-Test (Experimental)	Pre-Test (Control)	Post-Test1 (Experimental)	Post-Test1 (Control)	Post-Test2 (Experimental)	Post-Test2 (Control)	p-value
Attention Time	11.36	12.17	18.82	16.67	29.64	19.17	0.05
Intensity of Trainer Aids	21.91	22.5	10.09	18.83	8.64	16.83	0.0
Intensity of Stereotypies	2.64	2.87	2.91	2.43	3.0	2.86	0.001

Phase interaction showed signifi- cant effects (respectively, $F(2, 46) = 3.68$, $p < 0.05$, $\eta^2 = 0.09$ and $F(2, 46) = 8.91$, $p < 0.01$, $\eta^2 = 1.11$); with reference to trainer interventions, the Group X Phase interaction showed significant effects $F(2, 46) = 7.48$, $p < 0.00$, $\eta^2 = 1.03$. With reference to the intensity of stereo- typies (number of stereotypies that were registered in 5 min), compared with

the pre-test, there were statistically significant differences in post-test 1 and post-test 2 (respectively, $p < 0.001$; $p < 0.001$); Finally with reference to trainer interventions (the number of aids or containment in 5 minutes), compared with pre-test, there were statistically significant differences in post-test 1 and post-test 2 (respectively, $p < 0.001$; $p < 0.001$). These results suggest that patients with RTT showed a positive trend in improving selective attention across time with concomitant decreases of stereotypies and trainer interventions in both groups.

With reference to GAIRS (Table 4), mixed model ANCOVAs for repeated measures with a repeated factor Phase (T0–pre-intervention baseline, T1–5 weeks, post-test 1, T2–10 weeks, post-test 2), a factor Group (experimental, control) and RARS as a covariate were applied.

Table 4
The means and standard deviations of GAIRS' Areas.

Measures	Pre-Test Experimental	Pre-Test Control	Post-Test1 Experimental	Post-Test1 Control	Post-Test2 Experimental	Post-Test2 Control	p
Basic Behaviors	3.58 (0.47)	3.47 (0.59)	4.06 (0.42)	3.72 (0.53)	4.24 (0.27)	3.92 (0.41)	0.01
Neuropsychological Area	1.90 (0.28)	1.72 (0.48)	2.45 (0.40)	2.05 (0.55)	2.86 (0.50)	2.29 (0.66)	0.01
Basic Cognitive Area	2.29 (0.84)	2.06 (0.92)	2.79 (0.74)	2.45 (0.88)	3.22 (0.74)	2.79 (0.88)	0.001
Advanced Cognitive Area	1.00 (0.00)	1.08 (0.18)	1.01 (0.03)	1.10 (0.17)	1.03 (0.07)	1.15 (0.17)	0.08
Communication Area	2.16 (0.44)	1.87 (0.59)	2.60 (0.37)	2.23 (0.69)	2.81 (0.35)	2.37 (0.79)	0.001
Emotional Area	2.88 (0.58)	2.92 (0.74)	3.38 (0.74)	3.25 (0.82)	3.66 (0.76)	3.42 (0.75)	0.01
Hand motor Area	2.82 (0.67)	2.87 (0.56)	3.27 (0.83)	3.20 (0.52)	3.52 (0.78)	3.41 (0.61)	0.01
Graphomotor Area	1.36 (0.41)	1.24 (0.37)	1.33 (0.36)	1.38 (0.36)	1.58 (0.56)	1.48 (0.37)	0.09
Global Motor Area	2.61 (0.32)	3.01 (0.40)	2.85 (0.32)	3.19 (0.30)	2.94 (0.32)	3.26 (0.29)	0.01
Autonomy in Daily Life Area	2.37 (0.41)	2.10 (0.70)	2.42 (0.38)	2.15 (0.74)	2.47 (0.39)	2.17 (0.77)	0.08
Total Score GAIRS	2.29 (0.23)	2.30 (0.44)	2.66 (0.27)	2.51 (0.44)	2.84 (0.26)	2.67 (0.49)	0.01

Regarding the prerequisite area, the factor Phase shows a significant effect, $F(2, 46) = 5.59$, $p < 0.01$ $\eta^2 = 1.05$. The interaction Group X Phase showed no signif- icant effect, meaning that the participants of both groups significantly improved their performances in the basic behavior area, which is characterized by prerequisite behaviors for learning and communication. With reference to the neuropsychological area, the factor Phase showed a significant effect, $F(2, 46) = 4.37$, $p < 0.01$,

$\eta^2 = 0.99$. The interaction of Group X Phase showed no significant effect, meaning that the participants of both groups significantly improved performances in the neuropsychological area, which is character- ized by brain-based skills that are needed in acquisition of knowledge, manipulation of information, and reasoning. Regarding the basic cognitive area, the factor Phase showed a significant effect, $F(2, 46) = 39.04$, $p < 0.001$, $\eta^2 = 0.88$. The interaction Group X Phase showed no significant effect, meaning that the participants of both groups significantly enhanced performances in the basic cognitive area, which is characterized by the basic cognitive concepts that allow the understanding of reality (spatial concepts, topological concepts, etc.).

Regarding the advanced cognitive area, neither the factor Phase nor the interaction showed significant effects; this result may be due to the fact that not all the patients with Rett Syndrome can have access to this area as the basic cognitive area was not reached. Actually, this area evaluates the concepts of school learning that include the sub-areas of writing and mathematics. Regarding the communication area, the factor Phase showed a significant effect, $F(2, 46) = 8.99$, $p < 0.001$, $\eta^2 = 1.23$. The interaction Group X Phase showed no significant effect, meaning that the participants of both groups significantly enhanced performances in the communication area, which evaluates the development of any type of language by measuring responses to environmental sounds and speech, as well as the production of sounds and words. With reference to the emotional area, the factor Phase showed a significant effect, $F(2, 46) = 4.06$, $p < 0.01$, $\eta^2 = 0.88$. The interaction Group X Phase showed no significant effect, meaning that the participants of both groups significantly enhanced their performances in the emotional area, which evaluates a person's abilities to identify emotions and express emotions. With reference to the hand motor area, the factor Phase showed a significant effect, $F(2, 46) = 5.70$, $p < 0.01$, $\eta^2 = 0.88$. The interaction Group X Phase showed no significant effect, meaning again that the participants of both groups significantly enhanced performances in the hand motor area, which evaluates the ability to make movements using the small muscles in hands and wrists.

Regarding the graphomotor area, neither the factor Phase nor the interaction showed a significant effect, this may be due to the fact that not all the patients with Rett Syndrome can have access to this area as it evaluates fine motor skills incorporating graphomotor skills (GS) which, in turn, involve strength and control of finger muscles.

Regarding the global motor area, the factor Phase showed a significant effect, F (2, 46) = 4.33, $p < 0.01$, $\eta^2 = 0.93$. The interaction Group X Phase showed no significant effect, meaning that the participants of both groups significantly enhanced their perfor- mances in the global motor area, which evaluates gross-motor skills which are important

for an upright posture, walking and running.

Regarding autonomy in daily life area, neither the factor Phase nor the interaction shows a significant effect, for some patients with Rett Syndrome it is too hard to reach adaptive and self-help behavior at home, as well as social behavior that develops through early adult-child interactions and therefore the level of autonomy in the praxis of daily life. With reference to the total score of GAIRS, the factor Phase showed a significant effect,

F (2, 46) = 6.87, $p < 0.01$, $\eta^2 = 0.88$. The interaction Group X Phase also showed a significant effect F (2, 46) = 9.87, $p < .001$, $\eta^2 = 1.11$, meaning that the participants of the experimental group significantly enhanced their performances in all areas more than the control group.

With reference to VABS and RARS total scores, Table 5 shows that the scores at the pre-test were significantly different compared with those at post-test 1 and post-test 2 ($p < 0.001$). Only the Phase factor showed significant effects (VABS, F (2, 46) = 9.87, $p < 0.01$, $\eta^2 = 1.01$; RARS, F (2, 46) = 5.99, $p < 0.01$, $\eta^2 = 0.94$). These results indicate an increase in the global functioning in the three phases of treatment and a general decrease in the level of severity of RTT.

Table 5
Means and Standard Deviations of Vineland and RARS total score.

Measures	Pre-Test Experimental	Pre-Test Control	Post-Test1 Experimental	Post-Test1 Control	Post-Test2 Experimental	Post-Test2 Control	p
Vineland Score	98.70 (26.95)	95.25 (4.57)	120.50 (25.23)	97.25 (7.50)	105.60 (26.30)	95.24 (6.44)	0.001
RARS Score	67.70 (5.90)	67.00 (8.80)	65.75 (6.58)	66.30 (9.10)	64.60 (5.80)	65.90 (9.70)	0.01

4.1.4 Discussion

Telerehabilitation (TR) delivers rehabilitation and therapeutic services with the support of diverse technologies. Concerning the field of neurodevelopmental disorders (NDDs), a recent systematic review has demonstrated that TR is an effective tool in improving the adaptive skills of

children with NDDs (Woolf et al., 2016).

Although the use of TR for NDDs has grown rapidly, few studies use TR intervention for children with Rett Syndrome (RTT; Caprì et al., 2020; Dovigo et al., 2020; Iannizzotto et al., 2020; Fabio et al., 2018; Fabio et al., 2018; Fabio et al., 2020; Fabio et al., 2021; Gangemi et al., 2018). The main aim of the present study was to determine whether the use of TR with advanced technology (ATR) in patients with RTT leads to greater improvements in motor and cognitive functions than with the use of basic TR (BTR).

Both the ATR and BTR groups increased time of attention while the educational interventions and intensity of stereotypies decreased. Regarding the results of the GAIRS assessment, it was again shown that both the ATR and BTR groups increased their performances. Since both groups equally benefitted from ATR and BTR, our hypothesis is not confirmed. The advanced system (with eye-tracking and 3D-mapped in real-time) did not empower the performances of the girls and women with RTT any better than the basic system. We think that the explanation may be related to the complexity of RTT; the treatments for these girls and women require a multidisciplinary team, with specialists coming from different areas—medical, psychological, social, educational, and occupational—and are directed toward symptoms and providing support in different areas of daily life.

One of the most important factors in cognitive and motor rehabilitation is the high intensity and frequency of treatment; to reach functional educational goals, frequency is a critical factor (Fabio et al., 2018; Gangemi et al., 2018). In both the ATR and BTR groups, the training frequency was three times a week. The parents were also invited to follow the training with practical exercises as described in the procedure section.

Unlike other studies that were carried out in past years, the results of rehabilitation improvement were also demonstrated by clinical evaluation using the RARS and Vineland Scales. The RARS scale showed a reduction in score that was more marked for the ATR group, which is equivalent to a reduction in the severity of the syndrome, and the Vineland scale emphasized an improvement in the overall functioning of the participants at the end of the training. These results show a generalization of skills that are learned outside the purely rehabilitative field, as demonstrated by GAIRS (Fabio et al., 2021).

The results show that the improvements have been progressive and continuous throughout the project, as demonstrated by the results in post-test 1 and post-test 2 for the GAIRS, RARS, and Vineland scores, where the improvement of the parameters was more marked in post-test 2.

These results should be interpreted in the light of a number of limitations. The present study involved 20 subjects, which is not a small group for RTT, given that RTT is a rare disorder; but caution is required in the interpretation of results for potential problems in generalization. In addition, we didn't use a waiting group; it might be worthwhile having a waiting group who are ready and who, therefore, would not require training. Given that learning times in cognitive and motor disabilities are longer compared to typically developing subjects, it is important to set up a longer training period and include follow-up phases to show maintenance of the results in the medium- and long-term.

4.1.5 Conclusions

The main aim of the present study was to determine whether the use of TR with advanced technology (ATR) in patients with RTT leads to greater (or at least equal) improvements in motor and cognitive functions than basic TR (BTR).

The results indicated that telerehabilitation is an effective mode of rehabilitation for Rett syndrome as it allows high intensity and high frequency interventions, which has proven to be the most critical factor. The results did not reveal a substantial difference between the two groups with regard to the overall rehabilitation prospects that were assessed through GAIRS and the overall functioning of the participants that was assessed through RARS and Vineland; however, a more marked increase was seen for the ATR group compared to the BTR group for behavioral parameters with an increase in sustained attention to the task and a reduction in educational interventions and stereotypies. The results demonstrate an important aspect of generalizing the skills that are learnt from training, as there have also been increases in RARS and Vineland scores.

As stated in the introduction, few studies have used TR in patients with RTT and no study has compared the effectiveness of basic and advanced TR in this population, so future research is necessary to better understand the characteristics of effective TR interventions in these patients and to determine how these characteristics may differ for specific populations and outcomes.

INTRODUCTION TO EXPERIMENTAL STUDIES 2,3 AND 4

Experimental studies 2, 3 and 4 focus on the integration of virtual reality as a tool in motor rehabilitation for RTT. Before delving into the two research projects, a general overview of the use of Virtual Reality (VR) in the rehabilitation of Rett syndrome and similar conditions is provided below.

Among the new technologies in the rehabilitation field there is the VR, whose potentialities are known and discussed in literature since a long time. The effectiveness of VR emerged from studies in literature is precisely linked to the immersiveness of learning that it allows and determines. VR is not an out-of-body experience (Cho, Hwangbo, & Shin, 2014). Rather, when taking part in VR, patients are provided with visual and auditory feedback generated while they are performing specific tasks presented in the form of games. The feedback enables them to start efficiently controlling their movements (Hohmann, Paluch, Krueger, Meis & Grimm,2020). Recent studies show that the association of traditional rehabilitation programs with the VR tool leads to a greater effectiveness of rehabilitation (Ferreira dos Santos, Christ, Mate et al., 2016); this happens because one of the main problems of traditional rehabilitation is the lack of motivation on the part of the participant. VR allows inserting rehabilitation in a game environment and therefore to increase the participation of patients in rehabilitation programs not only in the short term but also in the long term (Kaplan, Cruit, Endsley et al., 2021). Mantovani and colleagues (Mantovani, Zucchella, Bottiroli, Federico, Giugno, Sandrini & Tamburin 2020) examine the key concepts that define VR: immersion (i.e., the extent to which the user perceives himself in the virtual environment rather than the real world), sense of presence (i.e., the subjective experience of the user as being in the virtual world), and the possibility to interact with the computer-generated environment.

The advantages of VR can be summarized (Mantovani et al., 2020: a) provides immediate feedback, b) allows the adaptation to patient's performance, c) highly engaging, high level of ecological validity, d) it can be combined with other tools/devices (e.g., electroencephalography, physiological activity registration tools).

The use of VR allows for a reproducible, objective assessment of cognitive processes underlying attention, memory, information processing, logical sequencing, and problem-solving (Ventura, Brivio, Riva & Baños, 2019; Zhang, Abreu, Masel, Scheibel, Christiansen, Huddleston &

Ottenbacher, 2001). VR also provides a safe environment in which to assess skills that might be too dangerous or risky to perform in the real world and the tested subjects are able to make mistakes without suffering the real consequences (Zhang, Abreu et al., 2001) the stimulating effect of VR on the human mind is highly beneficial for cognitive rehabilitation. In multi-disabilities' picture often display impairments of attention, memory, affectivity, behavior, planning, or executive functions (Maggio et al., 2019).

Significant improvements in learning following a VR exercise program are thought to be associated with changes in neuronal plasticity that enhance the working memory (Maggio et al., 2019). VR also significantly increases cognitive flexibility, shifting skills, and selective attention, leading to better behavioral outcomes in brain-injured patients. Improvement in selective memory processes and problem-solving skills facilitate social reintegration and leads to better vocational outcomes (Man, Poon & Lam, 2013).

VR provides a safe, controlled environment for performing customizable, engaging rehabilitation activities that promote learning of motor skills (Aida, Chau & Dunn, 2018) too. Furthermore, because VR is fun and enjoyable, it motivates children to participate in the rehabilitation interventions (Shen, Xiang, Luna, Grishchenko et al, 2020). The therapeutic effect of VR can be easily combined with computer assisted cinematic analysis of motor deficits (Bortone, Barsotti, Leonardis et al., 2020). This allows for reliable documentation of the degree of motor impairment in brain-injured patients undergoing rehabilitation therapy. Because the virtual environments are highly interactive, they strongly activate visual, vestibular, and proprioceptive systems during the execution of a virtual task, such as playing a video game. The main therapeutic effect of VR on upper limb motor activity is to increase the active range of motion (AROM) of the shoulder, elbow, and wrist (Georgiev, Georgieva, Gong et al., 2021). The most important feature of VR interventions, however, is that the improved upper limb motor function recovers activities of daily living (ADL) of brain-injured patients and enhances their quality of life (Jeon, Moon & Cho, 2019). Multiple disabilities may have impairments in the gait, maintenance and adaptation of balance, or postural control for a range of activities of daily living (Soares, Bacha, Mello et al., 2020; Beani, Filogna, Martini et al., 2022). Because the working load on the lower limbs during walking also includes support of the person's body weight, gait rehabilitation is greatly assisted by

robotic devices, which allow a smaller workforce and a longer exercise session with greater intensity compared to traditional treatment (Maggio et al., 2020).

Only one study (Mraz, Eisenberg Diener et al., 2016) experienced the use of VR for rehabilitation purposes with Rett patient. Mraz et al. (2016) demonstrated that a 12-week VR intervention using body-controlled games improved upper limb functionality, increased self-care abilities, and reduced stereotypical hand movements in patients with Rett syndrome. The approach was engaging and adapted to their cognitive levels.

Given the limited literature in relation to the use of technologies by patients with RTT syndrome, the following studies are reported in relation to the use of VR in subjects with different pathologies but who share with Rett Syndrome clinical and rehabilitative aspects in the motor and cognitive picture.

For example, many studies report research with children with cerebral palsy (PCI). Although PCI and RTT have different etiology and trends, they share some characteristics such as the presence of epileptic pictures, development of scoliosis, motor impairment, dyspraxia and intellectual disability with learning difficulties (Sadowska, Sarecka-Hujar & Kopyta, 2020). The study of Aran and colleagues (2020) had the aims to evaluate the effectiveness of VR-based rehabilitation program for children with hemiplegic cerebral palsy on cognitive functions. Ninety children with hemiplegic cerebral palsy were randomized to either study or control groups. The study group received VR intervention in addition to Traditional Occupational Therapy intervention, and the control group received Traditional Occupational Therapy for 20 sessions. Both groups were evaluated by blinded assessors with Dynamic Occupational Therapy Cognitive Assessment for Children to collect information on cognitive functioning. Both groups' cognitive functions were improved after 10 weeks of interventions. The between-group comparison revealed significantly greater improvements in all subtests of cognitive functions in the VR group than in the Traditional Occupational Therapy group. Another study (Cho, Hwang, Hwang, & Chung, 2016) investigated the effects of treadmill training with VR on gait, balance, muscular strength, and gross motor function in children with CP. Eighteen children with spastic CP were randomly divided into the VR treadmill training (VRTT) group and treadmill training (TT) group. The groups performed their respective programs as well as conventional physical therapy 3 times/week for 8 weeks. After

training, gait and balance was improved in the VRTT compared to the TT group, Muscular strength was significantly greater in the VRTT group than the TT group, except for right hamstring strength. The improvements in GMFM scores were greater in the VRTT group than the TT group. In conclusion, that study underlines that VRTT programs are effective for improving gait, balance, muscular strength, and gross motor function in children with CP. Another recent study (Chang, Ku, Park et al., 2020) investigated the effects of the use of VR on the motility of the upper limb in children with PCI. In that study, ten children received VR-based rehabilitation, which utilized RAPAEL Smart Kids and video games combined with conventional occupational therapy (COT). Seven children received COT alone, which was provided by a trained occupational therapist and focused on their upper extremities. Clinical outcomes were determined using the Quality of Upper Extremity Skills Test (QUEST) and Pediatric Evaluation of Disability Inventory (PEDI), which were administered before and 8 weeks after the first intervention session. Results of that training showed that VR group showed significant improvements in all QUEST domains and five PEDI domains, whereas the COT group showed a significant change only in total QUEST scores. A comparison between both groups revealed that the VR group had significantly greater improvements in five QUEST domains and two PEDI domains. Studies have also been carried out on patients with Stroke. Stroke (Wang, Reps, Kostka et al., 2020) is the leading cause of adult disability, with 65% of the nearly four million people in the United States who have survived a stroke living with minor to severe impairments; it is different for ethology and progression, but it is characterizing by impairment of the overall area of attention, concentration, memory, spatial processing skills, language, problem-solving skills, and planning skills. Such alterations are common to Rett syndrome. A study (Kim, Chun., Kim, & Park, 2011) involved twenty-eight patients with cognitive impairment following stroke. The VR group received both VR training and computer- based cognitive rehabilitation, whereas the control group received only computer-based cognitive rehabilitation. To measure, activity of daily living cognitive and motor functions, the following assessment tools were used: computerized neuropsychological test and the Tower of London (TOL) test for cognitive function assessment, Korean-Modified Barthel index (K- MBI) for functional status evaluation, and the motility index (MI) for motor function assessment. The VR group showed greater improvements than the other group, in visual and auditory continuous

performance tests (CPT), forward digit span test (DST), forward and backward visual span tests (VST), visual and verbal learning tests, TOL, K-MBI, and MI scores. Another condition that shares rehabilitative aspects common to Rett syndrome is multiple sclerosis. Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disorder of the central nervous system which progresses clinically over time but with a variable course (Rojas, Romano, Patrucco, & Cristiano, 2018). The ethology and progression are different but the symptoms that characterize the daily functioning of these people are partly in common with the RTT; these aspects are double vision, blind- ness in one eye, muscle weakness, trouble with sensation, fatigue, and paresthesia or motor coordination impairments (Noseworthy, Wolinsky, Lublin et al., 2000). Ultimately, the study of Ebrahim et colleagues (Norouzi, Gerber, Pühse, Vaezmosavi & Brand, 2021) examined the effectiveness of three interventions for learning a bimanual coordination task: VR training (VRT), conventional physical training (CPT), and the combination of VRT and CPT (COMB). A total of 45 women with MS were randomly assigned to one of the following study conditions: VRT, CPT or COMB. Bimanual coordination was assessed at baseline, eight weeks later at study completion, and 4 weeks after that at follow-up. Bimanual coordination improved over time from baseline to study completion and to follow-up. Compared to the VRT and CPT conditions, the COMB condition led to higher coordination accuracy and consistency. The combination thus appears to have the potential to speed up the recovery of motor control and rehabilitation of women with MS.

Given that the use of VR-based interventions for motor rehabilitation in patients with RTT is limited, and, since VR has been demonstrated to be effective in the improvement motor skills activity and functions of patients with neurological diseases (Georgiev et al., 2021), it becomes necessary to perform specific research with VR technological intervention for RTT.

4.2 Experimental Study 2: Effects of Immersive Virtual Reality with Treadmill in Subjects with Rett Syndrome: A Pilot Study

Gross motor function in RTT is consistently limited (Stahlhut, Downs, Leonard et al., 2017). During the regression phase, patients typically develop coordination deficits and instability, manifesting as uncontrolled movements of body segments and partial loss of trunk and balance control (Hagberg, 2002; Katz, Bird, Coenraads, Gray et al., 2016; Lee, Leonard, Piek & Downs, 2013; Neul, Kaufmann, Glaze et al., 2010; Leonard, Cobb & Downs, 2017).

Residual gross motor functions are often maintained into adulthood, with most patients able to walk with support and nearly half capable of walking independently or with minimal support (Monteiro, Savelsbergh, Smorenburg et al., 2014; Downs, Bebbington, Jacoby, et al., 2008). However, ambulation is frequently compromised by apraxia, fluctuating muscle tone, and poor coordination. More complex motor skills, such as climbing or descending stairs, are typically underdeveloped, with only a small percentage of patients able to perform these tasks independently. Postural changes are particularly challenging due to apraxia, although more than half of patients can sit and stand from a chair, and slightly fewer are able to get in and out of bed without using their arms (Downs et al., 2008).

Studies have documented a decline in motor function quality and level from around age 13, with a progressive increase in the need for support to complete motor tasks (Downs et al., 2008). Muscle tone fluctuations, often beginning in late childhood (Lotan, 2006), are frequently accompanied by the onset of parkinsonian rigidity (Humphreys & Barrowman, 2016). The variability in the severity of motor disability, neurological patterns, and overall impairment in RTT is striking, with individuals presenting diverse clinical profiles (Hagberg, 2002).

Musculoskeletal abnormalities are a common complication in RTT, often associated with neuromuscular impairments. Spinal and foot deformities are prevalent, although other joints may also be affected (Smeets, Pelc, & Dan, 2012). A large study of 909 females with RTT, aged 1–66 years, reported scoliosis in 80% of subjects over the age of 13 (Killian, Lane, Lee et al., 2017).

