23

Behavioral and Electrophysiological Characterization of Induced Neural Plasticity in the Autistic Brain

Jaime A. Pineda[1,2] et al.[*]
[1]*Departments of Cognitive Science, University of California,*
[2]*Neuroscience Group, University of California,*
San Diego,
USA

1. Introduction

Despite extensive research, the causes of Autistic Spectrum Disorders (ASD) are still unknown and no single explanation has been proposed that can account for the heterogeneous profile (Muller, 2007). The DSM-IV diagnostic criteria for ASD include deficits in social and communicative skills such as imitation, empathy, and shared attention, as well as restricted interests and repetitive patterns of behaviors. Elucidating the neuroetiology of these symptoms has been a challenge because the behavioral manifestations vary both in severity (high, medium, or low) as well as expression (Autistic Disorder, Asperger Syndrome, or Pervasive Developmental Disorder-Not Otherwise Specified) (Matson, 2006; Volkmar et al., 1994). Although a growing body of work has raised questions about the role of mirror neurons in human social behavior (Hickok, 2009; Lingnau et al., 2009), many recent studies suggest that a dysfunction in the frontal human mirror neuron system (hMNS) could potentially account for the social deficits in autism, including problems with imitation, understanding actions, emotion recognition, and empathy (Williams et al., 2001; Oberman et al., 2005; Dapretto et al., 2006; Williams et al., 2006).

Mirror neurons were initially reported by Rizzolatti and colleagues (di Pellegrino et al., 1992) in the premotor cortex of macaque monkeys (area F5), an area thought to be analogous to Broca's area (Brodmann's area 44) in humans (Buccino et al., 2001; Buccino et al., 2004; Petrides et al., 2005). Some cells in this region increase firing during the execution of an action as well as during observation of a similar action performed by others. This execution/observation matching feature is hypothesized to provide a mechanism for

[*]Heather Pelton[1], Oriana Aragon[3], Jia-Min Bai[4], Matt Erhart[1], Dane Chambers[1], Burcu Darst[1], Ernesto Enrique[5], Steven Gilmore[1], Stephen Johnson[1], Albert Anaya[1], Alicia Trigeiro[1], Dan T. Lotz[1], Nicholas Pojman[1], Tom Gamage[6] and David Linderman[1]
3 Department of Psychology, Yale University, USA
4 Department of Psychology, University of Illinois at Urbana-Champaign, USA
5 Departments of Biology, San Diego State University, USA
6 Department of Psychology, Virginia Commonwealth University, USA

mapping seeing into doing and vice versa – a mechanism capable of internally mirroring the action it perceives and performing a "simulation" of the action without accompanying motor execution (Rizzolatti & Craighero, 2004). Furthermore, the existence of auditory mirror neurons in the same region make it likely that a system exists that responds to implied action or attention to movement in the absence of discrete visual perception of the action (Umilta et al., 2001; Kohler et al., 2002; Iacoboni et al., 2005).