Considering walking abilities, Layne and colleagues (Layne, Lee, Young et al., 2018) observed that freezing, veering, foot dragging, parkinsonian shuffling, antepulsion or retropulsion, and hand stereotypes characterize overground and treadmill walking of subjects with RTT. Isaias

(2014) also underlined that subjects with RTT show a medium-lateral displacement during gait initiation, with a low activation of dorsiflexor of the ankle, a wide base of support, and compensatory rotational movements of the trunk. The scarce representation of movement and the lack of feedforward control may be associated with sensory integration alterations that may impact the walking function (Downs et al., 2015; Drobnyk et al., 2019). Lifelong motor rehabilitation is fundamental in subjects with RTT to maintain and improve their abilities and their quality of life through all the phases of the syndrome.

According to a systematic review by Fonzo et al. (2020), the interventions performed for patients with RTT include education, traditional physiotherapy, environmental enrichment, hydrotherapy, treadmill training, sensory-based treatment, computerized systems, and music therapy. Specific balance exercises, eye/feet coordination, walking on different surfaces, and crossing obstacles are some of the goals in motor rehabilitation (Isaias, Dipaola, Michi et al., 2014). Regarding gait training, there is some evidence that walking on a treadmill may improve physical and aerobic fitness in subjects with RTT (Layne et al., 2021; Lotan et al., 2004; Larsson, Julu, Engerström et al., 2018). In these studies, kinematic data were not reported.

As described in the introduction to the experimental studies, since the advent of virtual reality (VR), it has been extensively applied in the field of neurorehabilitation. The primary reason for using VR lies in its ability to encourage and motivate subjects to participate in rehabilitation processes, allowing them to train in a safe environment that enables repeated learning trials (Dvorkin, Shahar & Weiss, 2006). When combined with treadmill training, VR has increased engagement and provided enhanced feedback, improving motor function, movement kinematics, quality of life, and participation in children with cerebral palsy and adults with chronic stroke (Cho et al., 2016; Gagliardi, Turconi, Biffi et al., 2018).Considering the feasibility of using a treadmill with RTT and the engaging capabilities of VR, in this study we decided to assess if a short treadmill training coupled with VR activity is feasible in subjects with RTT. Specifically, the main aim of the present study was to investigate (1) the suitability of the system and the motivation of the patients during the training and (2) the motor responses of subjects with RTT in a setting of immersive virtual reality treadmill walking.

4.2.1 Materials and Methods

4.2.1.1 Participants

Subjects affected by RTT were recruited through the Italian Association of Rett Syndrome (AIRETT). Inclusion criteria were diagnosis of RTT and ability to walk autonomously or with a support (Rett Syndrome Gross Motor Scale, Item 8 from 1 to 3 points). Exclusion criteria were inability to walk and deep cognitive disability with absence of cause–effect understanding, according to therapists with expertise on Rett syndrome. The protocol was approved by the ethics committee of Scientific Institute Medea (protocol N. 72/21-CE approved on the 3 August 2021) and conducted in accordance with the Declaration of Helsinki. Participants' parents or caregivers provided written informed consent. The trial was registered on clinicaltrials.gov (NCT05691582).

4.2.1.2 GRAIL System

The GRAIL is a system developed for gait analysis and rehabilitation in a semi immersive VR environment. It is composed of a treadmill with two belts that can be driven at different speeds (0.1 to 3.6 Km/h) and equipped with two force platforms. The system integrates a motion platform that allows movements of antero-posterior pitch and lateral sway. To guarantee the patient's safety, it is also equipped with two handrails and a harness for body weight support. The treadmill is surrounded by a 180◦ screen, on which it is possible to project different scenarios and visual feedback. The GRAIL includes a Vicon motion capture system (10 optoelectronic cameras, sample frequency 100 Hz) and 3 video cameras to perform motion detection and gait analysis, and this allows the integration of multi-sensorial feedback (visual, proprioceptive and auditory) during the training

4.2.1.3 Study Design

Participants underwent an initial assessment of the cognitive and motor aspects using the Modified Adjusted Raven's Colored Progressive Matrices, the Rett Assessment Rating Scale (RARS) and the Rett Syndrome Gross Motor Scale (RSGMS). These baseline assessments were performed by an experienced therapist prior to the access to the GRAIL. The project consisted of 4

sessions of GRAIL activities in 1 week. During the first 3 sessions, exergames and augmented feedback activities were proposed. During the fourth session a gait analysis was conducted for the subjects with a suitable motor and behavioral level. In every session, before the beginning of GRAIL activities, an adaptation time of five minutes was proposed in order to allow an acclimatization to the GRAIL environment. Moreover, a further adaptation time was conceded anytime the exercise changed, according to the need of each subject. During all the sessions, a video recording was conducted to detect facial expression of the subjects, in order to use these data to compute the "happiness index". The exergames were administered until the therapists detected any sign of discomfort by the subject. The exercises to be used during the sessions were defined by two therapists that have experience with GRAIL training and two therapists with expertise on Rett syndrome. The protocol included 4 exergames that were administered every session to all the subjects. The order of execution of the activities changed at every session. The chosen exergames had different aims, as follows:

APP1—INTERACTION WITH VIRTUAL REALITY: this application aims to verify the ability to deal with the visual feedback and to use it to interact with the exergame. This game (licensed by Motek with the name "MM Christmas 2014") is a simulation of a slalom between snowmen. Subjects wear virtual skis and must move their center of mass (detected by passive reflective markers located on the pelvis) on the platform to interact with the environment. The treadmill is stationary. Particularly, they have to move their pelvis forward to increase the speed or backward to reduce it, while they have to shift their pelvis right or left to let the skis slide correspondingly. From the therapist console it is possible to set different task difficulties, modifying the distance between each snowman or adapting the sensitivity to the movement of the patient in order to increase or reduce the speed (Figure 7a). APP2—WALKING: this application (licensed By Motek with the name "MM Christmas 2012") was chosen to promote engagement during linear walking. In this exergame, subjects are immersed in a snowy wood, with joyful background music. During this game it is possible to set different speeds, according to participant abilities (Figure 7b). APP3—DYNAMIC BALANCE: this application aims to stimulate the capacity to modulate gait characteristics of participants in relation to the slope they encounter. In this application (licensed by Motek with the name "rope bridge") the subjects walk over a rope bridge. According to the capacity

shown by the patient, the therapist can set different walking speeds and different degrees of slope. Moreover, it is possible to deliver lateral sway perturbation, simulating blowing wind, in order to train the ability of the subject to react to external stimulations (Figure 7c). APP4—STATIC BALANCE: this application, developed at IRCCS Medea, aims to stimulate the balance reaction of the subject to external perturbations. The game presents a marine environment in which the subject must stand over a boat (projected on the GRAIL platform) rolling over the waves of the sea. During this exercise, the platform pitches forward and backward, with a movement amplitude related to the height and speed of the waves, as set by the therapist (Figure 7d). Exercise parameters were customized regarding the patients' engagement and need, modifying speed, inclination and difficulty of the exercise.

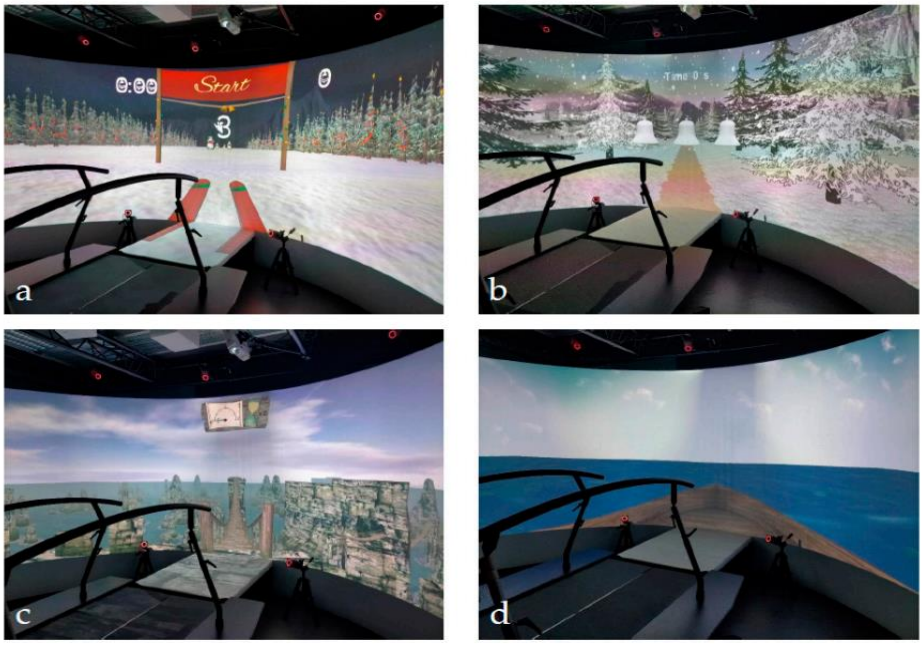


Figure 7. GRAIL environments. (a) APP1, interaction with virtual reality; (b) APP2, walking; (c) APP3, dynamic Balance; (d) APP4, static balance.

4.2.1.4 Baseline Evaluation

At baseline, the following data were collected.

- Rett Syndrome Gross Motor Scale (RSGMS) (Downs, Stahlhut, Won et al., 2016). This scale aims to assess gross motor abilities in individuals with RTT. It has 15 items, subdivided into three subscales (sitting subscale, standing and walking subscale and

challenge subscale) and includes abilities such as sitting, standing, transitions, walking and going up and down the stairs. The score is assigned according to needed assistance to complete every action, from 0 (meaning no assistance) to 4 (meaning maximum assistance or not being able to complete the action). RSGMS has demonstrated strong internal consistency both for the total score and for each subscale (Cronbach's alpha coefficient for total score = 0.96, for sitting subscale = 0.83, for standing and walking subscale = 0.97, for challenge subscale = 0.85) and a high repeatability (correlation coefficient = 0.99, 95% CI 0.93–0.98).

- Modified Adjusted Raven's Colored Progressive Matrices (Burke, 1958) was used in this study to assess general cognitive abilities of the participants. Only Series A was administered to the subjects, and the items were placed separately in front to the subjects. Every item was presented three times to every subject, randomizing position of the target. The subsequent table was presented after two correct consecutive answers. The test was interrupted if the subject gave three wrong answers. The score of the test was achieved by summing the number of right answers for each item
- Rett Assessment Rating Scale (RARS) (Fabio et al., 2005) is an RTT-specific scale that aims to evaluate the severity of the syndrome. It includes 31 items, which cover many aspects of the clinical features, such as behavioral, emotional, motor and communication aspects, and investigates in depth the presence and intensity of the main characteristics of RTT, such as anxiety, epilepsy, breathing dysregulation, bruxism and muscular tone. Every item can be rated from 1 (within normal limits) to 4 (strong abnormality). It showed high internal consistency for both total and subscale rates (Cronbach's alpha coefficient for total score = 0.91, for the subscales = 0.81–0.93).

4.2.1.5 Outcome Measures

The following outcome measures were collected during the three sessions of training (hereinafter, T1, T2 and T3): happiness index, endurance time, performance speed and attention focus on scenario. At the end of the training, parents were asked to fill in a modified version of the

Suitability Evaluation Questionnaire (SEQ) and the patients underwent a 3-dimensional gait analysis if possible.

- Happiness Index. This parameter is derived from Van der Maat's taxonomy (Van der Maat, 1992) which contains an analysis of the behavior of people with severe intellectual disabilities. Only seven behaviors out of the twelve main categories of the taxonomy of Van der Maat were taken into account. The behaviors were: gaze direction, sounds, mouth movements, physiological reactions and hand gestures. They were recorded by a camera in front of the subjects. Scores were assigned as 0 if the parameter (behavior) was not present in the record and as 1 if it was present. The overall score of the happiness index was defined as the sum of the scores. This assessment was performed during every session (i.e., at T1, T2 and T3).
- Endurance Time. During all the sessions, the time of execution of each exergame was registered. The activity was stopped when the participant showed discomfort or excessive inquietude, according to the experimenters.
- Performance Speed In APP2 and APP3, during which subjects had to walk over the treadmill, we recorded the maximum speed it was possible to reach. Performance speed was measured at T1, T2 and T3.
- Attention Focus on Scenario. The number of seconds of attention on each scenario was measured through the video recording analysis. The attention span started when the subject looked at the object of focus requested by each APP and continued until the subject looked away from the object and stared into space, as previously performed (Fabio, Semino, Giannatiempo et al., 2022). The type of requested action for each APP is described in the study design section. Suitability
- Suitability Evaluation Questionnaire (SEQ). At the end of the training, parents were asked to complete a modified version of the Suitability Evaluation Questionnaire (SEQ). Particularly, we modified the SEQ developed by Gil-Gómez and colleagues (Gil-Gómez, J. A., Gil-Gómez, H., Lozano-Quilis et al., 2013) to allow the parents to assess the suitability of the GRAIL system for the subjects. SEQ was indeed developed by Gil-Gómez and collaborators to assess usability, acceptance and

security of use of VR environments/devices. It includes 13 questions rated as a 5-point Likert scale and 1 open question. The SEQ assesses enjoyment, sense of being in the system, feeling of success and control, realism, easy-to-understand instructions and general discomfort of the system. Furthermore, the SEQ measures specific symptoms related to cybersickness such as nausea, eye discomfort and disorientation. Finally, one question assesses the perceived usefulness of the GRAIL system for patient rehabilitation. The total SEQ score ranges between 13 and 65 (excellent suitability).

4.2.1.6 Gait Analysis

On the last day of training, a gait analysis was conducted when it was considered feasible by experimenters. The criterion to access to this assessment was that the subject had to show a minimum of 5 min of autonomous walking. Subjects could use their hands to hold or reach the support, but no physical interactions with other persons was allowed during this period. To accomplish the analysis, 26 passive reflective markers were located over the patient according to the model proposed by Vicon (HBM2 trunk and lower limb) for the analysis of lower limb and trunk movements. For their safety, all the subjects had to wear the harness provided by the GRAIL manufacturer. The recordings were executed after a period of adaptation to the movement of the treadmill of at least 3 min. An adaptation time of 10 min is considered to be more reliable, but considering the reduced attention and walking resistance shown by these patients, it was decided to require a shorter adaptation period. For every subject, at least 4 blocks of gait acquisition were collected, each consisting of a minimum of 20 steps. In this study, the final gait acquisition was considered for further data analysis since it was evaluated as the most reliable. Gait data were extracted by using the Gait Offline Analysis Tool (GOAT by Motek). GOAT synchronizes 3D motion capture, force plate data and video recordings that are displayed, allowing identification of reliable steps. GOAT filters GRAIL data with a lowpass 2nd order Butterworth filter, with a cut-off frequency equal to 6 Hz, and performs gait event detection and standardized step selection. A visual inspection was then performed to eliminate incorrect steps. Specifically, a step was eliminated if the foot is located both on the left and the right belt of the treadmill, preventing a correct detection of

forces. Finally, GOAT normalizes the steps on 100 samples, returning a single value for the space–time parameters and time series for kinetic and kinematic parameters.

4.2.2 Statistical Analysis

Data were analyzed using SPSS version 24.0 for Windows. The descriptive statistics of the dependent variables were computed as median and interquartile range (IQR). Considering the small sample size, a non-parametric analysis was performed. Accordingly, the Friedman test was used to test if scores of parameters (happiness index, endurance time, performance speed and attention focus on scenario) were significantly different among the three VR environments (APP2, APP3 and APP4) and among the three sessions (T1, T2 and T3). Alpha level was set equal to 0.05. If the Friedman test highlighted statistically significant differences, a post hoc analysis with the Wilcoxon paired test with Bonferroni correction was used (considering p-values < 0.016 as significant).

4.2.3 Results

Nine female subjects (age range 6–44 years old, median (IQR) = 16 (4)) were recruited according to the inclusion criteria. Data are reported in Table 6. As shown in Table 1, the patients had a level of severity of syndrome from severe to mild (RARS total scores), with a median (IQR) value of 60.5 (8.5), meaning that they showed severe physical, language and social impairments. This clinical feature was expected because RTT is a severe disorder associated with deficits in several domains. With regard to motor functioning, it was moderately associated with the degree of severity of RTT (according to the RSGMS total scores). All participants performed the three training sessions (T1, T2 and T3) in one week according to the protocol.

Table 6
Characteristics of participants.

Participants	Age	Level of Severity[1]	RARS Total Scores[2]	RSGMS Total Scores[3]	Raven Total Scores[4]
P1	11	Moderate	57	9	0
P2	17	Mild	52.5	37	1
P3	16	Moderate	54	32	3
P4	16	Moderate	60	29	2
P5	44	Mild	53.5	30	1
P6	14	Moderate	68	32	1
P7	18	Moderate	57.5	34	3
P8	35	Moderate	61	32	2

P9	6	Severe	84	35	1

After the first training, it was decided to remove APP1 from the list of the exergames. This app was selected to assess the ability of subjects to understand the motor request and to interact with the system by moving their body left or right to perform the exergame. Unluckily, this task was too difficult for the subjects and, when this game was not conducted properly, the visual biofeedback was too confusing. All parents completed the SEQ. Gait analysis was possible only for four subjects. The following sections show results related to outcome measures, i.e., happiness index, endurance time, performance speed, attention focus on scenario (Table 7), SEQ and gait analysis.

Table 7

Median (M) and interquartile range (IQR) of happiness index, endurance time, performance speed and attention focus for each scenario in the three training sessions.

Parameters	Scenario	T1 M (IQR)	T2 M (IQR)	T3 M (IQR)
Happiness index[1]	APP2	4 (2)	4 (2)	3 (2)
	APP3	3 (2)	3 (1)	3 (0)
	APP4	2 (1)	2 (1)	3 (1)
Endurance time (s)[2]	APP2	180 (160)	280 (180)	240 (180)
	APP3	240 (230)	180 (80)	240 (90)
	APP4	300 (210)	300 (120)	300 (60)
Performance speed (m/s)[3]	APP2	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
	APP3	0.3 (0.1)	0.4 (0.1)	0.4 (0.1)
Attention focus (s)[4]	APP2	120 (125)	91 (118)	206 (198)
	APP3	54 (154)	160 (148)	195 (175)
	APP4	120 (93)	105 (92)	165 (90)

[1] Presence or absence of seven behaviors related to Van der Maat's taxonomy (min. 0–7 max). [2] Time of execution of each exergame measured in seconds. [3] Meters per second. [4] Attention time on each scenario measured in seconds.

Happiness Index

The happiness index scores significantly differed in the three environments ($p = 0.013$, Friedman test). Particularly, there were statistically significant differences in the level of happiness index between APP3 (dynamic balance) and APP4 (static balance) ($p = 0.014$) with participants showing more happiness-promoting behaviors in the bridge scenario proposed in APP3. In general, the snow scenario of APP4 was the one with lower values of the happiness index. With reference to happiness index scores observed during T1, T2 and T3 for each scenario, there was only a trend in the waves scenario proposed in APP4 ($p = 0.066$, Friedman test). This means that only in APP4 is it possible to observe a positive trend of happiness-promoting behavior among the three sessions.

Endurance Time

The Friedman test did not show statistically significant differences among environments in terms of endurance. However, there is a trend in data suggesting that participants performed the scenario of APP4 for a longer time, before showing discomfort signs, with respect to the other environments. There were no differences among T1, T2 and T3 for all the three applications.

Performance

Speed Considering the performance speed for APP2 and APP3, no significant difference was found. This means that participants walked on the treadmill in these two scenarios using similar speed. Considering the sessions, the Friedman test highlighted a statistically significant difference for APP3 ($p = 0.009$). The comparison between time points with the Wilcoxon test resulted in $p = 0.042$ (T1 vs. T2), $p = 0.027$ (T1 vs. T3) and $p = 0.498$ (T2 vs. T3) suggesting that performance speed increased after T1, although p-values were not statistically significant considering the Bonferroni correction ($p < 0.016$). Attention Focus

With reference to the attention focus on each scenario, no significant difference was found among them. This means that participants looked at each scenario for similar numbers of seconds. With reference to attention focus scores observed at T1, T2 and T3 for each scenario, there were statistically significant differences for APP3 ($p = 0.005$, Friedman test) and APP4 ($p = 0.013$, Friedman test) among sessions. Regarding APP3, T1 differed from T3 ($p = 0.021$, Wilcoxon test). Considering APP4, differences between T1 vs. T3 and T2 vs. T3 (Wilcoxon: $p = 0.021$ and $p = 0.028$, respectively) were observed. Although p-values were not statistically significant according to Bonferroni correction, these results suggest that attention focus improved over time.

SEQ

Parents reported a median SEQ score of 56 (IQR = 13) (range 44–62) (median normalized suitability score of 0.82 (IQR = 0.2)), highlighting a good suitability of the GRAIL system according to parents' opinion. Particularly, patients enjoyed the system (Q1, median (IQR) = 5 (2)), they did not have nausea or dizziness (Q8, median (IQR) = 1 (0)) and were not confused or disoriented (Q10, median (IQR) = 1 (1)). Parents thought that GRAIL could be helpful for their daughters' rehabilitation (Q11, median (IQR) = 4 (2))

Gait Analysis

Considering the four patients that were able to perform the gait analysis, they showed different gait patterns, as shown by kinematics graphs (Figure 2) and spatio-temporal and kinematic data reported in Table 3, but with some common aspects: as a whole, the group analyzed seems to show an increased stiffness, a marked reduction in the ankle range of motion (ROM) and a lack of plantar flexion during the push-off phase.

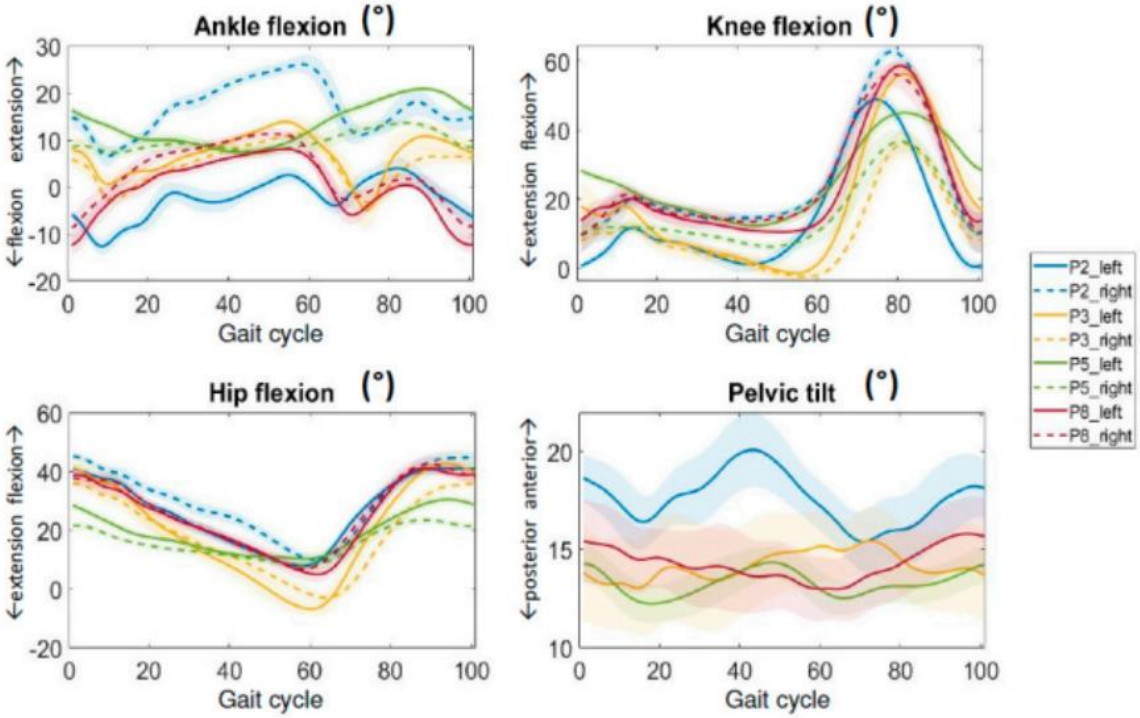


Figure 8. Gait analysis during flat walking over the GRAIL. Ankle, knee, hip and pelvis kinematics in the sagittal plane are reported. Blue lines refer to P2, yellow lines to P3, green ones to P5 and red ones to P8. Continuous lines refer to the left side while the dashed ones to the right side. The lines show the mean values of the steps collected while the colored shadows represent the standard deviation ofthe curves.

Table 8.
Mean and standard deviation (M ± std) of spatio-temporal and kinematic parameters of each participant performing gait analysis over the GRAIL (P2, P3, P5 and P8) are reported. The last column reports the median values and interquartile ranges of spatio-temporal and kinematic parameters considering all participants and legs. R: right (leg); L: left (leg); ROM: range of motion; IQR: interquartile range; ◦ degrees.

Gait Parameter	Side	P2	P3	P5	P8	Median (IQR)
Walking speed		0.66 ± 0.03	0.50 ± 0.02	0.25 ± 0.02	0.50 ± 0.04	0.50 (0.10)
Stance phase %	R	79.59 ± 1.45	73.79 ± 1.43	71.20 ± 2.36	67.50 ± 1.99	70.77 (3.27)
	L	66.43 ± 1.24	71.92 ± 2.30	70.34 ± 1.98	69.65 ± 1.72	
Stride time (s)	R	1.31 ± 0.05	1.57 ± 0.06	0.88 ± 0.07	1.35 ± 0.07	1.33 (0.20)
	L	1.32 ± 0.05	1.57 ± 0.06	0.87 ± 0.06	1.34 ± 0.08	

Step length (m)	R	0.42 ± 0.05	0.39 ± 0.02	0.12 ± 0.02	0.36 ± 0.04	0.37 (0.13)
	L	0.44 ± 0.03	0.39 ± 0.04	0.10 ± 0.02	0.31 ± 0.03	
Ankle ROM °	R	20.22 ± 3.21	15.63 ± 1.65	7.15 ± 1.36	21.48 ± 2.19	18.85 (5.82)
	L	17.48 ± 2.16	20.82 ± 4.51	13.76 ± 1.06	21.54 ± 1.90	
Knee ROM °	R	55.87 ± 3.42	38.99 ± 3.42	31.38 ± 3.33	49.21 ± 4.10	49.24 (13.93)
	L	49.98 ± 3.26	58.59 ± 3.76	33.10 ± 2.12	49.28 ± 1.86	
Hip ROM °	R	36.64 ± 2.05	40.04 ± 1.87	16.24 ± 1.98	36.04 ± 2.46	36.25 (6.29)
	L	34.62 ± 2.66	51.54 ± 3.81	20.94 ± 1.82	36.46 ± 1.70	

P2 was the subject who could reach the fastest speed amongst the analyzed group. In fact, P2 could walk at a mean speed of 0.66 m/s, with a mean stride length of 0.87 m. We measured 33 steps. P2's gait pattern was characterized by a marked asymmetry between the left and right lower limbs, as reported in Table 8. P2 used the right side more as a support and the left side for advancing, as evidenced by the comparison between the 79% of stance phase on the right side and 66% on the left. Correspondingly, from the kinematics graphs detailed in Figure 8, it is possible to notice that the right side was more flexed, both at the hip and the knee, and the ankle more dorsiflexed during the whole gait cycle. In contrast, the left side remained more extended, and it was characterized by stiffness of the knee during the swing phase and ankle plantarflexion throughout the cycle of gait. This is further confirmed by the difference in the ROM data between left and right lower limbs. P3 walked with a mean speed of 0.5 m/s and a mean length of the stride of 0.77 m. We measured 33 steps. Kinematics data show a reduction in the ROM of all the sections of the two lower limbs (consistent with a global stiffness), but it was more evident on the right side. Among the analyzed subjects, P3 was able to produce the greatest extension both in hip and knee as observable in the graph in Figure 2. P5 could walk only with a very low speed, equal to 0.25 m/s. We measured 57 steps. P5's mean stride length was only 0.20 m. The gait pattern was characterized by parkinsonian shuffling and severe stiffness, with reduction of the active ROM in all lower limb sections and, as a consequence, very small and fast steps. Data from kinetics report the same reduction of any activity of push over the ground. P8 walked with a mean speed of 0.5 m/s and a mean stride length of 0.68 m. We measured 39 steps. Differently from the other subjects, kinematics data from the lower limbs of this subject were more similar to kinematics of healthy subjects. The

hips appear to be slightly more flexed and showed an external rotation during the stance phase. From kinetics a reduction during distal push-off is evident.

4.2.4 Discussion and Conclusions

In the literature, several studies have been carried out to describe the feasibility and efficacy of walking training using a treadmill in populations affected by RTT (Layne et al., 2021; Lotan et al., 2004; Larsson et al., 2018). Layne et al. (2021) analyzed the gait pattern of 17 patients with RTT walking on a treadmill with a gradual increase in speed (0.1 m/s every 20 seconds). The authors underlined that participants could rely on sensory feedback to adapt their gait to changes in treadmill speed. Similarly, Larsson et al. (2018) demonstrated that, while walking on a treadmill, subjects with RTT can adapt their own speed until reaching their maximum. These results suggest the potential to use a treadmill for training to increase walking speed in RTT populations.

Lotan et al. (2004) proposed a low-intensity daily treadmill program for two months involving four subjects with RTT. The intervention improved the participants' physical and aerobic fitness, suggesting that treadmills can support cardiopulmonary training in this population. These studies measured physiological signals but did not report kinematic data.

Conversely, several studies have explored the interaction of RTT subjects with a VR environment (Mraz et al., 2016; Fabio et al., 2022; Fabio et al., 2021), focusing on upper limb functions and cognitive interaction. Therefore, this is the first study in which a short training combining treadmill and VR environment is proposed for subjects with RTT. The study investigated (1) the suitability of the system and the motivation of the patients during the training and (2) the motor responses of subjects with RTT during training. The first aim was fulfilled considering the adherence of patients to the training, the SEQ, the happiness index and the attention focus to scenarios. The second aim was achieved by means of the endurance time and performance speed measures as well as gait analysis data when available. Considering the feasibility, all the subjects recruited were able to complete the three GRAIL sessions and with four of them it was possible to conduct a gait analysis.

Moreover, the parents of participants showed a good satisfaction and considered the integration of the treadmill and VR a good possibility for future rehabilitative projects. Considering

the happiness index, the participants showed greater satisfaction in APP2 and APP3 during which they walked over the treadmill, in comparison to APP4, during which the task was performed in static conditions. Interestingly, our preliminary data may suggest that patients with RTT increase their at tention toward the scenario and task during training sessions, hypothesizing the feasibility of longer trainings with a treadmill and VR. Considering the second aim of the paper, according to data collected, the most durable activity was APP4. This may be due to the fact that it was the only activity that did not require walking and it is possible that patients got tired more slowly. Regarding gait speed, data reported in this manuscript may suggest that the gait speed can gradually increase during the training. The gait analysis was feasible in four patients. The median walking speed was 0.5 ms−2 , a value higher than the mean speed reached during the three sessions. This is due to the fact that only patients that showed greater endurance and safety during walking could accede to gait analysis, while data for mean speed during training were collected from all the subjects recruited. With this median speed, participants had a median stride time equal to 1.33 s that is longer than the one reported by Layne et al. [9], where the median stride time at a similar speed was between 1.21 s and 1.17 s. The four subjects that underwent the gait assessment showed some common characteristics, such as a global stiffness and, accordingly, the active ROM reduction in lower limbs, the lack in push-off activity in the pre-swing phase and an increase in the stance time. Considering the ROM, the study of Layne et al. included participants with severe stiffness that limited hip movement to a maximum ROM of 12◦ and knee ROM to a maximum of 10◦.

In contrast, the participants that underwent the gait analysis in this study had a median hip ROM of 36.25◦ and a median knee ROM of 49.25◦. This means that the subjects that could undergo the gait analysis showed a less impaired gait pattern. The lack of plantar flexion during the pre-swing phase (healthy subjects usually exhibit 15◦ of plantar flexion [27]) seems to suggest the deficiency in push-off activity of gastrocnemius and soleus muscles, and this can contribute to the reduction in stride length and in walking speed.

Finally, the increase in stance time, directly related to the duration of single-limb support, is a consequence of the difficulty in properly managing the balance of center of mass. On the other hand, additionally to these common behaviors, each subject showed peculiar characteristics. The mean walking speed, for example, was very different between P2 (0.66 m/s) and P5 (0.25 m/s). P2

was markedly asymmetrical, while P8 showed similar kinematics for left and right lower limbs; P5 was the most symmetric, but presented the most limited ROM, and this can explain this symmetry. During walking, P3 could achieve hip and knee extension, particularly in the late stance, while P8 remained with the knee flexed during the whole gait cycle. These results suggest that in addition to some common characteristics, subjects with RTT show specific gait pattern alteration.

For a correct interpretation of these results, we have to consider the limitations of this study. This is indeed a pilot study with a small simple size. Therefore, it is necessary to increase the sample size to confirm the results obtained. In addition, the age range of the sample is broad (6–44 yo); nevertheless, this is common in research papers about subjects with RTT. However, our results must be verified in a larger sample. On the other hand, it is important to consider that RTT is a rare syndrome, limiting the possibility to recruit a big sample with a narrow age range. Future studies could use the methodologies of this pilot study with a large sample of patients, taking into account their age, to replicate our findings.

Secondly, considering that for safety reasons the presence of any person in close proximity of the device was not possible, we decided to take a cautious approach (e.g., stopping the treadmill as soon as the subjects showed signs of discomfort or started veering). This limited the possibility to determine the effective maximum speed during the training.

Finally, although the purpose of this study was to verify the suitability of a treadmill training plus VR in patients with RTT and not its efficacy, we must consider the short duration of each session and the reduced dose of the therapy. Indeed, this study was preliminary to future research that will recommend more intensive training with this system as previously performed.

Nevertheless, this system may have strong clinical implications in the rehabilitation practice of children, adolescents and adults with RTT. Indeed, enhanced motivation and engagement fostered by exergames in virtual reality can support longer training sessions that may cause improved walking endurance. This may consequently boost autonomy in daily life activities, thus improving quality of life of patients with RTT. It is therefore possible to hypothesize that treadmill training combined with VR could be a valid treatment for motor functions in RTT. Future research will be conducted to validate this type of treatment in a randomized controlled trial.

4.3 EXPERIMENTAL STUDY 3: The use of virtual reality in Rett Syndrome rehabilitation to improve the learning motivation and upper limb motricity: A pilot study

Studies have thoroughly documented the impact of RTT on motor skills, including loss of hand function, apraxia, altered muscle tone, tremors, and repetitive stereotypical movements (Downs et al., 2016; Fabio, 2018). Early motor abnormalities are evident in infancy, with deviations in general movements (GMs) and fine motor control. Einspieler et al. (2005) reported that infants with RTT achieve some basic motor milestones, such as midline head alignment and supported sitting, but their movements lack coordination and fluidity. In a detailed analysis of newborns, Einspieler, Kerr, and Prechtl (2005) found no evidence of normal GMs, with many infants exhibiting jerky, disorganized, or excessively slow movements. These early motor abnormalities, while not specific to RTT, highlight the pervasive impact of the disorder on neurodevelopment.

The regression phase, typically occurring between 3 months and 4 years, marks a significant decline in motor and cognitive abilities, with fine motor skills particularly affected. During this period, children lose previously acquired hand functions, including the ability to grasp objects or perform purposeful movements. Downs et al. (2010) reported that 30% of individuals with RTT cannot grasp objects, and others exhibit significant impairments in their ability to manipulate items. This phase also introduces stereotypical hand movements, such as wringing, clasping, and hand-to-mouth gestures, which persist into later stages and remain a defining feature of RTT (Percy, 2008).

In later stages of RTT, hand functionality continues to decline, particularly in individuals with severe motor impairments or higher clinical severity. Studies by Carter et al. (2010) and Monteiro et al. (2014) demonstrated that while some individuals maintain basic self-care skills, such as eating with their hands, these abilities are significantly reduced over time. For example, Monteiro et al. (2014) found that only 3.4% of individuals in advanced stages could independently lift a glass using both hands. The reduction in hand functionality is accompanied by difficulties in fine motor coordination, attributed to apraxia and fluctuating muscle tone, further limiting independence in daily activities.

Hand function in RTT is significantly impaired, with individuals exhibiting a progressive decline in purposeful hand use. Umansky and colleagues (2003) examined hand preferences and functions in a cohort of RTT patients, finding variability in hand use for tasks such as scratching

and self-care. Approximately 40.7% demonstrated a right-hand preference, 33.6% a left-hand preference, and 25.7% no clear preference. This variability underscores the challenges of designing effective interventions for improving hand functionality.

Hand stereotypies, another hallmark of RTT, persist throughout life and are influenced by emotional, sensory, and environmental factors; these movements, which often include wringing, clasping, hand-to-mouth gestures, flapping, and hair twirling, emerge early in life and persist into adulthood (Percy, 2008; Vignoli, Fabio, La Briola et al., 2010). Hirano and Taniguchi (2018) noted that emotional states, such as pleasure or displeasure, significantly impact the frequency of these movements. Displeasure increased stereotypies in 63.8% of cases, while somnolence and sensory input reduced them. Managing these repetitive movements is crucial, as they can lead to secondary complications such as joint contractures and skin issues.

Studies have categorized these movements into distinct types, noting variations in their prevalence between typical and atypical RTT. For example, flapping is more common in atypical RTT, while wringing is predominant in typical cases (Chin Wong, Hung, Jan et al., 2017). Despite their persistence, these stereotypies tend to stabilize over time, although new movements can occasionally develop during follow-up (Vignoli et al., 2010).

The role of emotional, sensory, and environmental factors in modulating stereotypical movements has been explored extensively. Hirano and Taniguchi (2018) highlighted those emotional states, such as pleasure and displeasure, are primary factors influencing the frequency of these movements. Displeasure increased stereotypies in 63.8% of cases, while pleasure did so in 48.5%. Conversely, factors such as somnolence and specific sensory inputs were associated with a decrease in these movements, suggesting potential therapeutic strategies for managing stereotypies. However, studies like Wales et al. (2004) underscore the complexity of this phenomenon, indicating that repetitive movements in RTT may be maintained by intrinsic neurochemical processes and are less likely to be influenced by environmental manipulation.

Interventions targeting stereotypical movements and hand functionality have focused on mitigating their impact on daily life and preventing secondary complications such as skin damage or joint contractures (Hirano & Taniguchi, 2018). However, the efficacy of traditional therapies is limited, highlighting the need for innovative approaches. Virtual reality (VR) has emerged as a

promising tool in this context, offering immersive environments that encourage active participation and provide opportunities for targeted motor skill interventions. VR's potential to deliver personalized, engaging, and repeatable therapy sessions makes it particularly suited for addressing the unique challenges of RTT rehabilitation.

Since the advent of virtual reality (VR), it has been extensively applied in the field of neurorehabilitation and the rationale of using VR is that it encourages and motivates the subjects to participate in the rehabilitation processes, while training in a safe environent which allows repeated learning trials (Dvorkin, Shahar & Weiss, 2006). Only one study (Mraz, Eisenberg Diener et al., 2016) experienced the use of virtual reality for rehabilitation purposes with Rett patients: Virtual Reality has been used in this population in the rehabilitation of the upper limb in the Rett patients; 6 patients were involved, who trained with online games using a Microsoft Kinect camera and a FAAST software (Action and Articulated Skeleton Toolkit) that allowed you to interact with the virtual game through body movements and without the need for cursors, remote controls or controllers. Pre- and post-assessments were administered to examine any changes in upper extremity function. The participant completed a VR intervention 3 times per week for 12 weeks, each session lasting 60 minutes. In the first session, the targeted movement was set as forward reaching, requiring elbow extension and shoulder flexion/extension of the right arm. The movement threshold was set based on the participant's range of motion. YouTube videos used during the intervention were chosen by the participant. The participant was required to reach forward in order to cause the YouTube video to play. After approximately 10-15 seconds, the primary investigator or the caregiver would pause the video, requiring the participant to reach forward to play the video again. Once a video was completed, the participant would point to the next video she wanted to play. As the participant progressed throughout the intervention, the target movement was addressed to keep the activity a challenge. The forward reaching target was kept the same, but the arm required for the task was switched to the left, forcing the participant to use her other upper extremity. Interviews and observation revealed successful game play when games were motivating, clearly established cause and effect, and matched the level of cognitive ability of the participant. The VR intervention led to improvements in use of the upper extremities to complete self-care

activities, an increased number of reaches completed in a 15-minute period, and decreased time engaged in stereotypical hand movements.

As outlined in the general introduction to the studies 2 and 3, the use of VR-based interventions for motor rehabilitation in patients with RTT is limited, and, VR has been demonstrated to be effective in the improvement of upper limb motor activity and functions of patients with neurological diseases (Georgiev, Georgieva, Gong, Nanjappan, & Georgiev, 2021); it becomes necessary to perform specific research with VR technological intervention for RTT. The study we conducted aimed to address specific research questions regarding the use of virtual reality (VR) in Rett syndrome (RTT), including: 1) Can learning and exercising in a VR environment be motivating and emotionally positive for patients with RTT? and 2) Is the motor reaction speed and activation of patients with RTT higher in a VR environment compared to a concrete environment? To answer these research questions, the main aim of this pilot study was to develop a specific VR environment for patients with RTT to stimulate learning, motivation, and motor skills and to compare it with concrete environments.

4.3.1 Methods

4.3.1.1 Participants

Seven patients with a diagnosis of RTT, ranging from age 5 to 38 years old (mean age 15.86 ± 11.27 years), were recruited from the Italian Rett Association (AIRETT). Patients with RTT were classified as clinical stage III (characterized by prominent hand apraxia/dyspraxia, preserved ambulation ability, and some communicative ability, mainly eye contact) or stage IV (late motor deterioration, with progressive loss of ambulation ability), according to the criteria for classic RTT by Hagberg and colleagues (Hagberg, Witt-Engerström, Opitz, & Reynolds, 1986). A general assessment was conducted by a psychologist before starting the experimental sessions, by using Downs' scale for the level of purposeful hand function (Downs, Bebbington, Jacoby et al., 2010) to define the level of purposeful hand function as an evaluation of the functional level of the use of the hands, and by using the Rett Assessment Rating Scales (RARS; Fabio, Martinazzoli, & Antonietti, 2005) to evaluate the severity of the disease in patients with RTT. Table 9 shows the characteristics

of the groups. The MECP2 mutation was seen in 100% of the sample; patients with the FOXG1 syndrome and CDKL5 disorder were excluded from the sample.

Table 9
Characteristics of participants

Participants	Name	Clinical stage	Age	Level of severity (RARS)	Level of purposeful hand function (DOWNS' SCALE)
1	D.D.	IV	31	67.5	3
2	E.T.	IV	18	71	4
3	C.B.	III	5	85	2
4	E.B.	III	6	70.5	2
5	V.D.	III	7	64	2
6	D.B.	III	6	75	2
7	A.C.	IV	38	64	3

4.3.1.2 Measures

During the evaluation, the following data were collected.

- RARS (Fabio et al., 2005) is a standardized scale used to evaluate the severity of the disease in patients with RTT. The total score allows us to measure the severity of the disease along a continuum ranging from mild to severe symptoms. Skewness and kurtosis values in our data set, which were calculated for the distribution of the total score, were .110 and .352, respectively. Distribution was found to be normal. Cronbach's alpha was used to determine the internal consistency for the whole scale and subscales. Total alpha was .912, and the internal consistency of the sub-scales was high (from .811 to .934).
- Downs' scale for the level of purposeful hand function (Downs, Bebbington, Jacoby et al., 2010) is a scale that defines the level of motility of the hands of patients with RTT by assigning a score from 1, the minimum of manual functionality, to 8, the maximum of manual functionality; in particular the score is given as follows: 1) No observed hand function; 2) Able to hold at least one large object (cup, spoon, small ball or toy) >2s; 3) Need of assistance to grasp but able to pick up and hold at least one large object >2s; 4) Able to grasp, pick up, and hold at least one large object >2s; 5) Able to grasp, pick up, and hold at least one large

object >2s and use a raking grasp to grasp, pick up and hold a small object (e.g. sultana, sweet, or small piece of sandwich) >2s; 6) Able to grasp, pick up, and hold at least one large object >2s and use the radial side of the hand to grasp, pick up, and hold a small object >2s (can be a pair of scissors, inferior pincer, or superior pincer grasp); 7) Skills for level 6 and able to transfer an object from one hand to the other (accurate pre-shaping of the hand is not seen); 8) Skills for level 7 and when hand is approaching an object, hand orientation and size recognition closely approximate the position and size of the object.

4.3.1.3 Parameters

The following parameters were collected during the three sessions:

- Motivation Index (MI): to assess motivation, we used a multiple parameter, defined as a "happiness index". This parameter comes from the taxonomy of Van der Maat (1992), which is based on an extensive analysis of communication activity of people with deep intellectual disabilities with their usual caregivers (Petry & Maes, 2006). This taxonomy includes twelve main categories of behavioral form: (1) gaze direction, (2) facial expression, (3) sounds, (4) head posture, (5) head movement, (6) body posture, (7) movements of the lower limbs, (8) movements of the upper limbs, (9) mouth movements, (10) physiological reactions, (11) aggression and (12) conventional gestures. To create the multiple parameter, only five behaviors out of the twelve categories described were considered, i.e. gaze direction, sounds, mouth movements, physiological reactions (regarding the physiological reactions of the body, such as blushing or sweating) and hand gestures. They were recorded by a camera that was placed in front of the subjects during the three experimental conditions. Two independent blinded observers watched the video recordings and marked a cross on a checklist if the five behaviors were present/absent, assigning value 1 to "present" and value 0 to "absent". The MI was defined as the sum of the score for each behavior. In this study, the agreement between the two independent blinded observers was 96%.

- Coincident Timing (CT): CT (Fooken, Yeo, Pai & Spering, 2016) is the parameter used to see the reaction times of the patient to the incoming stimuli recorded in tenths of a second. Coincident timing is defined as the perceptual motor ability to perform a motor response in synchrony with the arrival of an external object at a given point. This task uses a chronometer, which is displayed on the computer screen.
- Length of reaching movement: the surface of the table is covered with graph paper. The participant's hand is placed on the lower edge of the table, which corresponds to distance 0. The computer and stimuli are arranged at a distance that corresponds to half the length of the participant's arm. The distance is measured in cm.
- Retrieval Memory (RM): memory is evaluated at the end of the previous session (session 3), proposing the recognition of stimuli (presented for 3 times) and their discrimination from different distractions. Discrimination occurs between the concrete targets presented, between photos of the targets loaded on power points and between the three-dimensional representation of stimuli in the virtual room. The presentation is random

4.3.1.4 VR environment

A virtual system was created by AIRETT's engineering team. This system can detect the position of the body in real space and replicate it in the virtual world. In this way, the girl's movements are detected and then reproduced in a virtual context, resulting in an interaction with virtual objects. Artificial vision techniques are used to reconstruct a simplified version of the body (skeleton) and body movements are detected through a stereo camera to reconstruct them faithfully in a virtual space. To monitor the interaction between the girl and the virtual environment, AIRETT's team developed a Web application composed of three parts. First, a computer vision component was designed to be able to detect and represent the skeleton of the participant. The skeleton was further analyzed to recognize the reaching movement of the participant. This component was based on Google MediaPipe [https://arxiv.org/abs/1906.08172]. Second, a virtual environment that represented the scene in which the objects appeared, was implemented with Unity

(unity.com). Third, an interface was designed to set the movement parameters, with respect to the physical conditions or preferences of the participant (e.g., the magnitude of the shoulder angle to activate the reaching movement, the choice between the left and right arm), or to choose an object that appeared in a scene from a list of objects, or finally to record the computer screen and, at the same time, the participant, for further analyses.

4.3.1.5 Stimuli

The reinforcing stimuli were a toy and a piece of food. The target stimuli, which the participants were familiar with, were a red ball and a bouquet of flowers. For each stimulus, a real object and its reconstruction in 2D and 3D, was used. Figure 1 shows an example of the images of the three experimental conditions.

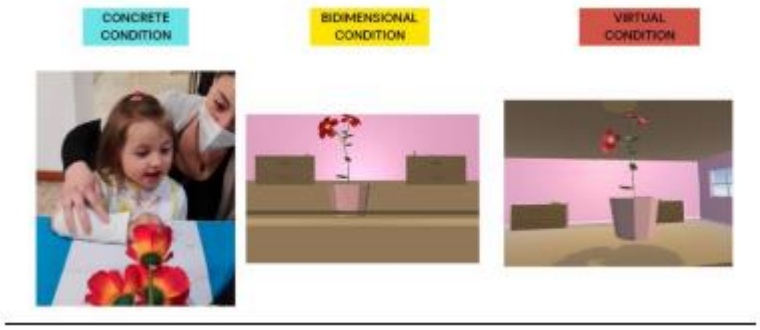


Figure 9. Example of the three experimental conditions

4.3.1.6 Procedure

Each participant went to the AIRETT Centre for 3 consecutive days in a week. Each session for each participant was videotaped with a front camera and a side camera positioned at 45 degrees and at a distance that framed the participant from head to toe. In all the experimental conditions, the sequence of sessions had the same structure, as follows: the participant was seated on her own orthopedic chair at a table with a central recess and that was adjustable in height (the height of the table was defined to ensure the elbow support of the dominant limb). The non-dominant limb was held under the table. For each session, a video recording of the patient was made for 1 minute in a neutral condition and in the absence of requests, to evaluate the motivation index before administration. The stimulus was thus presented to the participant and the therapist gave the

following instruction "go get it". The therapist waited 1 minute so that the participant could start the body movement. If motor activation did not occur, the therapist repeated the verbal request, and the maximum range of time was set at 3 minutes, after which the stimulus was removed; the session was then considered finished for the specific stimulus. Stimuli were sequentially and randomly presented in the three conditions; the order of conditions was also varied between sessions. A break of two minutes was left between the three conditions. In the concrete condition, real stimuli were placed on the table at the level of the midline. If the participant reached for the stimulus, the distance reached by the hand towards the target was marked on graph paper. In the 2D and 3D conditions, a computer was placed on the table and, through the software created by Airett engineers, stimuli were shown in 2D and 3D versions. When the participant reached for the stimulus shown on the screen, the virtual system was able to recognize the movement performed and the object shown in the virtual environment moved towards the participant as a feedback that it has been grasped.

4.3.2 Statistical analysis

The data was analyzed using the SPSS 24. The Shapiro-Wilk test was used to verify whether the distribution of the sample with respect to the dependent variables was normal. The results indicated that data was normally distributed ($p = .200$). Based on this outcome, analysis of variance (ANOVA) was performed assigning the type of experimental condition as the independent variable and the parameters as dependent variables. The Bonferroni correction was applied for multiple comparisons. Alpha level was set to $p < .05$ for all statistical tests. A Pearson's correlation analysis was performed to analyze the correlation between the MI and the dependent variables. 4. Results Concerning the first aim of this pilot study, Table 2 shows the mean and standard deviation of the MI of participants in the three experimental conditions (concrete, 2D, 3D) in relation to the presented stimuli (ball, flowers, personal object, food). The experimental condition variable showed statistically significant effects ($F(2.12) = 4.05$, $p < .04$), indicating that the different conditions in which the same stimuli were presented brought to differences in emotional reaction. As shown in Table 9, a high level of MI was reported in the 3D condition. The post hoc comparison also showed statistically significant differences between happiness in the 2D condition and 3D condition ($t(7) =$

3.40, $p < .014$). This indicates that participants showed more positive emotions in the virtual condition compared with concrete and 3D conditions.

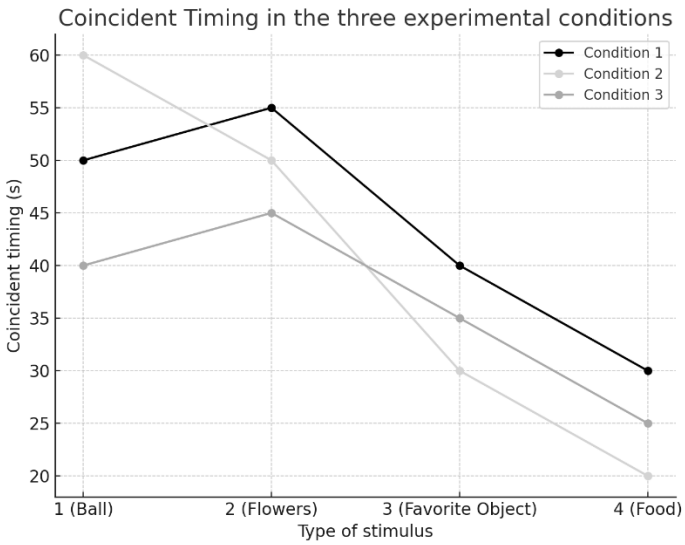
Table 9.
Mean and standard deviation of the Motivation Index of participants in the three experimental conditions

	Condition 1 (real condition)	Condition 2 (two-dimensional)	Condition 3 (three-dimensional)
Ball	17.38 (1.70)	15.07 (3.29)	18.11 (3.06)
Flowers	17.39 (1.71)	15.07 (3.31)	18.12 (3.02)
Favorite object	17.40 (1.73)	15.08 (3.31)	18.12 (3.02)
Food	17.45 (1.73)	15.09 (3.30)	18.13 (3.06)

Regarding the second aim of this pilot study, Table 10 shows the mean and standard deviation of the CT in the three experimental conditions (concrete, 2D, 3D) in relation to the presented stimuli (ball, flowers, personal object, food). Also in this case, the experimental condition variable showed statistically significant effects ($F(2.18) = 18.07$, $p < .001$). The CT of all participants was higher in concrete and 2D conditions than 3D conditions, indicating that the participants were activated more quickly when stimuli were presented in the 3D condition (Fig. 2). The type of stimuli also showed significant effects ($F(3.36) = 6.89$, $p < .003$). As shown in Figure 10, the participants showed a higher level of activation when their favorite objects were presented.

Table 10
Mean and standard deviation of the Coincident Timing of participants in the three experimental conditions in relation to the stimuli presented (ball, flowers, personal object and food)

	Condition 1 (real condition)	Condition 2 (two-dimensional)	Condition 3 (three-dimensional)
Ball	39.73 (20.99)	57.07 (15.41)	15.33 (4.67)
Flowers	50.33 (27.46)	53.33 (17.30)	26.50 (2.02)
Favorite object	34.07 (11.17)	27.29 (16.60)	20.93 (7.11)
Food	26.80 (13.63)	36.38 (8.95)	23.00 (9.37)



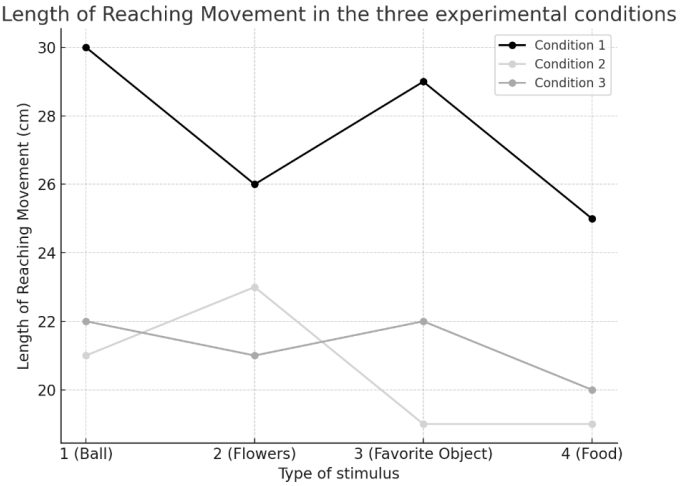
Legend: Black line represents the real condition, light grey line represents the 2D condition, and dark grey line represents the 3D condition. The numbers on the x-axis correspond to the following stimuli: 1 = ball, 2 = flowers, 3 = favorite object, 4 = food.

Figure 10. Coincident Timing in the Three Experimental Conditions

There was a significant interaction between experimental condition X type of stimuli ($F(6.36) = 3.77$, $p < .005$), indicating that the participants increased their activation in the 3D condition rather than in the 2D and concrete conditions when every stimulus was presented. Table 11 shows the mean and standard deviation of the Length of Reaching Movement parameter in the three experimental conditions in relation to the presented stimuli (ball, flowers, personal object, food). The experimental condition showed again significant effects ($F(2.18) = 3.66$, $p < .05$). As shown in Figure 11, the range of the length of reaching movement was greater in the concrete condition than in both virtual conditions.

Table 11
Mean and standard deviation of the Length of Reaching Movement in the three experimental conditions in relation to the stimuli presented (ball, flowers, personal object, and food)

	Condition 1 (real condition)	**Condition 2 (two-dimensional)**	**Condition 3 (three-dimensional)**
Ball	30.39 (7.33)	21.10 (10.31)	22.02 (8.00)
Flower	26.05 (10.85)	23.52 (12.11)	21.24 (5.72)
Favorite object	29.19 (7.52)	19.93 (8.67)	22.40 (8.43)
Food	25.75 (6.90)	19.00 (7.27)	19.88 (7.66)



Legend: Black line represents the real condition, light grey line represents the 2D condition, and dark grey line represents the 3D condition. The numbers on the x-axis correspond to the following stimuli: 1 = ball, 2 = flowers, 3 = favorite object, 4 = food.

Figure 11. Length of Reaching Movement in the Three Experimental Conditions

Table 12 shows the mean and standard deviation of RM. No significant differences between performance in the three experimental conditions were found. This result is probably due to high variability, but trend data showed that participants remembered a greater number of targets in the 3D condition as opposed to the other two conditions (2D and concrete).

Table 12

Mean and standard deviation of Retrieval Memory in the three conditions

	Condition 1 (real condition)	**Condition 2 (two-dimensional)**	**Condition 3 (three-dimensional)**
Retrieval Memory	2.57 (0.78)	2.00 (1.00)	3.00 (0.58)

A Pearson's correlation analysis was performed to analyze the correlation between the MI and the dependent variables. The results showed a positive and statistically significant correlation between MI in the concrete condition and the length of reaching movement in both the 2D and 3D conditions, respectively ($r = .806$, $p = .05$; $r = .803$, $p = .05$). There was also a positive statistically significant correlation between MI in the 2D and 3D conditions Condition 1 Condition 2 Condition 3 ($r = .819$, $p = .05$). Probably, these results can be related to the fact that the order of conditions was random between sessions and the MI was higher in the 3D condition

4.3.3 Discussion

These are fundamental prerequisites of motor rehabilitation in patients with RTT, as the dyspraxia component usually makes the beginning and maintenance of movement difficult, and stereotypies interrupt the sequencing of voluntary and controlled movement. Hence, both studies demonstrated the possibility of using VR in the rehabilitation of RTT, increasing target abilities and motivation.

The present research has some limitations that a pilot study can highlight. The results obtained cannot be generalized due to the small sample size, so we suggest caution in the interpretation of the results. However, RTT is a rare genetic disorder and it can be difficult to recruit a large data set with similar age or other features. Moreover, pilot studies are not a hypothesis testing study evaluating the efficacy of an intervention. They produce pilot date that can be used as a guide in the design and implementation of future larger scale efficacy studies (Leon, Davis, & Kraemer, 2011).

4.3.4 Conclusions

In conclusion, given the complexity of RTT as well as the limited state of knowledge concerning the methods to implement VR in patients with RTT, the present pilot study can be considered a significant phase of the research process suggesting that learning and exercise in a VR environment is more motivating and emotionally positive for patients with RTT rather than in a concrete environment. Hence, this pilot study is a necessary first step in exploring the use of VR as a novel and potential application of interventions in RTT and it offers support concerning the feasibility of use, considering the necessity of planning and of developing larger efficacy trial studies.

4.4 Experimental Study 4: Virtual Reality as a Tool for Upper Limb Rehabilitation in Rett Syndrome: Reducing Stereotypies and Improving Motor Skills

Experimental Study 4 serves as a continuation of Experimental Study 3, as VR is used in this study to design a rehabilitation training program for upper limb motor skills.

Building on the results obtained from Experimental Study 3, the present study aims to:

1. To evaluate the impact of a virtual reality (VR) training program on improving upper limb motor skills in patients with Rett Syndrome (RTT), focusing on exercises involving reaching and hand opening movements. Significant differences are expected between the experimental and control groups following the training in motor parameters, including Temudo's analysis of stereotypy intensity, Temudo's analysis of stereotypy frequency, Time to Satisfy Request for the first reaching task, Time to Satisfy Request for the hand-opening task, and Correct Performances in both tasks.

More in detail we await a significant empowering trend in all the ten phases of training. Within the training program, it is expected that Temudo's Analysis of Intensity, Temudo's Frequency Analysis, Time to Satisfy Request, Maximum Arm Extension, Correct Performances, and Time to Open Hands will show measurable improvements in motor performance and stereotypy reduction

2. To assess the transferability of the results obtained from the virtual reality (VR) training program to real-world (ecological) contexts, with a focus on upper limb motor skills in experimental group patients. No significant differences are expected between the virtual and ecological conditions for both tasks (reaching and open hand) and for parameters including Time to Satisfy Request, Length of Reaching Movement (in cm), Correct Performances, as well as Time to Open Hands, Maximum Opening of Hands, and for stereotypy reduction.

3. To evaluate the role of VR in enhancing motivation and engagement during the execution of motor tasks, addressing the main challenges of the rehabilitation process for

this population. Specifically, it is anticipated that the experimental group will demonstrate improvements in attention and motivation throughout the training sessions.

Based on previous research, this study aims to provide new insights into the application of VR in RTT rehabilitation, bridging the gap between traditional methods and emerging technological solutions.

4.4.1 Method

4.4.1.1 Participants

Twenty patients with Rett Syndrome, aged between 5 and 33 years (M: 16.80; SD: 10.81), were recruited by the Italian Rett Syndrome Association (AIRETT). The participants were divided into two groups: a control group consisting of 10 participants and an experimental group consisting of 10 participants. Rett patients were classified according to the classical Rett criteria by Hagberg (2002) as clinical stage III (characterized by prominent hand apraxia/dyspraxia, apparently preserved walking abilities, and some communicative skills, mainly through eye contact) or stage IV (late motor deterioration with progressive loss of walking ability). All participants displayed pervasive upper limb stereotypies. Depending on their age, all participants attended schools or socio-educational centers, except for one participant (age 33). The AIRETT team conducted a general assessment of the sample using the GAIRS Checklist (Fabio et al., 2022), the Rett Assessment Rating Scales (RARS) (Fabio et al., 2005), and the Downs Scale (Downs et al., 2016). Table 13 presents the characteristics of the two groups and their test equivalence. The Mecp2 mutation was identified in 100% of the sample. Patients with FOXG1 and CDKL5 mutations were excluded from the sample.

Table 13
Characteristics of participants.

Participants	Name	Clinical Stage	Age	MeCP2 Mutation	Level of Severity (RARS)	Functional Ability Level (GAIRS)	DOWNS' SCALE
EG 1	L.M.	III	10	C965C	58	1,85	1

2	L.A.	IV	27	T158M	69,5	2,45	1
3	L.G	IV	27	T158M	69,5	2,26	1
4	V.S.	II	5	C538C	67,5	1,8	2
5	D.D.	IV	33	R306C	67,5	2,48	2
6	B.C.	II	8	C965C	89,5	2,07	3
7	B.A.	III	16	P152R	57	2,1	1
8	A.G.	IV	28	R270X	67	2,43	1
9	H.G.	II	7	T158M	71	1,84	1
10	B.M.	II	7	C502C	79	2,1	1
CG							
1	C.A.	II	8	C502C	58	2,55	2
2	C.M.	III	10	T158M	65,5	1,9	1
3	A.A.	III	13	C763C	85,5	1,5	1
4	A.S.	IV	18	C1156	57	2,34	2
5	G.B.	IV	27	P152R	63,5	2	3
6	L.G.	III	15	R106W	64	2,7	3
7	L.S.	III	12	T158C	67,5	1,4	2
8	S.D.	IV	18	P133C	72	2,19	4
9	D.F.	IV	28	R255X	64,5	1,69	2
10	B.E.	II	9	C880C	58	2,15	1

To be included in the study, participants needed to meet specific criteria. They had to be registered with the Italian Rett Syndrome Association and fall within the age range of 3 to 35 years. Eligible individuals were required to have confirmed genetic mutations such as Mecp2, CDKL5, or FOXG1. They also needed to demonstrate sufficient postural control to sit independently and exhibit motor intentionality, as well as behavioral capabilities like maintaining eye contact, engaging in joint attention, and tracking visually.

In addition, participants were selected based on a high frequency and intensity of stereotypies, particularly behaviors like “hands together” or “hand-clapping on the midline.” Exclusion criteria included severe structural retractions or deformities that restricted upper limb movement, as well as cases of drug-resistant epilepsy. Finally, participation was contingent on the availability of trained teachers who could provide ongoing support within the school setting.

Participants caregivers were contacted to provide a detailed explanation of the study, its objectives, procedures, and potential benefits and risks. Before starting the activities, their caregivers were asked to sign the informed consent form. This document confirmed their willingness to participate in the study and certified that they fully understood the details, participation methods, and participants' rights.

For the selected participants, during the evaluation phase before the start of the training for both groups, AIRETT therapists recorded the following demographic and clinical variables: age, gender, type of mutation, syndrome severity using the Rett Assessing Rating Scale (RARS), the participant's functional level using the Global Assessment and Intervention in Rett Syndrome (GAIRS) scale, and manual functional motor skills level using the Downs protocol (2010).

- Rett Assessing Rating Scale (RARS) (Fabio et al., 2005) is a standardized scale used to evaluate the severity of the disease in patients with RTT. The total score allows to measure the severity of the disease along a continuum ranging from mild to severe symptoms. It provides a comprehensive assessment of core symptoms across various domains, including motor skills, communication abilities, social engagement, and behavioral aspects.
- Downs' scale for the level of purposeful hand function (Downs, Bebbington, Jacoby et al., 2010) for the level of purposeful hand function (Downs et al., 2016) is a scale that defines the level of motricity of the hands of patients with RTT by assigning a score from 1, the minimum of manual functionality, to 8, the maximum of manual functionality.
- Global Assessment and Intervention in Rett Syndrome (GAIRS) (Fabio et al., 2022), scale is a checklist that gives an overview of the different areas and is intended for use in the functional analysis of the overall abilities of the patient with RTT. The GAIRS checklist is composed of 10 macro-areas, for each area, different sequential skills, hierarchically structured, are evaluated. A total of 85 skills are evaluated. Each skill has a numerical score ranging from 1 to 5, where 1 is the minimum level of capacity and 5 is the maximum level of capacity to perform a specific activity.

4.4.1.2 *Experimental Design*

This study employed a pre-test 1, training, post-test design. The design included a between-subjects variable (Groups: Experimental with virtual reality and Control with usual activities) and a within-subjects variable (Phases: pre-test and post-test).

Data were analyzed using SPSS version 24.0 for Windows. Descriptive statistics were calculated for each dependent variable, and the alpha level was set at .05 for all statistical tests. To determine whether scores obtained at times T1 and T2 differed significantly, the Wilcoxon signed-rank test was applied. The Bonferroni correction was used to account for multiple comparisons, with the significance level adjusted according to the sample size

4.4.1.3 *Instruments for evaluation*

Pre-Post Test Measures (T1-T2)

The measurement parameters for the test phases were collected in two distinct settings: virtual reality and ecological (real-life) environments. The experimental test phase was conducted within the VR software, while the ecological test phase took place in a real-world setting, replicating the movements proposed in the experimental setup.

Stereotypy analysis, based on Temudo's checklist, was performed throughout the entire test phases, repeated at both T1 and T2. Additional measures analyzed focused on two specific tasks required during the test phases: reaching movements and hand opening movements. These measures, detailed in Table 14, include the Time to Satisfy the Request for both tasks (Fooken, Yeo, Pai & Spering, 2016) and the Correct Performances recorded for each task.

Table 14
Pre-Post Test Measures.

Measure	**Description**	**Data recording**

Temudo's Checklist	Temudo's checklist is a systematic tool used to assess the presence, frequency, and characteristics of motor stereotypies, a distinctive symptom of Rett syndrome. It categorizes stereotypies based on the nature of movements, such as hand-wringing, hand-clapping, bringing hands to the mouth, and other repetitive behaviors. The checklist documents the type, frequency, intensity, and duration of these movements, analyzing how long they persist and how strongly they manifest.	Recorded by the therapist.
Time to Satisfy Request	Used for both exercises this is the parameter needed to assess the patient's reaction times to incoming stimuli, recorded in tenths of a second.	Automatically recorded by the software.
Correct Performances	This variable quantifies the number of correct responses performed by the participant during a series of trials. A "correct response" is defined by the accurate execution of an assigned task, without errors or assistance. This parameter is used to evaluate the effectiveness of training in increasing response accuracy and precision, providing an indicator of the participant's ability to follow instructions and reduce errors associated with stereotypical behaviors.	Automatically recorded by the software.

4.4.1.4 Training Measures

During the training, only for the experimental group, various fine-motor parameters were automatically recorded by the system. The parameters monitored for each session (4 sessions per week) were: Time to Satisfy Request **for** reaching movements and hand opening movements and the number of Correct Performances **for** reaching movements and hand opening movements, all automatically recorded by the system.

In addition to these parameters automatically recorded by the software, therapists recorded the frequency and intensity of stereotypies, following the model of Temudo (2007).

Behavioral aspects were also monitored during the training by the therapists. Specifically, the therapists, for the entire training session, recorded the Attention time, the Number of Aids Given and the Happiness Index, according to the scales by Van der Maat (1992) and Garant, Brivio, Riva, Baños (2019). Table 15 describes the parameters recorded during the training sessions for the experimental group.

Table 15
Training Measures for experimental group.

Measure	**Description**	**Data recording**
Analysis of stereotypies according to Temudo's checklist	Described in Table 3	Recorded by the therapist.
Time to Satisfy Request for **reaching movements and hand opening movements**	Described in Table 3	Automatically recorded by the software.
Correct Performances for **reaching movements and hand opening movements**	Described in Table 3	Automatically recorded by the software.
Number of Aids Given for all session of training	The Number of Aids Given refers to the total count of assistance provided to participants during all training sessions.	Recorded by the therapist.
Attention time for all session of training	Attention Time refers to the total duration participants maintained focus during all training sessions.	Recorded by the therapist.
Motivation index for all session of training	To assess motivation, a "happiness index" was used, derived from Van der Maat's (1992) taxonomy, which analyzes communication behaviors in individuals with profound intellectual disabilities interacting with their caregivers (Petry & Maes, 2006). The taxonomy includes twelve behavioral categories, but the index focused on five: gaze direction, sounds, mouth movements, physiological reactions (e.g., blushing or sweating), and hand gestures. These were recorded via a front-facing camera during three experimental conditions. Two independent blinded observers evaluated the videos, marking behaviors as present (1) or absent (0) on a checklist. The motivation index (MI) was calculated as the sum of scores for the five behaviors.	Recorded by the therapist.

4.4.1.6. Instrumentation

The following equipment was used to support the experimental setup and facilitate interaction with the virtual environment for participants with motor and cognitive disabilities:

- **ZED Mini Camera**: The ZED Mini, developed by Stereolabs in 2017, was an RGB-D camera with dual sensors and lenses, allowing it to capture both color frames and depth maps of the scene. This depth information, along with the images, was used for

purposes like detecting people and reconstructing their 3D poses. The camera was positioned in front of the participant during each session to capture the upper body and arms, ensuring stable body tracking.

- **PC**: A computer with appropriate hardware was used to capture data from the camera and run the exergame. Required specifications included an NVIDIA GPU with CUDA support, a USB 3.0 port for camera connection, and an HDMI port for monitor output.
- **Display**: A large display (between 50 and 65 inches), positioned behind the camera, was used to show the virtual environment to the participants. This could be a standard television of the indicated size, requiring at least one HDMI port for connection to the computer.
- **Click4All**: Developed by ASPHI in 2013, Click4All was a customizable interface kit that enabled individuals with complex motor and cognitive disabilities to access PC, smartphone, and tablet technology. The device was a 15x10x3 cm rectangular interface with sensor connection ports, allowing for the creation of up to 18 touch-sensitive buttons, adjustable for each user's abilities. Touch sensors were crafted using conductive materials and connected via Bluetooth or USB. For interacting with the virtual environment, two 30x21 cm Forex tablets were used, one covered with a copper adhesive sheet and the other with a soft synthetic fabric with copper wire sewn in. Both surfaces detected touch and were secured to a table with suction cups.

Considering the non-immersive nature of the Virtual Reality application, the training setup was carefully designed to ensure an optimal environment for the participants. The layout involved seating the participants at a table positioned approximately 150 cm away from the monitor, with the monitor centered horizontally relative to them. This arrangement aimed to provide a clear view and align the participants' eye level with the center of the screen. Above the monitor, conductive pads for input transmission were placed as needed, adding to the seamless integration with the VR application. To capture movements accurately, a ZED Mini camera was positioned about 50 cm in front of the monitor, making it roughly 100 cm away from the participants. The camera was mounted on either a standard tripod or a tabletop tripod, allowing flexibility in positioning based on available furniture.

This careful placement and height adjustment ensured an unobstructed view of the screen and minimized potential occlusions or visual interference. In terms of height, an eye-level alignment of approximately 120 cm with the center of the monitor was recommended, with a camera height of about 100 cm and a table height around 80 cm. These proportions aimed to provide a comfortable, adaptable setup, ensuring an effective and user-friendly training experience for all participants. In Figure 12, the setup, equipment and the hardware connection diagram is explained are presented.

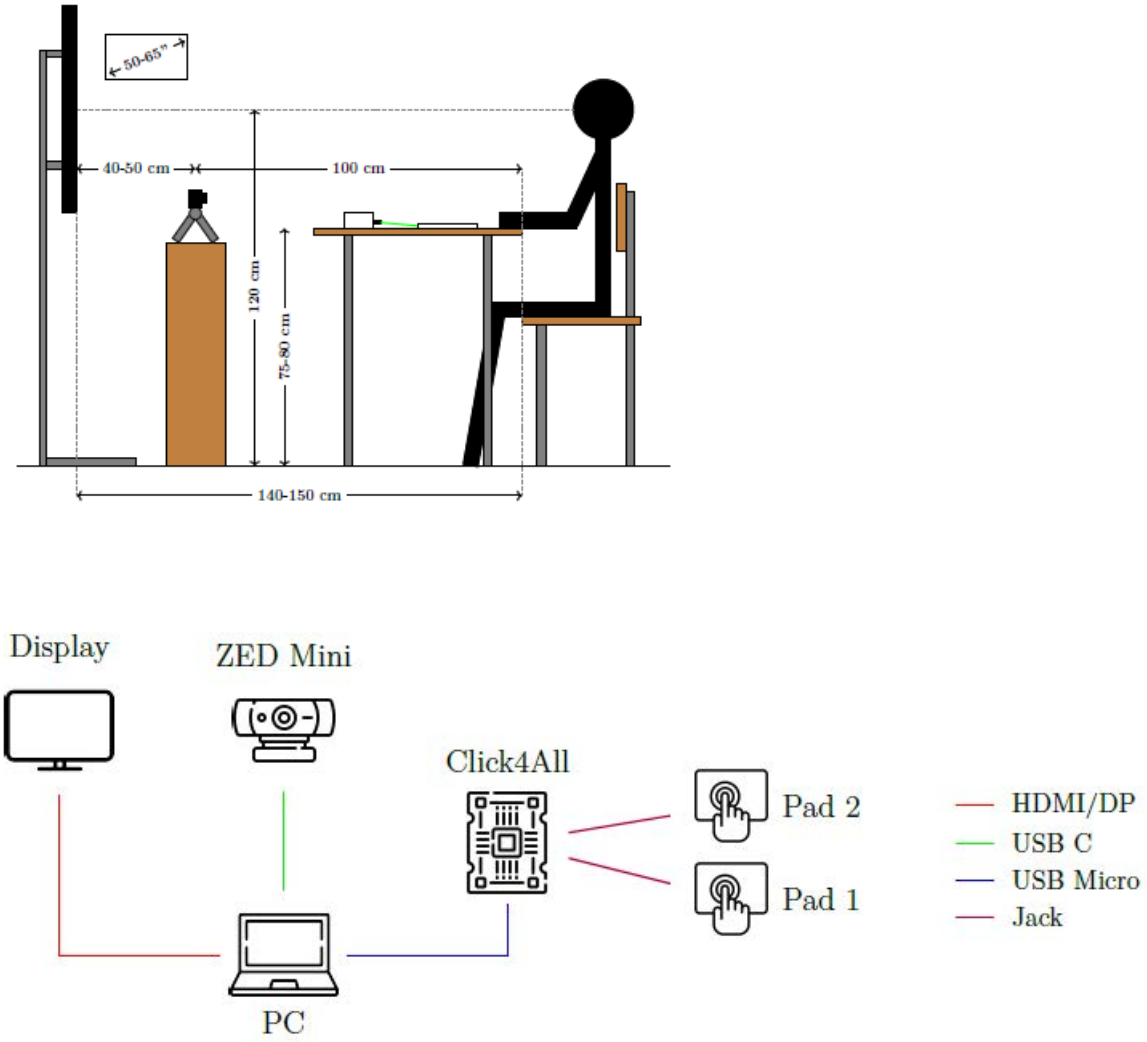


Figure 12. Setup, equipment and Hardware

- ***Virtual Room 2.0* software**.

The Virtual Room 2.0 software served as the tool through which participants in the experimental group engaged in virtual exercises during sessions. Hardware requirements for the system included a computer to run the software, a monitor to display the virtual environment, a ZED Mini camera for subject tracking, and a Click4All device to detect inputs. Either a desktop or portable computer could be used, as long as there was direct access to the keyboard and mouse to allow caregiver intervention during sessions. Developed using Unity 3D, the software leveraged the capabilities of the ZED Mini camera and machine learning models to detect the participant's pose. Each captured frame identified a predefined set of body joints (shoulders, elbows, nose, ears), enabling the creation and updating of a virtual skeleton that mirrored real movements. This skeleton animated a first-person avatar, visible only in its arms and hands, which the participant controlled directly through eight specific movements. Frames, used solely for movement analysis, were deleted immediately after processing without being saved.

- **Rehabilitation exercises for reaching and touch actions** took place within a virtual room designed as a playroom.
    - At the center of the room was an oval table, surrounded by two shelves containing toys and dolls.
    - In front of the table, a large window offered a view of the garden, featuring small trees, an outdoor table with chairs, and a bean bag chair.
- **First block of reaching exercises**:
    - The participant was positioned in the room, facing the table.
    - On the table, there was a musical instrument: a keyboard, a pair of maracas, or a snare drum.
    - A guiding voice instructed the participant to touch the instruments to produce music.
- **Second block of reaching exercises**:
    - Set in the garden, the participant encountered one of three cartoon-style animals: a German shepherd, a dachshund, or a cat.

- The narrative voice suggested moving the hand toward the animal to pet it, triggering an auditory and visual response.

- **Exercises for conditioning stereotypies**:
  - These took place within a small virtual theater.
  - In the background, personalized videos selected for each participant's preference were projected.
  - Viewing and hearing were partially obscured by a curtain, allowing only central visibility and reducing sound volume.
  - The guiding voice suggested that participants separate their hands to improve viewing and hearing of the video.
  - The separation gesture opened the curtain, enhancing video quality.
  - The curtain remained open as long as the hands stayed sufficiently apart and did not touch other parts of the body.

Contact with targets was recorded based on the training phase:

- **Phase 1**: Visual alignment is maintained, and contact is confirmed only when the real hand touches one of the two pads connected to the Click4All device.
- **Phase 2**: No physical intermediaries are used; contact is confirmed when the virtual hand touches the 3D target model.
- Success with each target is marked by golden stars.

Hand separation is determined solely through camera data, using preset minimum distance and separation time parameters.

In Figure 13, the exercises are shown.

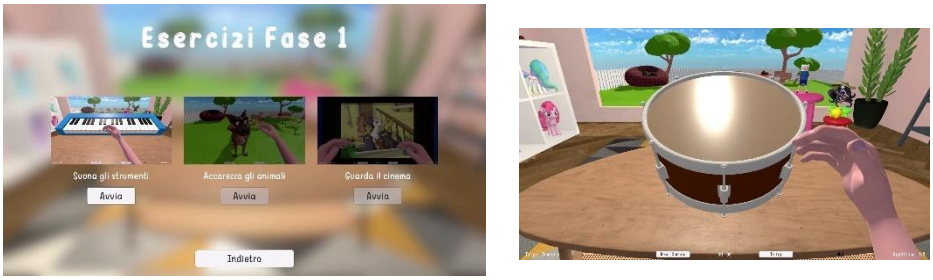


Figure 13. Exercises for Phase 1 and Phase 2

- **Phase 0 (Familiarization Exercises)**: **Exercise 1**: In the garden setting, balloons float up from the ground; participants pop them by hitting them with their hands. **Exercise 2**: In a room with a large whiteboard, participants are prompted to "draw" by the narrative voice, guiding hand positions to create colorful traces on the board, with colors changing randomly. **Exercise 3**: In a bathroom setting, participants interact with bubbles released by their virtual hands in a tub filled with floating toys. In Figure 14, the progression of Phase 0 is shown.

Figure 14. The Progression of Phase 0

The software provided a simple and minimal interface that allowed caregivers to start, pause, resume, and monitor the progress of each session. During exercises, caregivers observed participants closely, initiating each repetition based on the participants' behavior and level of focus.

Upon launching the exergame by double-clicking the executable file "Stanza Virtuale.exe," the main menu appears, as shown in Figure 15. From the main menu, accessing the settings via the "Settings" button opens the configuration interface (Figure 16), with options grouped by category and accessible through buttons on the left-hand column.

Figure 15. The Main Menu

The general settings menu (Figure 16) includes a dropdown for selecting a reminder sound and the progression mode. The reminder sound plays periodically during patient

inactivity to regain attention. Further down, there are specific settings for the two exercise categories in the study. For the reaching exercises, users can specify the patient's dominant hand. The avatar displays only the corresponding arm to focus attention and avoid visual artifacts caused by obscured limbs.

For stereotypy-conditioning exercises, the settings (Figure 16) define thresholds for success, requiring patients to keep their hands separated by at least a specified duration and distance (e.g., 20 cm for at least 1 second by default).

Figure 16. General Settings, Reaching Exercises, and Stereotypy Conditioning Exercises

When an exercise is started using the "Start" button, the program connects to the camera and shows a black loading screen. Once connected, a calibration screen appears (Figure 15), displaying a child's silhouette. This screen helps align the camera to ensure the patient's face and upper limbs are visible. Adjustments can be made to align the patient's face with the silhouette and ensure the arms are visible on the table.

If conductive pads are needed, the system requires calibration of their position. Using the interface (Figure 17), the software displays a virtual pad represented as a gray block. By dragging a slider, the virtual pad's position is adjusted to match the physical pad on the table. The calibration ensures the avatar's hand aligns visually with the physical pad, enabling proper interaction. Once calibration is complete, the exercise can be started by clicking "Play."

Figure 17. The Steps to Start the Exercises

After completing each exercise, the software automatically generated a summary file containing measurements for further analysis. Caregivers could adjust various parameters within the system, such as the minimum time required for participants to keep their hands separated to register a successful task, and they could upload custom videos for use in stereotypy conditioning exercises. The software also offered the option to enable continuous movement logging, allowing specific joints to be tracked with results saved in CSV format for offline analysis or visualization. At the start of each session, the software prompted caregivers to enter the participant's name, creating a settings file that would load automatically in subsequent sessions and could be updated as needed. Additionally, the main menu included an option to initiate an initial assessment session, which determined the appropriate set of exercises for each participant based on their individual needs.

The exercises are described in Table 16.

Table 16
Description of the exercises that comprised the training sessions.

Phase 0 **Familiarization Exercises**	**Exercise 1:** In a garden setting, balloons float up from the ground; participants pop them by hitting them with their hands.	
	Exercise 2: In a room with a large whiteboard, participants are prompted to "draw" by the narrative voice, guiding hand positions to create colorful traces on the board, with colors changing randomly.	
	Exercise 3: In a bathroom setting, participants interact with bubbles released by their virtual hands in a tub filled with floating toys.	
	Reaching Exercises	Hand opening movements **for Contrasting Stereotypies Exercises**

	The exercises took place within a virtual room designed as a playroom. At the center of the room was an oval table, surrounded by two shelves containing toys and dolls. In front of the table, a large window offered a view of the garden, featuring small trees, an outdoor table with chairs, and a bean bag chair.	The exercises took place within a small virtual theater. In the background, personalized videos selected for each participant's preference were projected. Viewing and hearing were partially obscured by a curtain, allowing only central visibility and reducing sound volume.
Phase 1 Visual alignment is maintained, and contact is confirmed only when the real hand touches one of the two pads connected to the Click4All device.	**Participants trained with 2 types of targets, musical instruments and pets, as follows.**	The guiding voice suggested that participants separate their hands to improve viewing and hearing of the video.
Phase 2 No physical intermediaries are used; contact is confirmed when the virtual hand touches the 3D target model. Success with each target is marked by golden stars. Hand separation is determined solely through camera data, using preset minimum distance and separation time parameters.	**Musical Instruments:** The participant was positioned in the room, facing the table. On the table, there was a musical instrument: a keyboard, a pair of maracas, or a snare drum. A guiding voice instructed the participant to touch the instruments to produce music.	The separation gesture opened the curtain, enhancing video quality. The curtain remained open as long as the hands stayed sufficiently apart and did not touch other parts of the body.
	Pets: Set in the garden, the participant encountered one of three cartoon-style animals: a German shepherd, a dachshund, or a cat. The narrative voice suggested moving the hand toward the animal to pet it, triggering an auditory and visual response.	

4.4.1.7 Procedure

The study's procedure was systematically organized to ensure a thorough approach from sample selection to training phases, providing a structured framework for participant assessment and skill development. The process included:

- **Step 0: Sample Selection and Consent Form Signing**

The sample selection was conducted by identifying participants who met the inclusion criteria defined for the study, such as age, diagnosis, and willingness to participate in the training.

After identification, participants and/or their legal representatives were contacted to provide a detailed explanation of the study, its objectives, procedures, and potential benefits and risks. Before starting the activities, their caregivers were asked to sign the informed consent form. This document confirmed their willingness to participate in the study and certified that they fully understood the details, participation methods, and participants' rights, including the right to withdraw at any time without consequences.

- **Step 1: Pre-Post Test Phase (T1-T2)**

After a comprehensive assessment of participants' functioning, both the experimental and control groups underwent the test phase, which was then repeated at the end of the training. The VR-based test session involved asking the child to perform 2 arm-opening movements and 4 touch movements (2 movements with 2 different targets). In VR, scenarios were designed to simulate opening a curtain behind which filmed scenes appeared and approaching and touching pleasant elements corresponding to an animal and a musical instrument (randomized across 4 target variants). The ecological test session was conducted by asking the child to perform 2 arm-opening movements and 4 approach movements. These movements corresponded to real-life actions, such as opening arms to move curtains and approaching and touching a musical instrument or an animal (realistic or a puppet). For each exercise, the therapist performed a demonstrative trial, fully assisting the participant to complete the task, reinforcing their understanding of the request. The measure used was the total number of correct responses, with a range from 0 to 4. More specifically, each behaviour was scored as follows: 1 if the child performed it independently, 0.5 if performed with partial assistance, and 0 if the goal was not achieved. The total score (maximum 6) was obtained by summing the performance of the 2 movements, repeated twice per target.

- **Step 2: Caregiver Training**

Alongside the test phase, equipment was provided to the caregivers. Airett therapists visited the designated facilities to set up the environment together, conducted an initial training session, and provided a technical and instructional guide. The manual included essential information for managing the PC and VR, as well as appropriate pedagogical strategies and methods.

- **Step 3: Training**

The training phase was conducted in everyday life setting and lasted for 2 months, focusing on practicing two target skills: reaching and opening arms to separate the hands. Five sessions per week were conducted at school. Based on literature on VR intervention durations, it was determined that 720 minutes of treatment should be sufficient for experimental VR training (citations). From this information, the patients were scheduled for 40 sessions of 20 minutes each over an 8-week period. Given the wide variability in the clinical profiles of patients with Rett syndrome, session durations were individualized for each child based on their average attention span. The training included the following phases: **Phase 0** served as an introductory cause-and-effect learning stage where participants were exposed to three distinct scenarios. During this phase, generalized upper-body movements triggered specific animations on the screen, such as popping balloons, painting a canvas, and seeing butterflies in a meadow. Each scenario included three movement and oculomotor integration tasks, totaling nine learning activities per session, with each session lasting 20 minutes. Building on the foundational skills established in Phase 0, **Phase 1** introduced Virtual Reality (VR) with interactive objects and was divided into two parts. In the first part, participants practiced reaching for four different objects (2 musical instruments and 2 animals), repeating each object interaction three times. In the second part, participants performed three exercises of opening and separating the hands, with each hand movement represented by a curtain that revealed a favorite video behind it. Each session in this phase lasted 20 minutes, with the order of parts randomized to maintain engagement and flexibility. These parts could be completed consecutively or with a break in between. Progressing from object-based interactions in Phase 1, **Phase 2** continued with VR but removed the use of physical objects, focusing instead on pure movement tasks. Similar to the previous phase, this stage was split into two parts: in the first part, participants reached for four virtual objects (again, 2 musical instruments and 2 animals), and in the second part, they practiced three opening and separating hand movements with curtains revealing a favorite video. Like Phase 1, each session in Phase 2 lasted 20 minutes, with a randomized order allowing for consecutive or paused completion of the two parts.Each phase was carefully structured to build upon the skills learned in the

previous stage, guiding participants progressively from cause-effect interactions to targeted motor tasks, with the ultimate goal of fostering skill mastery through VR-assisted activities.

During the training, strategies based on applied behavior analysis (ABA) were used (Lotan, Shavit, and Merrick, 2015; Fabio, Antonietti, Castelli, and Marchetti, 2009; Mraz, Eisenberg, Diener, Amadio, Foreman, and Engsberg, 2016).

Each exercise was initially conducted with total physical guidance and verbal instruction provided by the caregiver. After this first phase, the exercise was then repeated three additional times using only verbal instruction. Every 10 seconds, the verbal prompt was repeated, and after 120 seconds, if no response emerged from the participant, the exercise session was considered concluded. The verbal prompt was configured by the software system.

The training followed a progressive sequence between the various phases: the participant moved to the next phase of training when the trained skill reached a mastery criterion of three correct performances across three consecutive sessions (Fabio, Semino, and Giannatiempo, 2022).

Each session lasted 20 minutes. The training was supervised by Airett therapists, who connected remotely and monitored the entire session. Supervision frequency followed a gradual schedule: two supervisions per week for the first two weeks, then once per week for the following two weeks, and finally, one every 15 days in the subsequent weeks.

4.4.2 Statistical Analysis

Statistical analysis was conducted using non-parametric tests, as the data did not meet the assumptions for parametric analysis. Despite the control group (CG) and the experimental group (SG) being equivalent in baseline parameters, non-parametric methods were chosen due to the nature of the data. The Wilcoxon signed-rank test was used for within-group comparisons, while the Mann-Whitney U test was employed for between-group comparisons. To assess trends over time, the Friedman test was applied to data from the 10 training sessions. This approach ensured that differences between groups, as well as changes

over the training sessions, were appropriately tested while accounting for non-parametric distributions.

4.4.3 Results

Training was completed in all the subjects involved without any adverse events. No significant differences emerged at test phase (T1) between the two groups concerning demographic variables and psychometric/outcome scores (see Table 17).

Table 17
Clinical description of the experimental group and the control group

Patients	EG N=10	CG N=10	*t*	*p*-Value
Age	16.80 (10.81)	15.80 (7.05)		
Gairs Checklist	2.13 (0.26)	2.04 (0.43)	*0.61*	0.55
RARS scale	66.00 (6.82)	65.55 (8.42)	*0.13*	0.09
Downs' scale	1.40 (0.70)	2.10 (1.00)	*-1.82*	0.09
Right-Lateral Dominance	9	9		
Left- Lateral Dominance	1	1		

With reference to the first hypothesis, to assess the impact of a virtual reality (VR) training program on enhancing upper limb motor skills in patients with Rett Syndrome (RTT), the experimental group showed a significant reduction in the frequency and intensity of stereotyped movements after the training, whereas the control group did not show any notable changes in both tasks. A Wilcoxon signed-rank test confirmed significant improvements within the experimental group ($p < .01$ for frequency and $p=.03$ for intensity). The test also indicated a significant difference between groups, with the experimental group outperforming the control group for the opening hand and reaching task ($p < .01$ and $p=.03$ for intensity).

The experimental group significantly reduced their response time for both the first and second tasks after training, while no changes were observed in the control group ($p < .01$, Wilcoxon; $p < .01$, Mann-Whitney U). Additionally, the number of correct responses increased significantly in

the experimental group, with no improvements in the control group ($p < .01$, Wilcoxon; $p < .01$, Mann-Whitney U). No significant differences were found in the length of reaching movements between or within groups. Refer to table 18 for detailed statistics.

Table 18
Median, (range), and p-values (Mann-Whitney U test) for Experimental and Control Groups in Pre-Test and Post-Test phases

Parameter	Group	Pre-Test	Post-Test	p (Pre vs. Post)
Temudo's Analysis of Stereotypy Intensity	Experimental	24.00 (10–48)	15.00 (5–30)	.008**
	Control	25.00 (12–60)	24.00 (10–55)	.350
Temudo's Analysis of Stereotypy Frequency	Experimental	210.00 (120–350)	145.00 (70–240)	.005**
	Control	215.00 (90–390)	214.00 (100–380)	.240
Time to Satisfy Request for Reaching Task	Experimental	86.00 (40–140)	56.00 (30–98)	.012*
	Control	84.00 (45–130)	78.00 (40–110)	.870
Time to Satisfy Request for Opening Hands	Experimental	113.00 (90–140)	98.00 (85–125)	.030*
	Control	134.00 (90–140)	116.00 (80–125)	.780
Correct Performances (Reaching Task)	Experimental	0.40 (0.10–0.80)	0.80 (0.40–1.20)	.003**
	Control	0.45 (0.20–0.90)	0.46 (0.25–0.90)	.690
Correct Performances (Opening Hands Task)	Experimental	0.42 (0.15–0.70)	0.82 (0.60–1.00)	.002**
	Control	0.44 (0.10–0.85)	0.46 (0.20–0.80)	.620

*=p<.05; **p=.01

To analyze more il detail the training, the results of the Friedman test revealed significant trends over the 10 training sessions for the following parameters: Temudo's Analysis of Intensity, Temudo's Frequency Analysis, Time to Satisfy Request and Correct Performances. Specifically, the analyses indicated significant improvements across the sessions, supporting the efficacy of the virtual reality training program in enhancing motor skills and reducing stereotypies. Temudo's Analysis of Intensity ($\chi^2(9) = 35.76$, $p = .001$), Temudo's Frequency Analysis ($\chi^2(9) = 30.54$, $p = .002$), Time to Satisfy Request ($\chi^2(9) = 28.83$, $p = .003$) and Correct Performances ($\chi^2(9) = 33.15$, $p = .001$). These results suggest that, over the course of the training, significant improvements were observed in all measured motor parameters, which are indicative of the positive impact of the VR

training on upper limb motor skills and stereotypy reduction. This confirms that the program is effective in promoting continuous progress throughout the sessions (table 19).

Table 19
Comparison of Median and Range for Experimental Group during Training Sessions

Parameter	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9	Session 10
Behaviour Aspects										
Number of Aids Given	32.00 (12–64)	35.00 (14–70)	32.00 (16–68)	31.00 (13–64)	25.00 (10–60)	25.00 (11–60)	22.00 (10–60)	20.00 (8–56)	20.00 (8–56)	18.00 (7–48)
Reaching Task - Motor Aspects										
Temudo Intensity	120.00 (35–260)	125.00 (50–300)	130.00 (40–250)	120.00 (45–250)	130.00 (50–300)	120.00 (45–250)	115.00 (50–240)	115.00 (50–220)	105.00 (50–200)	80.00 (30–180)
Temudo Frequency	13.00 (5–30)	14.00 (5–40)	13.00 (5–35)	12.00 (5–30)	12.00 (5–30)	11.00 (5–30)	11.00 (5–30)	11.00 (5–30)	10.00 (5–20)	7.00 (4–15)
Time to Satisfy Request	55.00 (30–100)	50.00 (30–100	45.00 (30–90)	40.00 (30–80)	40.00 (20–80)	35.00 (20–80)	33.00 (20–70)	32.00 (20–70)	30.00 (20–60)	25.00 (15–50)
Maximum Arm Extension	0.40 (0.20–0.60)	0.45 (0.20–0.60)	0.40 (0.20–0.60)	0.40 (0.20–0.60)|	0.40 (0.20–0.60)|	0.40 (0.20–0.60)	0.40 (0.20–0.60)	0.40 (0.20–0.60)	0.40 (0.20–0.60)	0.40 (0.20–0.60)
Correct Performances	5.00 (3–9)	6.00 (3–9)	7.00 (4–9)	7.00 (4–9)	7.00 (4–9)	7.00 (4–9)	7.00 (4–9)	7.00 (4–9)	8.00 (5–9)	8.00 (5–9)
Opening Hands Task - Motor Aspects										
Temudo Intensity	240.00 (90–400)	240.00 (100–400)	200.00 (90–400)	190.00 (90–400)	170.00 (70–400)	160.00 (70–350)	140.00 (50–250)	120.00 (50–250)	100.00 (50–250)	100.00 (50–250)
Temudo Frequency	15.00 (7–30)	16.00 (7–40)	14.00 (7–30)	14.00 (7–30)	14.00 (7–30)	12.00 (6–25)	11.00 (5–20)	9.00 (5–15)	9.00 (5–15)	8.00 (5–10)
Time to Satisfy Request	55.00 (30–110)	50.00 (30–100)	40.00 (20–100)	40.00 (20–90)	40.00 (20–100)	35.00 (20–90)	30.00 (20–80)	30.00 (20–80)	30.00 (20–70)	25.00 (15–50)
Correct Performances	1.00 (0–3)	2.00 (0–3)	3.00 (1–5)	4.00 (2–6)	4.00 (2–6)	4.00 (2–6)	4.00 (2–6)	5.00 (3–6)	5.00 (3–6)	5.00 (3–6)

The second hypothesis aimed to assess the generalization of the results obtained from the virtual reality (VR) training program to real-world (ecological) contexts, with a focus on motor skills parameters in reaching exercises. The results showed that, regarding Time to Satisfy Request, the experimental group exhibited a significant reduction in response time in the post-test phase compared to the pre-test phase, both in virtual and ecological conditions. Wilcoxon tests confirmed that this improvement was significant within the experimental group ($p < .01$), and the Mann-Whitney U test revealed a significant difference between the groups ($p < .01$).

Finally, Improvements were also observed in the Correct Performances parameter, where the experimental group showed a significant increase in correct performances, with greater effectiveness in virtual conditions ($p < .01$, Wilcoxon; $p < .01$, Mann-Whitney U).

These improvements were observed only in the virtual context, as they could not be replicated in the ecological setting (table 20).

Table 20

Median, (range), and p-values (Wilcoxon test) for Experimental Group in Virtual and Ecological conditions

Parameters	Phases	Virtual	Ecological	p (Virtual vs. Ecological)
Temudo's Analysis of Stereotypy Intensity	Pre-Test	24.00 (12–46)	28.00 (20–48)	.210
	Post-Test	15.00 (9–30)	16.00 (10–42)	.840
Temudo's Analysis of Stereotypy Frequency	Pre-Test	210.00 (120–320)	220.00 (120–350)	.130
	Post-Test	145.00 (100–220)	149.00 (110–250)	.790
Time to Satisfy Request for Reaching Task	Pre-Test	86.00 (38–140)	92.00 (40–140)	.120
	Post-Test	56.00 (30–110)	55.00 (42–130)	.770
Time to Satisfy Request for Opening Hands	Pre-Test	113.00 (88–142)	120.00 (90–170)	.140
	Post-Test	98.00 (80–128)	100.00 (92–135)	.560
Correct Performances (Reaching Task)	Pre-Test	0.40 (0.12–0.78)	0.40 (0.10–0.80)	.910
	Post-Test	0.80 (0.55–0.95)	0.72 (0.20–0.85)	.130
Correct Performances (Opening Hands Task)	Pre-Test	0.42 (0.10–0.72)	0.42 (0.15–0.70)	.980
	Post-Test	0.82 (0.50–0.85)	0.72 (0.20–0.72)	.180

For the third hypothesis, which aimed to evaluate the role of VR in enhancing motivation and engagement during the execution of motor tasks, addressing the main challenges of the rehabilitation process for patients with RTT, the trend analysis of the data reveals significant findings. Specifically, it was anticipated that the experimental group would demonstrate improvements in attention and motivation throughout the training sessions, as well as a decrement in the number of aids required.

Table 21 shows the medians and range for each phase.

Table 21
Median and (range) of Attention Time, Number of Aids Given During Task Performance, and Motivation Index Across Training Sessions

Parameter	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9	Session 10
Attention Time (seconds)	4 (4–6)	10 (6–12)	20 (12–30)	40 (30–50)	60 (50–70)	90 (70–110)	120 (100–140)	150 (130–170)	200 (180–220)	300 (250–300)
Number of Aids Given	32 (12–64)	35 (14–70)	32 (16–68)	31 (13–64)	25 (10–60)	25 (11–60)	22 (10–60)	20 (8–56)	20 (8–56)	18 (7–48)
Motivation Index	5.50 (4–7)	5.50 (4–7)	6.00 (5–7)	5.50 (4–7)	6.00 (5–7)	6.00 (5–7)	6.00 (5–7)	5.50 (4–7)	6.00 (5–7)	6.00 (5–7)

The analysis of the trend for the Attention Time parameter shows a clear positive trajectory over the training sessions, with an increasing number of seconds dedicated to attention, ranging from 4 seconds in the first session to 300 seconds in the last session. The non-parametric trend analysis (e.g., the Friedman test or the Cochran's Q test) reveals a significant increase in attention over the training sessions ($\mathbf{p} < 0.01$), indicating that participants became more engaged and sustained their attention over time.

In terms of the Number of Aids Given, a noticeable downward trend is observed, indicating a reduction in the number of aids provided as the sessions progressed. Starting with a median of 32 (range 12–64) in the first session, it decreased to a median of 18 (range 7–48) by the tenth session. The trend analysis (e.g., the Friedman test) confirms a statistically significant decrease in the number of aids required ($\mathbf{p} < 0.05$), suggesting that the experimental group was able to perform the motor tasks with increasing independence, requiring fewer aids as their motor and cognitive engagement improved.

Finally, regarding the Motivation Index, the data shows stability in motivation, with median values consistently around 5.5 to 6 (range 4–7) throughout the training sessions. This indicates that while motivation remained relatively high and stable across the sessions, the level of engagement did not significantly increase or decrease over time. The trend analysis indicates no significant change in motivation ($\mathbf{p} > 0.05$), suggesting that motivation levels were stable throughout the training period.

In conclusion, the results support the hypothesis, as both attention and motivation remained stable or slightly improved, while the number of aids required showed a significant decline, suggesting a positive impact of the VR training on task execution and reduced reliance on external support. The trend analysis supports these conclusions, with statistically significant findings in attention ($\mathbf{p} < 0.01$) and the number of aids ($\mathbf{p} < 0.05$), while motivation remained stable ($\mathbf{p} > 0.05$). Further analysis would be needed to explore the exact factors contributing to these trends, particularly in relation to the VR program's design and individual participant responses.

4.4.4 Discussion

This study aimed to evaluate the effectiveness of a Virtual Reality (VR) training program in enhancing upper limb motor skills, promoting the generalization of these improvements to real-world contexts, and fostering motivation and engagement during rehabilitation in individuals with Rett Syndrome (RTT). The findings support the efficacy of the VR intervention, providing promising evidence for its role in improving motor skills and reducing stereotypical behaviors, as well as enhancing the overall rehabilitation experience. These findings resonate with previous research emphasizing the potential of VR in treating RTT and other neurodevelopmental disorders.

Regarding the first hypothesis, our results indicate that the VR training program significantly improved the frequency and intensity of stereotypical hand movements in the experimental group. This aligns with findings from Stasolla et al. (2013, 2015, 2018) and Mraz et al. (2016), who also demonstrated that VR-based interventions could effectively reduce stereotypical behaviors and improve upper limb motor functions in RTT patients. The significant improvements observed in the experimental group, as evidenced by the Wilcoxon signed-rank test, highlight the potential of VR as a therapeutic tool for mitigating the motor challenges associated with RTT. Conversely, the control group showed no notable changes in these parameters, reinforcing the impact of the VR intervention as opposed to traditional therapies. Additionally, the trend analysis conducted using the Friedman test revealed significant improvements across all measured motor parameters, including Temudo's analysis of intensity and frequency, time to satisfy request, maximum arm extension, correct performances, and time to open hands. These findings are consistent with previous studies, such as those by Fabio et al. (2023), who emphasized the motivational benefits of immersive VR environments. Our results further demonstrate that the VR training was successful in promoting continuous progress throughout the sessions, supporting its use as a long-term intervention strategy.

The second hypothesis, which focused on the transferability of the VR training results to real-world contexts, yielded mixed findings. While the experimental group exhibited significant improvements in motor parameters such as time to satisfy request, correct performances, and hand opening capacity in the virtual context, these improvements did not always generalize to the ecological conditions. This observation aligns with the challenges noted by Franze et al. (2024),

who discussed the difficulty of transferring VR-based gains to real-world scenarios. Nevertheless, the improvements observed in both virtual and ecological contexts indicate the potential for VR to enhance functional abilities in diverse settings, a crucial factor for the success of rehabilitation programs for RTT patients. As highlighted by Caprì et al. (2020), such advances are vital, particularly for individuals in remote areas or with limited mobility.

For the third hypothesis, the results of the analysis of attention and motivation reveal a clear trend towards increased engagement in the experimental group. The data show a significant increase in attention time across the 10 sessions, suggesting that VR can foster sustained focus and active participation in rehabilitation tasks. This aligns with the findings of Fabio et al. (2023), who demonstrated the engagement benefits of VR for RTT patients. Additionally, the reduction in the number of aids required throughout the training indicates growing independence and motor proficiency, which aligns with the goals of the intervention. However, the motivation index remained relatively stable, suggesting that while participants were engaged, the VR environment did not significantly enhance motivational levels beyond baseline values. This stability in motivation could be attributed to the design of the VR program, which may require further adaptation to maximize engagement and motivation over time, as indicated by previous studies on VR-based interventions in neurodevelopmental disorders (Parsons et al., 2009; Vieira et al., 2021).

While the findings of this study are promising, several limitations must be acknowledged. First, the small sample size of 20 participants restricts the generalizability of the results to the broader population of individuals with Rett Syndrome. This limited sample also reduces the statistical power to detect subtler variations or effects. Second, the intervention duration of 40 sessions over eight weeks, while sufficient to observe initial improvements, may not provide a comprehensive understanding of the long-term impact of VR training. A longer intervention period might clarify whether these benefits can be sustained or amplified over time. Additionally, the study lacked a long-term follow-up phase. The absence of evaluations beyond the immediate post-training period makes it challenging to assess the durability of the observed improvements or to determine the extent to which skills learned in VR translate to real-world contexts over time. Incorporating follow-up assessments three months or more post-intervention would provide valuable insights into the lasting effects of VR rehabilitation. Another limitation pertains to the

transferability of skills. While participants demonstrated significant improvements in the virtual environment, these gains were not consistently mirrored in real-world (ecological) settings. This inconsistency highlights the need for further refinement of VR systems to better support the generalization of skills from virtual to everyday environments. Motivation also presented a limitation. The stability of the motivation index suggests a potential ceiling effect, as participants were already highly motivated at the start of the program. This limited the ability to assess whether VR could enhance motivation further, despite its engaging nature. Finally, the VR environment itself could be improved by incorporating more multisensory stimuli and dynamic design elements. These features could increase engagement, sustain motivation over longer periods, and make the VR experience more immersive, ultimately enhancing the rehabilitation process. Addressing these limitations in future research will be critical to maximizing the effectiveness of VR interventions for individuals with Rett Syndrome and ensuring their applicability to real-world rehabilitation scenarios.

4.4.5 Conclusion

The findings of this study suggest that VR training can be an effective tool for enhancing upper limb motor skills, reducing stereotypical behaviors, and promoting engagement in rehabilitation for individuals with Rett Syndrome. These results resonate with previous research on the potential of VR in RTT rehabilitation, as evidenced by studies such as those by Stasolla et al. (2013), Fabio et al. (2023), and Mraz et al. (2016). The significant improvements in motor performance and the reduction in the number of aids required indicate the potential of VR to address some of the core challenges faced by individuals with RTT in traditional rehabilitation settings, as noted by Lotan & Ben-Zeev (2006). While the generalization of these improvements to real-world contexts was not always observed, the overall results support the integration of VR into rehabilitation programs for this population, as advocated by Caprì and colleagues (2020).

Future research should focus on refining VR environments to enhance skill transfer to real-world contexts, incorporating multisensory elements and personalized tasks to improve motor outcomes and engagement. It should also investigate strategies to sustain motivation during extended rehabilitation, ensuring long-term benefits for individuals with RTT.

4.5 Experimental Study 5: Teaching Through Gaze: The Impact of Fiaba Interattivas on Learning in Patients with Rett Syndrome

As described in Chapter 2, cognitive rehabilitation using an eye tracker has proven effective in Rett syndrome. This experimental study aims to test a new tool for incorporating learning content while increasing motivation and reducing frustration.

Interactive fairy tales and Serious Games

Interactive fairy tales and Serious Games offer several educational tools to enhance motivation, communication skills, and improve motor and learning abilities. Fairy tales have been recognized as effective educational tools for addressing difficulties and stressful events, as well as for stimulating creative senses (Kuciapiński, 2014; Muthik et al., 2022). These narratives not only serve a psychological support function but can also be used for diagnostic and educational purposes (Duss, 1940;Apriliya, Nuraeni, Nurjanah et al., 2023). The literature has extensively explored the psychological significance of fairy tales and their therapeutic effects on children's minds (Otaboyeva, 2023; Von Franz, 2017). Therapeutic fairy tales allow children to identify with characters and find solutions to their problems, providing them with the awareness that they are not alone (Sheldon, 2004). The Sheldon's study focuses on a specific type of therapeutic fairy tale that stands out for its interactivity and realism. Interactive fairy tales enable children to directly and spontaneously engage with characters and story content, participating in imaginative play and identifying with various protagonists (Sindic et al., 2022; Kole, 2018; Bateman, 2021). One application of these interactive fairy tales is found in Serious Games, which are digital games designed to educate and instruct rather than solely entertain (Mori, 2012; Chiazzese, Tosto, Seta et al., 2023; Fabio et al., 2019; Papanastasiou, Drigas, Skianis et al., 2017). Serious Games incorporate educational elements through narrative-driven gameplay where users make consequential choices for the protagonist (Bateman, 2021). Starks (2014) highlights the pedagogical value of video games, noting that they can introduce evaluative and educational goals without sacrificing entertainment. Unlike commercial games, Serious Games are designed with well-defined educational objectives, considering aesthetic, narrative, and technical characteristics that foster engagement (Chiazzese et al., 2023; Stark, 2014; Poels et al., 2007). Serious Games offer significant benefits in rehabilitation, providing opportunities for treatment in various environmental contexts and allowing patients to

"play" from home under the supervision of a tutor or parent (Calleja, 2011; Jennett, Cox, Cairns et al., 2008). Interacting with a game that involves the patient allows them to immerse themselves in a daily situation and learn to make choice in specific situations in the most appropriate way, internalizing the experiences to modify their own behavior (Calleja, 2011; Jennet et al., 2008).

The ability to make choices is crucial for improving quality of life and increasing the sense of control in individuals with developmental neurological disorders (Castelli et al., 2013; Lancioni et al., 2018). Digital games are increasingly utilized in special education to support well-being, social skills, independence, and inclusion for students with special needs, such as autism spectrum disorders and learning difficulties (Durkin, Elsabbagh, Barbaro et al., 2015). Recent studies demonstrate that digital games, using eye-tracking technologies, can enhance motivation and attention in individuals with Rett Syndrome (Evans et al., 2013; García-Redondo, García, Areces et al., 2019).

Innovative Technologies meets the needs of patients with Rett Syndrome

As described in Chapter 3, eyetracker technology proves to be effective in the cognitive and communicative rehabilitation pathways of patients with Rett syndrome.

Building on the advancements of Serious Games, which are designed to provide educational and therapeutic benefits beyond mere entertainment, and the established efficacy of eye-gaze tracking technologies, there is promising potential for further enhancing the support provided to patients with RTT (Amoako et al., 2020; Fabio et al., 2020; Stasolla et al., 2021; Vessoyan et al., 2018). Serious Games can be tailored to include interactive elements that not only engage users but also facilitate learning and cognitive development through structured gameplay. When combined with eye-gaze tracking, these games can create customized experiences that accommodate the specific needs of individuals with RTT, offering a dynamic and interactive approach to therapy.

By integrating Serious Games with eye-gaze tracking technology, it is possible to develop tools that address both cognitive and motor challenges faced by patients with RTT. These advanced technologies can help bridge communication gaps, provide opportunities for skill development, and offer greater autonomy and self-expression. As research and development in this area continue to evolve, there is potential for creating more effective and personalized solutions that improve the overall well-being and functional abilities of individuals with RTT.

Aim of the present study

The present study aims to investigate the role of interactive eye-gaze digital games in improving attention and motivation in girls with RTT. We will analyze eye-tracking parameters, such as fixation length and their correlation with attentional abilities and learning (Kumazaki, Warren, Swanson et al., 2019).

Our research distinguishes itself by focusing on an interactive fairy tale presented through Serious Games, allowing participants to actively shape the narrative. This study explores an uncharted territory by examining the impact of interactive choices within a fairy tale on attention, motivation, and learning outcomes in girls with RTT. Eye-tracking parameters are used to monitor fixations, while motivation is assessed through non-verbal responses and a happiness index. Additionally, the discomfort index is used to gauge the tolerability of the interactive fairy tale, and learning outcomes will be measured through the recognition index of correct choices made during the interaction. The objective is to determine whether interactive eye-gaze fairy tales, structured as Serious Games, can enhance motivation, selective attention, and learning in individuals with RTT.

The significance of this study lies in its unique focus on the interactive elements of the fairy tale, allowing participants to influence the story's progression. This exploration of an under-researched area may lead to groundbreaking insights, highlighting the need for further research to understand the impact of interactivity on learning experiences for individuals with RTT. The underlying rationale of this study is twofold: first, to enhance learning predispositions by addressing motivation and engagement, and second, to emphasize the critical connection between increased motivation and the selection of appropriate technologies to facilitate the learning process. It is posited that if girls with RTT perceive their agency in shaping the outcome of a story—where their choices actively influence its direction—this participatory engagement may amplify their motivation. This study aims to provide novel insights into the interplay between motivation, active participation, and the strategic use of technologies in enhancing learning experiences for this population.

4.5.1 Methods

4.5.1.1 Participants

Fifty patients and young women diagnosed with RTT were recruited from the Italian Rett Association, ranging in age from 5 to 33 years. Twenty-two of them participate to the calibration phase, while twenty-eight participated to the experiment. All participants were born to non-consanguineous marriages, received regular vaccinations, and had normal birth weight and height. Patients with RTT were classified as belonging to clinical stage III (characterized by prominent hand apraxia/dyspraxia, apparently preserved walking ability, and some communicative capacity, mainly through eye contact) or stage IV (late motor deterioration, with progressive loss of walking ability), according to the criteria for classic RTT by Hagberg (1985). All participants exhibited pervasive hand stereotypies. They all attended schools or socio-educational centers. A psychologist conducted a general assessment using the Rett Assessment Rating Scale (RARS) (Fabio et al., 2005) and the GAIRS Checklist for Rett syndrome (Fabio et al., 2022). Table 22 shows the characteristics of the groups.

Table 22
Characteristics of the participants.

Partecipants	ID	Clinical Stage	Age	Mecp2 Mutation	Severity Level (RARS)	Functioning Level (GAIRS)
Experimental Group						
1	B.M	III	5	T158M	70.5	201
2	C.A.	III	6	2e.502	67.5	172
3	D.G.	III	7	T158M	85.5	204
4	D.E.	III	5	C916	81	183
5	D.G.	III	22	P152R	53.5	244
6	D.D.	III	14	C397	77	137
7	F.D	IV	19	T158M	55	120
8	G.L.	III	13	P152R	64	223
9	L.G.	IV	18	G226	51.5	196

10	M.A.	III	25	C965C	70	215
11	M.M.	IV	16	P133C	79	191
12	O.S.	III	22	R255X	79	229
13	R.E.	IV	33	C965C	91.5	199
14	R.E.	III	4	P152R	58	149
15	S.D.	IV	17	R255X	91.5	160
16	T.G.	IV	19	T158M	91.5	139
18	V.G.	III	22	P152R	58.5	292
19	V.S.	III	3	P152R	91.5	200
Control Group						
20	B.E.	III	7	T158M	65	283
21	B.A.	IV	14	C806	57.5	144
22	C.E.	II	22	P152R	44.5	167
23	D.D	III	33	R255	68.5	129
24	P.T.	III	11	T158M	64	92
25	E.L.	III	7	P152R	65	201
26	S.G.	III	14	R255X	57.5	215
27	O.M.	II	22	T158M	44.5	172
28	L.S	III	5	P152R	68.5	244
29	F.C	III	16	R255X	64	145
30	S.G.	IV	17	R255X	90.5	161
31	F.A.	III	7	P152R	65	201
32	R.A.	III	5	C916	81	183
33	D.L	II	22	P152R	44.5	167
34	D.G.	III	22	P152R	53.5	244
35	A.L	III	5	P152R	68.5	244
36	P.T.	II	22	T158M	44.5	172
37	B.E.	III	7	T158M	65	283
38	P.R	III	8	C965C	55.5	200

In relation to the eligibility criteria for participants with Rett Syndrome, individuals were required to be able to sit and observe the presented stimuli independently or with limited support. Exclusion criteria applied to patients with genetic mutations in FOXG1 and CDKL5.

The participants were divided into two groups: the experimental group and the control group. Additionally, the referring neuropsychiatrists were asked to provide a medical severity rating based on the typical characteristics of the syndrome (epilepsy, mood swings, seizures, aerophagia, scoliosis). The severity level ranged from 5 (mild severity) to 20 (severe severity). The mean severity index for the typical syndrome characteristics was 9.

4.5.1.2 Materials

For data collection, an eye-tracking system was used. This instrument recorded eye movements such as the position and duration of fixations (when the eyes stop on an object of interest). The participants were positioned at approximately 30 cm from the screen, and an infrared ray system recorded the patients' eye movements (Figure 18).

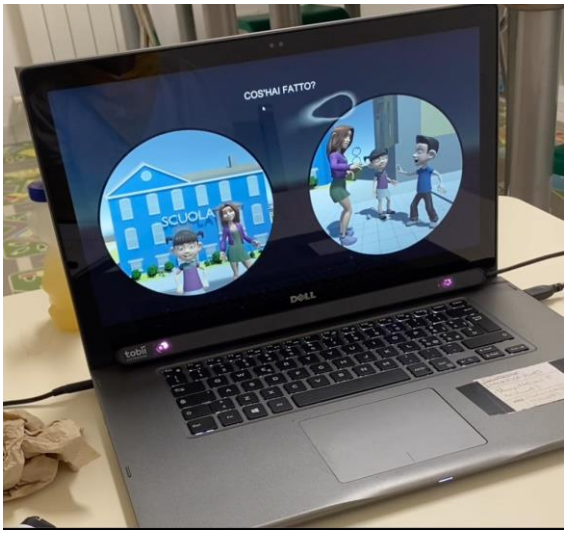


Figure 18. Example of the eye-tracking system used during the interaction with the fairy tale.

The Fiaba Interattiva was presented directly through this system and specifically designed and conceived for use with patients with Rett Syndrome. The selection and construction of realistic characters also considered the patients' interest in faces and facial expressions (Figures 19).

Annas's teacher

Anna

Anna and her friend

Figure 19. Example of the characters used

4.5.2 Study 1: Calibration Work Prior to Fairy Tale Development

Before the development of the Fiaba Interattiva, a rigorous calibration process was carried out with a cohort of 22 patients diagnosed with Rett Syndrome. This process aimed to assess their preferences in various aspects, which subsequently informed the design and content of the fairy tale. The key areas evaluated during this calibration phase included type of faces, colors, moving or static figures, scenes with or without accompanying music, scenes with or without many details (Figure 13).

Preference for Round and Close-Up Faces: The study investigated the patients' inclination towards round facial features presented in a close-up view. This preference was instrumental in shaping the visual design of the characters within the Fiaba Interattiva. The length of fixations was

the parameter. The round facial features were observed longer than the triangle and square facial feature, $t(21) = 3.45$, $p<.01$, $d=0.78$.

Preference for Soft Colors (Shades of Pink and Lilac): The research sought to determine the patients' attraction to soft and gentle color palettes, specifically shades of pink and lilac. This preference significantly influenced the color scheme and overall visual aesthetics of the fairy tale, the measurement parameter was again the length of fixation vs pink or lilac images and vs grey or green images, $t(21) = 4.11$, $p<.01$, $d=0.79$.

Preference for Observing Moving Figures: The study examined the patients' inclination toward observing dynamic and moving visual elements, with static picture observed lesser time the dynamic ones, $t(21) = 5.11$, $p<.01$, $d=0.81$. This insight was vital for incorporating animated components and actions within the Fiaba Interattiva.

Preference for Accompanying Music: The patients' inclination towards scenes accompanied by background music was investigated. This finding guided the incorporation of musical elements into the fairy tale to enhance the overall sensory experience. Again, when the patients watched scenes accompanied by background music, they focus longer time then when they watched the same scene without music, $t(21) = 7.43$, $p<.01$, $d=0.91$.

Preferring Scenes with Minimal Stimuli and Limited Details: the study examined the patients' inclination toward scenes with varying levels of detail. It was observed that the patients spent a longer duration viewing scenes with fewer details compared to scenes with a higher level of detail, $t(21) = 3.78$, $p < .01$, $d = 0.71$.

The results of this calibration work provided a foundation for crafting a tailored Fiaba Interattiva designed to meet the specific preferences and engagement factors identified in this cohort. These insights helped create a narrative that resonated with the patients and maximized their interest and participation in the storyline. The calibration phase ensured that the Fiaba Interattiva was not only engaging but also tailored to the unique preferences of the target audience, patients with Rett Syndrome.

4.5.2.2 Narrative Structure and Interactivity

Within the narrative of the fairy tale, the participants were placed in scenarios that mimicked everyday life, with a particular focus on the experiences of a typical school day. This narrative followed the fundamental concept of a narrative game with consequential choices. The story revolved around a central character, Anna, and her journey through various school activities.

In this interactive narrative, the user, using the eye-tracking system, had the opportunity to make decisions on behalf of the protagonist, Anna. The user was presented with two possible responses or choices at critical junctures in the story. By selecting one of these options through the eye-tracking system, the patients could influence the direction of the story. This interactivity allowed them to immerse themselves in a daily life scenario and actively shape the progression of the story.

To capture and maintain the attention of the patients with Rett syndrome, the narrative of the experimental condition incorporated four distinct "choice events." These events served as pivotal moments in the narrative where the user's decisions significantly influenced the storyline. These carefully designed interactive elements aimed to enhance engagement and foster a sense of agency within the narrative, making it a more immersive and personalized experience for the participants (Fig.20).

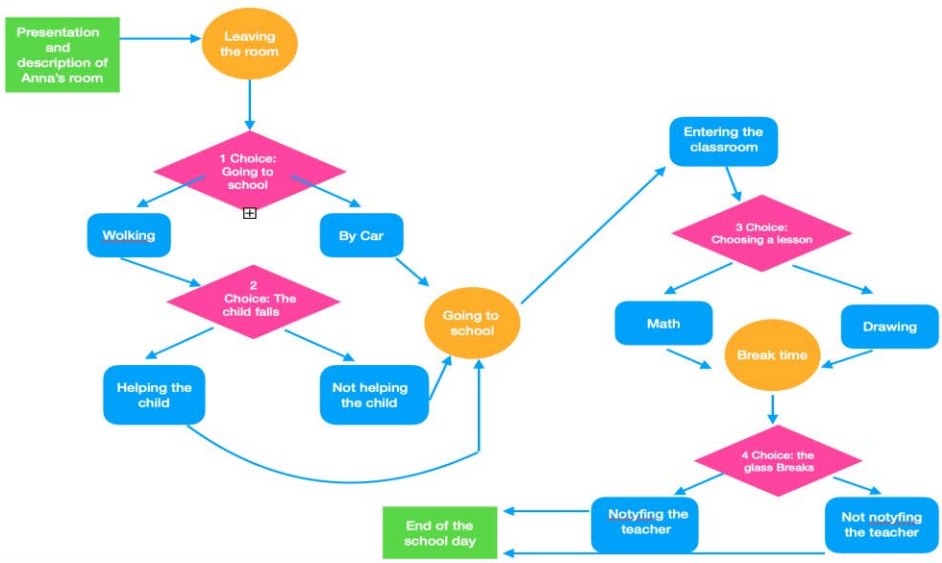


Figure 20. Development of the Fiaba Interattiva with various choice possibilities.

To accomplish this, a predefined storyline served as the foundation for the development of the narrative using the Unity 3D game engine, renowned for its capabilities in creating advanced graphical interfaces. A visually appealing 3D environment was crafted, incorporating captivating graphical elements to sustain a high level of engagement and maintain the patients' interest in the storyline. Special attention was devoted to colors, element dimensions, and camera viewpoints to enable the patients to identify with the protagonist and avoid distractions from other elements. Furthermore, facial expressions were integrated to establish a more lifelike scenario. The narrative featured multiple branches, prompting the patients to make decisions regarding its progression. Decision-making was facilitated through carefully designed eye-controlled buttons to ensure a high level of interaction.

4.5.3 Study 2

The study lasted approximately one month. Initially, a therapist conducted a global evaluation of the participants using the Rett Assessment Rating Scales (RARS) (Fabio et al., 2005) and the GAIRS Checklist (Fabio et al., 2023). Additionally, their discomfort and happiness indices were monitored to assess each subject's baseline reaction to the interactive story.

Subsequently, the therapist presented the fairy tale to both groups (interactive and non-interactive), with a minimum of 1 viewing and a maximum of 10 viewings. The experimental group (eighteen patients) was able to view the story while making choices, actively deciding what the characters would do, whereas the control group (ten patients) could only view the non-interactive version of the story.

Eleven patients' parents made them available to review the fairy tale up to 10 times. After watching the fairy tale, the therapist re-tested the participants' cognitive abilities to evaluate any improvements in both conditions (interactive and non-interactive).

4.5.3.1.1 Measures

The participants were exposed to the fairy tale a minimum of 1 time and a maximum of 10 times. Throughout all phases, a therapist recorded:

- Happiness and discomfort indices.

The *discomfort index* was coded based on behavioral responses of participants during exposure to the Fiaba Interattiva, including the presence or absence of certain parameters: gaze direction, facial expressions (disgust, anger, sadness), aggressive behaviors (for situation avoidance), psychological reactions (body stiffness), and the use of conventional gestures (to indicate whether to continue or suspend the activity). The discomfort index was determined by the presence of at least 4 out of the 5 considered behaviors. Regarding body movement and posture, the presence or absence of stereotypies was assessed. The discomfort index was deduced from the summation of the considered behaviors. The *happiness index* was derived from composite parameters consisting of six sub-parameters: gaze direction, sounds produced by participants, facial expressions, aggressive behaviors (such as situation avoidance), psychological reactions (blushing, body stiffness), and use of conventional gestures (to indicate whether to continue or suspend activities). The encoding of these composite parameters follows a specific methodology. The composite parameter related to the happiness index will be derived from the presence of at least 5 out of the 6 considered behaviors. The composite parameter related to the happiness index is derived from the summation of the considered behaviors.

- Cognition measurements.

The parameter related to cognition were: fixation length (FL) and the number of correct responses (CR). FL is the amount of time (in seconds) that the subject spent looking at relevant elements of the fairy tale (specific to each presented scene); RC refers to the number of correct responses that the subject provided in a recognition task; after the completion of the fairy tale viewing, the therapist asked the subjects in the experimental group to recall the choices they had made during the interactions. (e.g. “Do you remember if you chose to have the girl go to school on foot or by car?").

- Global evaluation assessment.

For the global evaluation, the RARS (Fabio et al., 2005) and the GAIRS Checklist (Fabio et al., 2023) were used. The RARS scale is a standardized scale used to assess patients with RTT. It is organized into seven domains: cognitive, sensory, motor, emotional, autonomy, typical disease characteristics, and behavior. The typical disease characteristics and behavioral domains measure the following features: mood swings, seizures, dyspnea, hyperactivity, anxiety, aggressiveness,

bruxism, oculogic crises, epilepsy, aerophagia, muscle tension, and food preferences. A total of 31 items were generated as indicative of the RTT profile. Each item is accompanied by a brief glossary explaining its meaning in a few words. Each item is assessed on a 4-point scale, where 1 = within normal limits, 2 = infrequent or low anomaly, 3 = frequent or moderate-to-high anomaly, and 4 = strong anomaly. Intermediate ratings are possible; for example, a response between 2 and 3 points is evaluated as 2.5. For each item, the evaluator circles the corresponding number that best describes the patient. After a patient has been assessed on all 31 items, a total score is calculated by summing the individual ratings. This total score allows the evaluator to identify the severity level of RTT, conceptualized as a continuum ranging from mild symptoms to severe deficits (Mild = 0-55; Moderate = 56–81; Severe = > 81). The standardization of the Rett Assessment Rating Scales (RARS) was conducted through a procedure involving a sample of 220 patients with RTT, demonstrating that the instrument is statistically valid and reliable. Specifically, analyses of the normal distribution of scores were calculated, and the scale's mean scores were similar to the median and mode. The skewness and kurtosis values, calculated for the distribution of the total score, were 0.110 and 0.352, respectively. The distribution was found to be normal. Cronbach's alpha is used to determine internal consistency for the overall scale and subscales. The total alpha was 0.912, and the internal consistency of the subscales was high (ranging from 0.811 to 0.934).

The second global evaluation assessment is the GAIRS is a global checklist for evaluating individuals with Rett syndrome. Through a comprehensive analysis, it provides an overview of different areas and is intended for the functional analysis of the patient's overall abilities. The GAIRS checklist consists of 10 macro-areas: basic or prerequisite behavior, neuropsychological skills, basic cognitive concepts, advanced cognitive concepts, communication abilities, emotional-affective skills, fine motor hand skills, graphomotor skills, gross motor skills, and level of autonomy in daily life. For each area, various sequentially structured skills are evaluated. A total of 85 competencies are assessed. Each skill has a numerical score ranging from 1 to 5, where 1 represents the minimum level of capability and 5 represents the maximum level of capability to perform a specific activity. Here are some examples. In the area of basic behavior, the first skill assessed is spontaneous eye contact. The score for this skill is as follows: 1 if the child is unable to establish spontaneous eye contact, 2 if the child can establish spontaneous eye contact 2-3 times out of 10, 3

if the child can establish spontaneous eye contact 4-6 times out of 10, 4 if the child can establish spontaneous eye contact 7-8 times out of 10, and 5 if the child always establishes eye contact. On the other hand, the sixth skill studied in the hand motor area is the ability to grasp, and the score is as follows: 1 if the child cannot grasp an object on the table, 2 if the child can grasp an object from 5 cm using a palmar ulnar grip, 3 if the child can grasp an object from 5 cm using a palmar grip.

4.5.3.1.2 Procedure

This study lasted from the end of August to the end of September 2022. Before the start of the study, professionals from the AIRETT center reached out to families via phone to confirm their willingness to participate and to conduct the GAIRS assessment. Subsequently, parents were invited to join an online session during which they completed the RARS. This assessment is instrumental in determining the severity of Rett syndrome in patients. After these sessions, the GAIRS assessment was administered by all professionals from the AIRETT Center (a physician, a speech therapist, and a psychologist). All professionals had certified specialized training in Rett syndrome. Some skill scores that could not be directly assigned during the assessment, such as the ability to use the bathroom, were evaluated through videos or interviews with the parents. Each skill was queried 3 times, but if the participant provided the first 2 correct responses, the skill was considered acquired; similarly, if the participant provided the first 3 incorrect responses, the skill was considered not acquired. The total administration time was approximately 4 hours (range: 3 to 7), but for severely affected patients, it was necessary to divide the checklist administration into multiple sessions (2 or 3). During the first phase, a therapist assessed the patients' skills using the RARS Scale and administered the GAIRS Checklist. The therapist recorded the seconds of selective attention from the beginning of the fairy tale until the patients averted their gaze and evaluated the discomfort and happiness indices of each subject during the activity. During the second phase, the therapist presented the fairy tale. During the presentation, the participants were exposed to the fairy tale according to the condition to which they belonged, either with or without interaction.

4.5.3.2 Results

The results were initially examined in relation to cognitive aspects. Figure 15 illustrates the fixation lenght for both the control group and the experimental group across all story elements throughout the narrative development. This analysis was particularly significant, as each subphase emphasized either the protagonist or a relevant element. The analysis also included calculating the proportion of attention directed by participants to each frame (Figure 21). For each scene, the exact duration was calculated—for example, 3 seconds for one scene and 6 seconds for another—and the fixation length was then determined proportionally. For instance, if a child observed the main character in a 3-second scene for the full 3 seconds, the proportion was calculated as 1. If she observed the main character for 2.5 seconds, the proportion was calculated as 2.5/3 = 0.83.

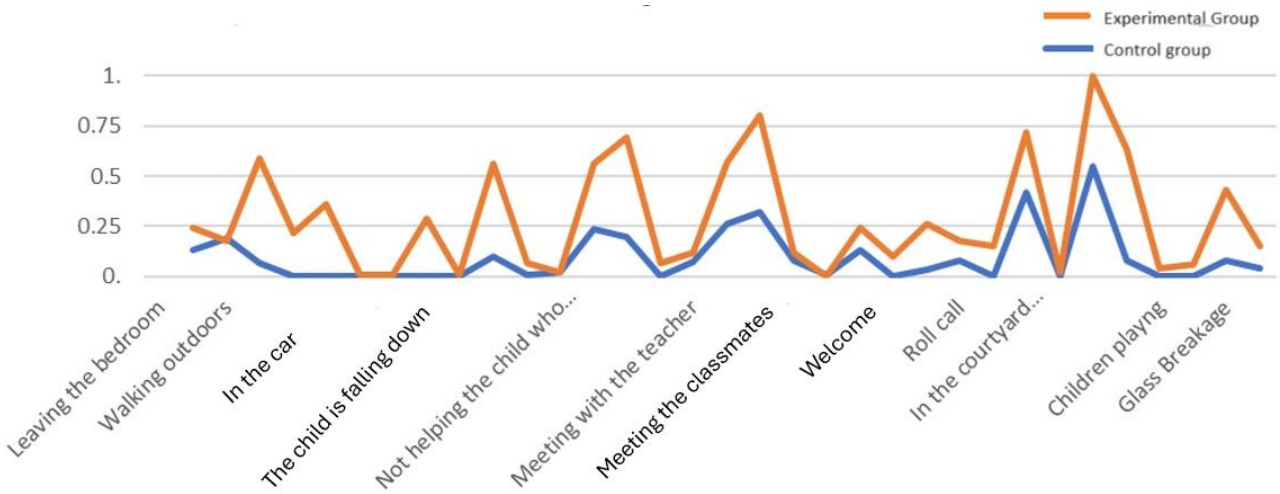


Figure 21. Proportion of attention (fixation length) to significant elements of the Fiaba Interattiva for each group

As emerged from the results, the experimental group showed higher level of fixation length compared to the control group throughout the viewing of the story, $F(1, 349) = 18.34$, $p < .001$, $\eta^2=0.76$. This trend remained consistent even during the 10 viewing of the story for both groups.

The results confirm the initial hypothesis that participants who could interact with the story and characters through eye tracking (experimental group) continued to have longer fixation

length in terms of seconds compared to the control group, and this higher fixation index persisted even during repeated presentations of the story.

Regarding the recognition task, the number of correctly recognized responses was considered in relation to the choices made during the interactive story (figure 22).

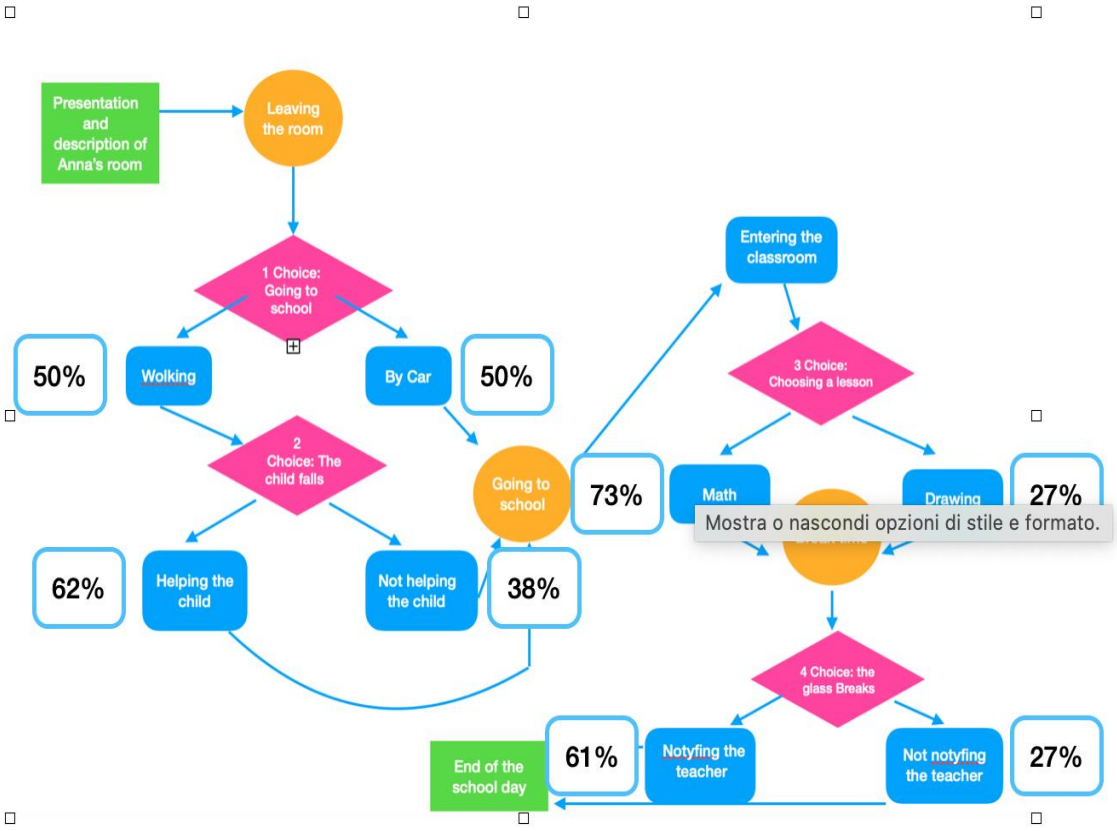


Figure 22. *Percentage of choices in one of the two directions made by the patients during the fairy tale presentation.*

The findings indicate that performance is above 50%, but on average, it hovers around 50% for repeated indices (Table 23).

Table 23
Means and standard deviations of the recognitions of the correct choices made for all repetitions of the fairy tale.

	Means	Standard Deviation
N° of the correct choices		
1° repetition	2.5	0.70
2° repetition	3.00	0.00
3° repetition	2.72	1.81
4° repetition	2.52	1.41
5° repetition	2.54	0.70
6° repetition	2.5	1.41
7° repetition	2.00	0.70
8° repetition	2.5	0.70
9° repetition	2.5	0.21
10° repetition	3.00	0.00

The factor phases does not show significant effects, $F(9, 112) = 15.58$, $p < .06$. This indicates a fairly stable performance across the repeated story trials, despite few participants completing all 10 repetitions. They remain consistent in remembering the choices they made.

This parameter was considered only in reference to the experimental group, which had the ability to make choices during the viewing of the story, while the control group did not have this opportunity.

Table 24 presents the means and standard deviations of the happiness index for the 10 presentations of the story.

Table 24
Means and standard deviations of the happiness index for the 10 presentations of the story.

		Mean	Standard Deviation
1° ripetition	Experimental Group	3.11	0.78
	Control Group	3.4	1.51
2° ripetition	Experimental Group	3.11	1.05
	Control Group	2.00	1.41
3° ripetition	Experimental Group	2.88	1.16
	Control Group	1.8	1.30
4° ripetition	Experimental Group	3.33	1.31
	Control Group	1.4	0.89
5° ripetition	Experimental Group	3.33	1.00
	Control Group	2.00	1.41
6° ripetition	Experimental Group	3.16	0.78
	Control Group	1.5	1.09
7° ripetition	Experimental Group	2.96	1.28
	Control Group	1.38	0.90
8° ripetition	Experimental Group	3.70	1.12
	Control Group	1.45	0.88
9° ripetition	Experimental Group	3.55	0.99
	Control Group	1.47	0.75
10° ripetition	Experimental Group	3.55	1.12
	Control Group	1.30	1.52

The group factor revealed significant effects, $F(1, 18) = 6.43$, $p < .02$, $\eta^2 = 0.81$, indicating that the patients in the experimental group exhibited higher happiness indices compared to those in the control group. Additionally, the interaction between group and story presentation phases was significant, $F(1, 18) = 3.81$, $p < .05$, $\eta^2 = 0.65$. This suggests that during the initial phase, the two groups did not differ significantly, as indicated by a non-significant t-test, $t(27) = 0.96$, $p = .22$.

However, from the second presentation of the story onwards, there was a notable increase in the happiness index among the patients in the experimental group compared to those in the control group. One participant from the control group was excluded from the analysis due to a constant happiness index irrespective of the stimulus presented (Figure 23).

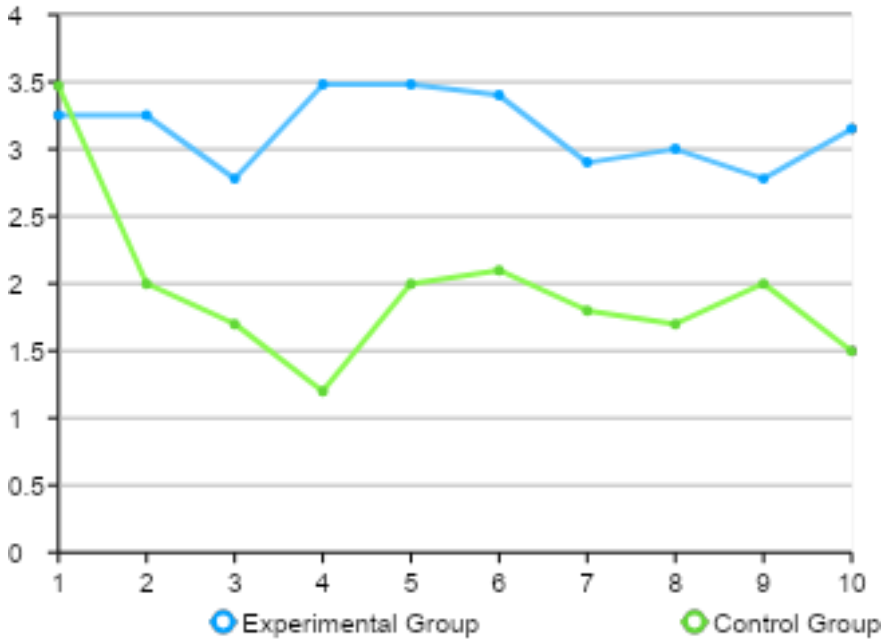


Figure 23. Happiness index during the 10 fairy tale presentations.

Regarding the discomfort index (Table 25), the data remain consistently low, indicating no significant differences between the two groups, suggesting that no instances of intrusiveness or avoidance were observed during the viewing of the story in both groups.

Table 25
Means and standard deviations related to the intolerance index.

		Media	Deviazione Standard
Intolerance Index			
1° repetition	Experimental Group	0.42	0.26
	Control Group	0.55	0.50
2° repetition	Experimental Group	0.68	0.49
	Control Group	0.88	0.36

3° repetition	Experimental Group	0.42	0.75
	Control Group	0.57	0.27
4° repetition	Experimental Group	0.53	0.27
	Control Group	0.41	0.34
5° repetition	Experimental Group	0.66	0.48
	Control Group	0.81	0.19
6° repetition	Experimental Group	0.56	0.43
	Control Group	0.61	0.28
7° repetition	Experimental Group	0.48	0.29
	Control Group	0.57	0.52
8° repetition	Experimental Group	0.43	0.31
	Control Group	0.44	0.56
9° repetition	Experimental Group	0.68	0.21
	Control Group	0.55	0.17
10° repetition	Experimental Group	0.70	0.18
	Control Group	0.51	0.22

4.5.3.3 Discussion

The present study aimed to explore the impact of interactive eye-gaze digital games, specifically structured as fairy tales, on improving attention, motivation, and learning in patients with Rett Syndrome (RTT). Grounded on previous research emphasizing the psychological significance and therapeutic effects of fairy tales on children's minds (Bettelheim, 1950; Von Franz, 2017), the study focused on an interactive and realistic fairy tale that facilitated direct and playful interaction with characters and storyline, fostering imaginative play and emotional identification (Sjndic et al., 2022; O'Brien et al., 2008). The study extended its investigation into the domain of Serious Games, where digital games serve not only for entertainment but also for educational and instructional (Douglas & Hargadon 2001; Mori, 2012; Chiazzese et al., 2023)

The findings demonstrate that interactive digital games, when designed with attention to the preferences and cognitive abilities of individuals with RTT, can significantly enhance engagement and selective attention. The experimental group, which was exposed to the interactive version of the

fairy tale, showed a marked improvement in fixation length and happiness compared to the control group, which viewed a non-interactive version of the story. These results suggest that the opportunity to make choices and directly influence the storyline, even though minimal interaction via eye-gaze, can foster greater cognitive involvement and enhance memory retention in patients with RTT.

The study also underscores the importance of designing digital content that resonates with the sensory and cognitive preferences of the target population. The calibration phase, which identified preferences for certain facial features, colors, movement, and sound, was crucial in crafting a narrative that maximized attention and engagement. This tailored approach appears to be instrumental in enhancing the therapeutic potential of Fiaba Interattivas, as it aligns the content with the innate interests and abilities of the participants.

Moreover, the use of eye-tracking technology provided valuable insights into the attentional patterns of the participants. The longer fixation times and increased accuracy in recalling narrative choices in the experimental group highlight the effectiveness of this technology in assessing cognitive engagement. The eye-tracking data not only served as an objective measure of attention but also revealed the participants' ability to process and interact with the digital content meaningfully, despite the severe motor impairments associated with RTT.

The significant difference in happiness indices between the experimental and control groups further supports the notion that interactivity within a digital narrative can positively influence emotional responses. The interactive group exhibited higher happiness indices, suggesting that the sense of agency and involvement in the story contributed to a more enjoyable and less distressing experience. This finding is consistent with the broader literature on the benefits of interactive media, which suggests that participation and agency in a narrative can enhance emotional well-being, particularly in populations with limited opportunities for self-expression and choice-making. The opportunity to make choices within these games can significantly enhance their quality of life and sense of control (Lancioni et al., 2018; Fabio et al., 2018; 2019).

The study's significance lies in establishing a connection between increased motivation and the selection of appropriate technologies to expedite the learning process. The results of the study align with existing literature (Sánchez-Peris & Sedeño, 2010) concerning the use of Serious Games

in rehabilitative contexts. Notably, patients who had the opportunity to use the interactive story and make choices exhibited increased attention to the story, as evidenced by longer fixation times on different presented situations, compared to those who could not make choices. Regarding the correlation between increased motivation and the ability to recall choices, only half of the sample accurately recalled the choices made. However, it was significant that the patients demonstrated a willingness to explore new paths during the presentations, despite changes in choices. Furthermore, the ability to recall choices correctly remained constant, indicating the potential for meaningful learning experiences within the Fiaba Interattiva. In conclusion, this study has expanded knowledge on the use of Serious Games in the context of Rett Syndrome. An important focal point is the patients' perception of controlling the narrative within the fairy tale, shedding light on the crucial role of perceived agency in influencing motivation and engagement. Nonetheless, further research with larger samples and a greater number of story presentations is required to delve deeper into the examined aspects and establish a more comprehensive understanding of the impact and potential of eye-gaze digital games in improving attention, motivation, and learning in individuals with RTT.

However, it is important to acknowledge some limitations of the study. The sample size, although adequate for initial findings, may not be representative of the broader population of individuals with RTT. Additionally, the study focused exclusively on patients with RTT, given the gender-specific prevalence of the disorder, which limits the generalizability of the results to other populations with similar neurodevelopmental challenges. Future research should aim to include larger and more diverse samples to validate and expand upon these findings.

In conclusion, this study provides compelling evidence for the potential of Fiaba Interattivas, delivered through eye-gaze technology, to enhance attention, motivation, and learning in patients with RTT. The integration of narrative interactivity within therapeutic games represents a promising avenue for improving the quality of life and cognitive engagement in individuals with severe neurological disorders. The insights gained from this research highlight the need for continued innovation in the development of digital tools that are both engaging and accessible to populations with special needs, offering new possibilities for therapeutic interventions that are both effective and enjoyable.

4.5.3.4 Conclusions

This work suggests that digital games have significant potential as educational tools for individuals with disabilities, particularly those with Rett Syndrome (RTT), and could be beneficial for rehabilitation efforts. Therapists and educators should consider integrating digital games into rehabilitation programs to improve cognitive and behavioral outcomes for individuals with RTT. The findings challenge previous assumptions about the irreversibility of cognitive and behavioral deficits in RTT, indicating that cognitive rehabilitation can achieve better outcomes than previously thought.

In practical terms, the use of Fiaba Interattivas to enhance motivation, attention, and memory provides actionable strategies for rehabilitation. Therapists are encouraged to explore interactive narratives, such as digital fairy tales, as a means to increase engagement and motivation during sessions, potentially leading to more effective rehabilitation outcomes. Furthermore, the observed improvement in happiness levels within the experimental group underscores the importance of incorporating emotional well-being into rehabilitation practices. Thus, therapists should integrate activities and interventions that foster emotional well-being and overall satisfaction as essential components of the rehabilitation process.

4.5 Ongoing Study: Serious Game for learning in Rett Syndrome: “Lettoscrittura” gaming.

Cognitive empowerment represents a central perspective in psychological and cognitive research, aimed not only at improving specific abilities but also at enhancing the modifiability of underlying cognitive processes (Feuerstein, Rand, & Rynders, 1992; Vygotsky, 1978). The theoretical principles that guide cognitive enhancement, including Vygotsky’s zone of proximal development and Feuerstein’s theory of modifiability, emphasize the crucial role of educational mediation and interaction with the environment (Tzuriel, 2021; Fabio, 2020). These approaches are based on the idea that, through appropriate experiences and stimuli, the brain can adapt its neural connections thanks to neuroplasticity (Jellinger, 2022).

Cognitive enhancement, even when applied to individuals with severe disabilities such as Rett Syndrome, demonstrates the potential for significant improvements in cognitive abilities (Fabio et al., 2016). Rett Syndrome, caused by mutations in the MECP2 gene, is characterized by motor, linguistic, and cognitive deficits (Amir & Zoghbi, 2000), but rehabilitative interventions using innovative techniques such as eye-tracking and serious games show promising potential (Fabio et al., 2018d; Fabio et al., 2021).

The”LETTOSCRITTURA” project is an example of the practical application of these theories. The software is a serious game designed to improve cognitive learning in patients with Rett Syndrome, combining the use of advanced technologies like eye-tracking with personalized teaching methodologies (Fabio et al., 2021).

4.6.1 Phase 1: Creation of the "Lettoscrittura" Serious Game for Learning

At the current stage, in collaboration with the engineering team, we have developed the *Lettoscrittura* software, an innovative and customizable tool designed to support the learning and rehabilitation of individuals with Rett Syndrome through an inclusive and playful approach. By combining gamification, eye-tracker interaction, and meticulous educational design, the software provides a motivating and engaging experience that respects the specific needs of the target audience and ensures flexibility in its use. With the aim of continually improving the product's

effectiveness and adaptability, the project focuses on developing new features and expanding the possibilities for customization.

4.6.2 Technical Features of the Software

The *Lettoscrittura* software is a cognitive serious game created for individuals with Rett Syndrome, with the objective of teaching them word recognition, the connection between words and their corresponding meanings, and their breakdown into syllables and letters. It was developed using the Unity game engine, and the interaction is managed through eye-tracking technology. The program runs on Windows computers and does not require particularly high computational power, thus maximizing its usability in various contexts. The only hardware requirement is the presence of an eye-tracker sensor, necessary for gaze tracking, which is used as an input source to control the game progression. The eye-tracker can be either integrated or external, as long as it is a model produced by Tobii.

As described in the previous study, a serious game is a specific form of video game designed to teach a cognitive task (such as performing arithmetic operations or memory tests). This type of software leverages the concept of gamification, which refers to incorporating elements traditionally associated with video games into applications not primarily intended for entertainment. By doing so, activities that are typically tedious and less engaging, such as rehabilitation, can become stimulating and exciting, enhancing the results obtained.

The project uses non-immersive Virtual Reality to create an experience that encourages greater patient engagement. Normally, applications of this type are delivered in an immersive mode, which involves using dedicated wearable headsets (such as Meta Quest 3 or HTC Vive Pro) that block most external stimuli and allow users to focus entirely on the content displayed internally. However, while this approach offers a higher level of immersion with its associated advantages, its use is generally avoided in the treatment of Rett patients due to limited tolerance and various safety concerns.

For these individuals, a non-immersive mode is preferred, where the content is displayed on a traditional monitor, preferably a large one, similar to other clinical and rehabilitation settings. This approach allows caregivers and medical staff to maintain greater control over the activity,

eliminates the need for dedicated hardware (thus reducing costs), and provides a more accessible solution for anyone with a computer.

The virtual environment consists of scenarios familiar to the subjects, such as rooms in a house or a school. The gaming experience is guided by a teacher who communicates with the players, offering praise and advice during the exercises. Although the application mode does not remove all real-world stimuli, the subjects' attention is generally captured by structuring the game appropriately to avoid boredom and by including engaging visual and auditory effects.

4.6.3 Structure of the Learning Pathway

The game is structured as a progressive eight-phase pathway (0–7) for each word to be learned, with each phase consisting of one to four sub-phases. The goal is to reinforce the association between the graphic representation of objects and their written form. In each phase, the required action involves making a choice by fixing one’s gaze on an option for a sufficiently long time. If the system does not correctly detect a selection, the caregiver can manually input the choice with a click. Additionally, if necessary, it is possible to skip one or more repetitions using a designated button.

Phase 0 serves as a review and focuses on object recognition in increasingly complex contexts. Initially, the object to be selected is presented alone in a room, then in a neutral environment alongside a distractor, and finally in a room with a variable number of 2 to 4 distracting elements. These distractors are typically chosen from a group of common objects not included in the list of learnable words and are contextually related to the specific environment in which they appear: bathroom, kitchen, garden, living room, or bedroom. An additional level of complexity is introduced when at least two other words, besides the current one, have been learned beyond Phase 0. In this case, the final scenario is revisited, incorporating previously learned objects as distractors to further verify and consolidate learning.

Starting from Phase 1, the exercises take place on a blackboard, and the selectable elements are cards representing entire words, images, syllables, or individual letters. The first phase focuses on training word recognition, with increasing levels of complexity: first, the word is presented in

isolation, then compared with a blank white card, followed by a non-word (symbols equal to the number of letters), and finally with another word of the same length.

In Phase 2, attention shifts to the one-to-one correspondence between a word and an image. In the first sub-phase, a reference card briefly displays the target word, requiring the selection of the corresponding image from two options (one correct and one distracting). In the following sub-phase, the roles reverse: starting from the image, the exact word must be identified.

Phase 3 introduces syllable learning, involving progressive recognition for each syllable: first, compared to a blank card, then against a non-syllable (a sequence of symbols of equivalent length), and finally against another syllable of the same length.

Phase 4 requires the reconstruction of the word using its syllables. The exercise involves a binary choice between the target syllable and a distractor, while the word, broken into syllables but missing the one to be selected, is displayed above.

Phase 5 mirrors the structure of Phase 3 but focuses on recognizing individual letters.

In Phase 6, the learning process becomes more complex: the word is reconstructed through backward chaining, starting with the last letter. The exercise requires choosing the correct letter from a distractor while a partial sequence of letters, followed by positions to be filled, is displayed. The difficulty increases progressively, as each completion requires adding more letters until the entire word is reconstructed.

The final phase, Phase 7, replicates the last iteration of Phase 6 but introduces a significant variation: the word is reconstructed by selecting the correct option from a set of ten different cards.

For every target to be recognized—whether an object, image, whole word, syllable, or letter—the exercise is repeated three consecutive times, randomizing the position of the elements on the screen. The only exception is the first presentation in Phase 0, Sub-phase A, where there is only one execution with a fixed central position.

Currently, the set of words included in the application is as follows: *casa* (house), *tablet*, *divano* (sofa), *quadro* (painting), *vaso* (vase), *mela* (apple), *piatto* (plate), *succo* (juice), *gatto* (cat), *zaino* (backpack), *palla* (ball), *fiore* (flower), *erba* (grass), and *uccello* (bird). The group was defined to include at least one occurrence of every letter in the alphabet. Additionally, the words

are either part of the domestic sphere or are already familiar to the target audience. If not, Phase 0 has been specifically included to reintroduce or introduce these concepts.

In Figure 26, examples of the learning phases are shown..

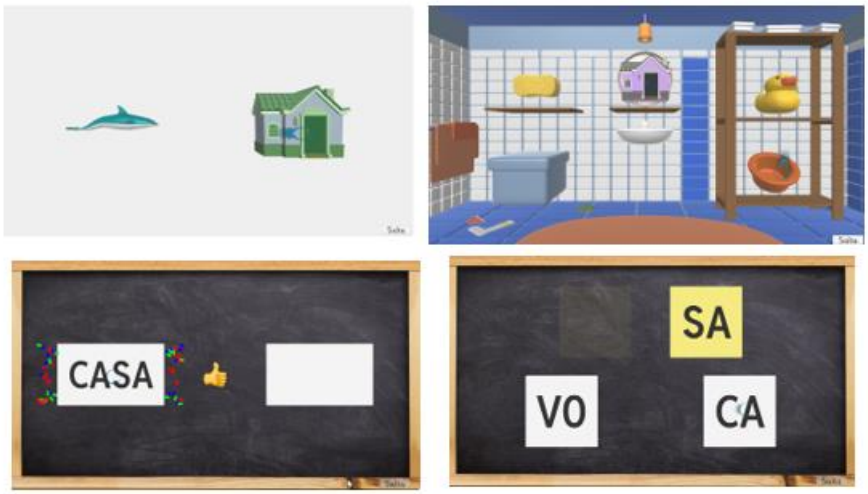


Figure 26. Examples of Exercise Phases

Given the needs of Rett patients for whom the product is designed, the future objective is to allow parents and caregivers to autonomously and specifically expand the set of words used in the exercises for the treated subject.

4.6.4 Customization and User Settings

When the software is launched, the main screen appears, where a username must be entered to access the settings or the section for creating the exercise plan. Through the settings, three key parameters for game execution can be defined, as shown in figure 24):

- Fixation time (in milliseconds): the minimum time an object on the screen must be fixed consecutively to be selected.
- Refractory time (in seconds): the time at the beginning of an exercise when eye-tracking is temporarily disabled to allow for preliminary exploration of the scenario.
- Number of distractors: the number of distractors (from two to four) to be used in Phase 0, Sub-phase C, in addition to the target.

Figure 27. Settings of Specific Parameters

The exercise plan can be generated in automatic or manual mode (Figure 25). In automatic mode, the set of exercises is created based on progress recorded during previous sessions. On the first use, entering the subject's name starts a new progress log, initially limited to Phase 0 of the word *"casa"*. At the end of each session, the log is updated, potentially including new words or advancing those already present to subsequent phases.

Figure 28. Learning Path Selection Screen.

For each word, a phase is considered passed when all corresponding exercise choices are correct for three consecutive sessions. In this case, the next phase is unlocked, and if the completed phase is Phase 0, a new word is added to the list, itself starting at Phase 0. The learning of words progresses independently across phases and is proportional to the quality of the responses. Once Phase 7 is also completed, a word is considered learned and is only used as a distractor in Phase 0, Sub-phase C.

Manual sessions allow for the creation of a customized exercise set. However, progress achieved in this mode is not recorded and does not affect the automatic session plan. Since the automatic protocol cannot be modified, personalized plans are particularly useful during the initial

uses. This approach allows the caregiver to work with already known words, facilitating familiarization with the game environments and reducing the cognitive effort required.

For each word, regardless of the phase, the first task always involves fixing the object representing the target, which is positioned at the center of the screen and, except in Phase 2, accompanied by a card displaying its corresponding textual representation.

Within the exercises, when a choice is made, visual and auditory feedback is provided. There are three cases, depending on the type of exercise:

In Phase 0 (object recognition), correct objects rotate around their axis while emitting confetti and may slightly lift. If the choice is incorrect, nothing happens.

In phases requiring a choice between two cards (Phases 2, 3, 5) without word completion, confetti is emitted from the correct choice, while the incorrect choice produces no effect.

In phases requiring word completion (Phases 4, 6, 7), the word is initially displayed on a whole card, which then separates into its components (syllables or letters), before disappearing and leaving the current target more prominently displayed. Upon a correct selection, an animation shows the fixed card being placed in the position of the missing element, emphasizing the composition of the word. In the case of an incorrect selection, this animation does not occur.

The feedback animation is designed to motivate and support learning through a gentle and encouraging approach. In the case of a correct choice, the animation features a positive gesture such as an "OK" or a thumbs-up, accompanied by a short motivational phrase that highlights success. Words like *"Top!"*, *"Super!"*, or *"Excellent! That's exactly it!"* are expressed enthusiastically, while the correct choice is clearly emphasized.

When the choice is incorrect, the approach is completely different. The character displays a doubtful and reflective attitude but uses a gentle and non-judgmental tone. Phrases such as *"I don't think it's this one…"*, *"I didn't understand…"*, or *"Hmm, maybe it's something else"* gently suggest the possibility of rethinking the answer. By doing so, the principle of "positive reinforcement" is followed: valuing attempts, encouraging the search for the correct solution, and always maintaining a high level of motivation while avoiding any form of discouragement or explicit criticism.

4.6.5 Data Collection and Analysis

Lastly, the software is designed to regularly save a specific set of information related to each session of use. In particular, the following data is tracked:

- Fixation point of the subject relative to the screen, recorded at 60Hz frequency
- Settings used during the session (fixation time for selection, refractory time, number of distractors for Phase 0C)
- Total session duration
- For each word practiced:
- Exercise duration
- Word
- Phase and sub-phase

For each repetition:

- Reaction time from the end of the request
- Repetition duration
- Set of displayed elements
- Fixation time for each object
- Outcome (correct, incorrect, skipped)
- Caregiver assistance

This dataset is sufficient to fully reconstruct the session's progress and can be used to perform statistical analyses on both individual subjects and across different subjects to assess the effectiveness of the tool. Additionally, it provides a starting point for studying the patient’s learning progression over time, adding valuable information beyond the simple evolution of the exercise plan.

4.6.2 Phase 2: Pilot Study on Usability and Feasibility of the "Lettoscrittura" Game

The current phase of the project focuses on a pilot study aimed at evaluating the usability and feasibility of the "Lettoscrittura" software. This innovative tool is designed to enhance cognitive and literacy skills in patients with Rett syndrome through an interactive and accessible approach.

The study involves 15 patients diagnosed with Rett syndrome, along with their caregivers, including both parents and teachers.

4.6.2.1 Study Phases

The implementation of the "Lettoscrittura" software follows a structured approach designed to ensure seamless integration into the rehabilitation process for patients with Rett Syndrome. The process begins with the installation of the software on personal computers, customized to meet the accessibility needs of the patients and their environments. This is followed by a targeted training session for caregivers, equipping them with the necessary knowledge to support and guide the patients effectively during their interactions with the software.

1. Software Installation: The software is installed on personal computers provided for the patients, ensuring accessibility and compatibility with their usage environments.
2. Caregiver Training: A concise training session is conducted for caregivers to familiarize them with the objectives and functionalities of the "Lettoscrittura" software. This step ensures that caregivers are prepared to guide the patients effectively during the exposure sessions (Brooke, 1996).
3. Exposure Sessions: Patients engage with the software for 10 exposure sessions, conducted twice a week under the supervision and support of their caregivers. These sessions are not structured as formal training but rather focus on introducing the patients to specific pre-defined targets within the software. The aim is to allow patients to explore and interact with the tool in a supportive environment (Sauro & Lewis, 2016). In the following figure, a session with a patient is shown.

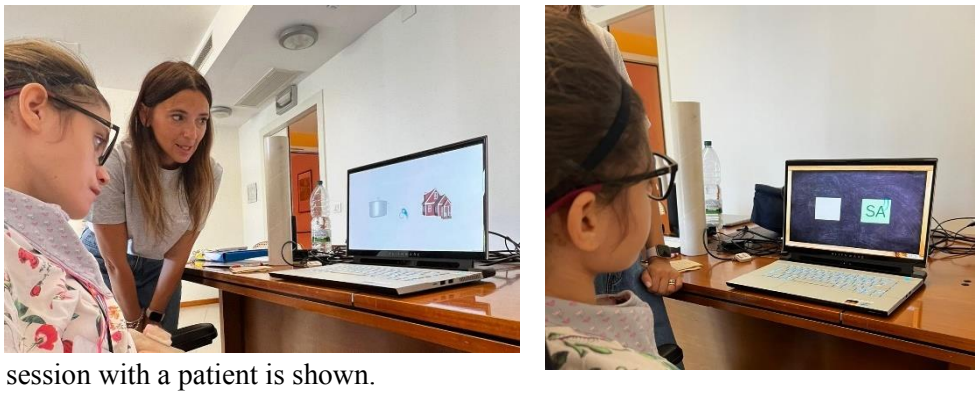


Figure 29. A session with a patient is shown.

4.6.2.2 Evaluation Metrics

Once the exposure phase is completed, caregivers will be asked to provide feedback using two standardized tools. The first is the System Usability Scale (SUS), which is designed to assess the technical usability of the software. This questionnaire focuses on key aspects such as how easy the software is to use, its intuitiveness, and the overall satisfaction with its design and functionality (Brooke, 1996).

The second tool is the Modified Subjective Evaluation Questionnaire (SEQ), which evaluates the feasibility and effectiveness of the software in relation to the patients' experiences. This includes assessing the patients' level of engagement, their reactions during the sessions, and their overall performance while using the software (Lewis, 2018). These tools ensure a comprehensive evaluation, helping to refine and optimize the software for its intended therapeutic use.

4.6.2.3 Objectives of the Pilot Study

The pilot study is designed to achieve several key objectives. First, it aims to evaluate the usability and technical reliability of the "Lettoscrittura" software, ensuring that it functions smoothly and is user-friendly for both patients and caregivers. Additionally, the study seeks to explore the feasibility of integrating the software into the daily routines of patients with Rett syndrome, assessing how well it fits within their existing therapeutic and educational activities.

A significant focus of the study is to collect both qualitative and quantitative data on the patients' interactions with the software. This includes examining its potential to support cognitive and literacy development, as well as understanding how patients engage with the tool. Furthermore, the study aims to identify areas for improvement in the software's design and application methodology, helping to enhance its effectiveness and user experience.

This pilot study represents an important step toward refining the "Lettoscrittura" software and preparing it for broader use in therapeutic and educational settings.

GENERAL CONCLUSIONS

This doctoral thesis highlights the transformative potential of innovative technologies in addressing the complex rehabilitation needs of individuals with Rett Syndrome (RTT). Through the development and implementation of experimental projects, the research explores the impact of advanced tools such as tele-rehabilitation platforms, immersive virtual reality (VR) environments, and customized Serious Games. These technologies not only tackle key challenges of traditional rehabilitation but also open new pathways for enhancing the effectiveness, accessibility, and personalization of interventions.

The thesis begins by establishing a comprehensive framework for understanding RTT as a paradigmatic example of multiple disabilities. It examines the clinical presentation, stages, and atypical forms of RTT, emphasizing the role of MECP2 gene mutations in advancing knowledge about the syndrome's etiology and clinical management. The discussion highlights the heterogeneous nature of RTT, which necessitates highly individualized and multidisciplinary therapeutic approaches.

Building on this foundation, the research explores the principles of learning and rehabilitation, focusing on neuroplasticity and mediated learning as theoretical underpinnings for cognitive and motor enhancement. Traditional and non-medical rehabilitation strategies are reviewed, including the use of assistive technologies, augmentative communication tools, and therapies such as music and sensory integration. While these approaches show significant promise, systemic challenges such as limited accessibility, high costs, and the lack of standardized protocols underscore the urgent need for innovative solutions tailored to RTT's specific challenges.

The thesis then introduces the transformative potential of assistive technologies (AT) in RTT rehabilitation. Tools like tele-rehabilitation platforms, eye-tracking systems, and virtual reality environments are presented as groundbreaking approaches to overcoming the limitations of conventional rehabilitation methods. These technologies enable enhancements in motor and cognitive functions while addressing barriers related to logistics, engagement, and sustained participation. By fostering greater inclusion and autonomy, these tools provide a more engaging and effective rehabilitation experience for individuals with RTT.

Collectively, these chapters lay the groundwork for the experimental studies detailed in the subsequent sections, demonstrating how an interdisciplinary approach combining clinical expertise and technological innovation can revolutionize rehabilitation for RTT and similar complex disorders.

The experimental studies followed an evolutionary trajectory, demonstrating the feasibility and efficacy of these technologies. From the design of an advanced tele-rehabilitation platform to the creation of VR environments for motor training and the development of interactive games for cognitive enhancement, the results highlight the potential of these approaches in improving attention, fostering active participation, and enhancing patients' skills. A common theme across all studies was the increase in motivation: the technologies utilized demonstrated their ability to reduce the psychophysical fatigue often experienced by this population, making rehabilitation sessions more stimulating and engaging. This is particularly important, as motivation is a key factor for the success of any rehabilitation intervention. The main findings are summarized below:

Tele-rehabilitation Platforms: The development and implementation of an advanced tele-rehabilitation platform marked a crucial starting point. This tool demonstrated its capability to overcome logistical barriers, such as the need for frequent travel to specialized centers, enabling more frequent and personalized rehabilitation interventions directly in domestic settings. The platform proved effective in optimizing therapeutic continuity while also reducing the burden on caregivers.

Immersive Virtual Reality (VR): Studies on virtual reality highlighted how immersive environments can promote motor recovery and the acquisition of specific skills. Through the GRAIL system and other VR solutions, patients showed significant improvements in attention, concentration time, and motor skills such as gait and upper limb use. These results suggest that VR can serve as a highly motivating tool, reducing the monotony of repetitive exercises typical of traditional rehabilitation.

Serious Games: An important contribution emerged from interactive games such as the “Interactive Fairy Tale” project and the “Lettoscrittura” game. The “Interactive Fairy Tale,” based on eye-tracking technology, demonstrated its effectiveness in improving parameters such as memory, attention, and social interaction through personalized and engaging narratives. Meanwhile,

the “Lettoscrittura” game aimed to develop linguistic and cognitive abilities in an accessible and innovative way, offering a playful yet educational platform that facilitates learning.

Despite these promising results, several challenges remain. It is essential to develop long-term evaluations to gain a deeper understanding of the sustainability of the observed effects. Equally important is ensuring the economic accessibility and scalability of these technologies to make them widely available to a larger patient population. Given the complexity of Rett syndrome, an interdisciplinary approach is crucial—one that seamlessly integrates clinical and technological expertise while fostering collaboration among professionals from diverse fields.

This doctoral thesis demonstrates that a combined approach incorporating traditional interventions and advanced technologies can revolutionize RTT rehabilitation, improving patients’ cognitive and motor abilities while reducing the burden on caregivers. This represents a significant opportunity to make rehabilitation not only more effective but also more inclusive and sustainable, offering new hope for the future of patients and their families.

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