While individual mirror neurons have not been studied directly in humans, the existence of an analogous system has been supported by indirect population-level measures including transcranial magnetic stimulation (TMS: (Fadiga et al., 1999), functional magnetic resonance imaging (fMRI: (Iacoboni et al., 1999), and electroencephalogram/magnetoencephalogram (EEG/MEG: (Pineda, 2005; Muthukumaraswamy et al., 2004; Muthukumaraswamy & Singh, 2008; Hari et al., 1997). These and many other studies strongly support the existence of a mirror neuron system consisting of interconnected regions, in the ventral premotor area (PMv) of the IFG, parietal frontal (PF) in the rostral cortical convexity of the inferior parietal lobule (IPL), and the superior temporal sulcus (Rizzolatti & Craighero, 2004; Iacoboni et al., 2005; Buccino et al., 2004). MEG and EEG studies have further suggested that mirroring activity is reflected in the mu frequency oscillations (8-13 Hz, 13-15 Hz, and 15-25 Hz) measured over the sensorimotor cortex (Hari et al., 1997; Cochin et al., 1999; Pineda et al., 2000; Muthukumaraswamy et al., 2004; Muthukumaraswamy & Johnson, 2004). Although some of these frequency bands overlap with the traditional alpha frequency band, the evidence argues for distinct neural sources (Niedermeyer, 1997). More specifically, traditional alpha oscillations reflect visual information processing in the occipital lobes and are readily affected by the opening and closing of the eyes. In contrast, mu rhythms are generated in more anterior sources and are not susceptible to eye closure. It is also assumed that their sources are in sensorimotor cortices, where neurons fire synchronously while at rest to produce high amplitude oscillations measured at the scalp. It is argued that input from premotor areas, presumably correlated with mirroring or simulation activity, produces asynchronous firing in the sensorimotor circuits during self, observed, and imagined movement resulting in suppressed or desynchronized EEG activity (Gastaut & Bert, 1954; Pfurtscheller & Aranibar, 1979; Pineda et al., 2000; Pineda, 2005). Such suppression to observed movement in the absence of self movement is hypothesized to reflect downstream modulation of sensorimotor circuits by premotor hMNS (Altschuler et al., 1998; Oberman et al., 2005; Pineda, 2005).

In short, hMNS is hypothesized to be engaged during the observation of actions and thought to be one of the neural mechanisms by which we comprehend such actions (Gallese et al., 1996; Rizzolatti & Fabbri-Destro, 2009), understand the goal or intentions of those actions (Blakemore et al., 2001), learn through imitation (Williams et al., 2001; Wohlschlager & Bekkering, 2002), interpret facial expressions (Carr et al., 2003), and exhibit empathy (Leslie et al., 2004). Given these relationships, Williams et al. (Williams et al., 2001) posited that a developmental deficiency in hMNS could lead to problems in imitation learning and account for the type of theory of mind deficits thought to occur in ASD individuals (Baron-Cohen et al., 1985; Baron-Cohen et al., 1997; Baron-Cohen, 2009). That is, an inability to "form and coordinate social responses of self and others via amodal or cross-modal representation processes" could impede early affective, social, and communication development (Ozonoff et al., 1991; Rogers et al., 2003).

Impairments in individuals with autism include deficits in behaviors that parallel the presumed functions of hMNS, (Rogers et al., 2003; Leslie et al., 2004; Williams et al., 2004; Buxbaum et al., 2005). Anatomical evidence provides support for such a link. Villalobos et al (Villalobos et al., 2005) found reduced functional connections between the inferior frontal cortex and primary visual area (V1) while Just et al (Just et al., 2004) found reduced functional connections between the inferior frontal cortex and other areas during language tasks. Recent neurophysiological studies provide further evidence for an hMNS dysfunction in individuals with ASD (Just et al., 2004). Oberman et al (Oberman et al., 2005) found that children with ASD, when compared to matched typically-developing controls, exhibit mu suppression for self but not observed action. In a study by Dapretto et al (Dapretto et al., 2006) comparing children with ASD to typically developing children it was found that ASD children did not show activation of the IFG during imitation of facial expressions.

Recent studies argue that many aspects of autism arise from atypical anatomical connections and therefore produce altered activity between different areas in the brains (Courchesne & Pierce, 2005). Such a 'disconnection syndrome' as a function of hypoconnectivity could lead to desynchronization and ineffective intra- and interhemispheric communication impacting many neural circuits. If hMNS is dysfunctional because of altered connectivity, it seems appropriate to consider whether it is possible to change cortical dynamics in this circuit to induce neural plastic changes that would normalize those connections and *inter alia* alleviate symptoms of the disorder. One approach in this regard is plasticity-inducing rehabilitation training (PIRT), which utilizes neurofeedback training of EEG signals as a form of operant conditioning (Lubar 1997). This type of training has been used clinically for many years and provides real-time feedback to the user allowing for alteration and enhancement of brain function (Hirshberg et al., 2005). One explanation of how such training produces its behavioral and electrophysiological effects is by gaining access to and control over regulatory mechanisms that increase or decrease synchronous or desynchronous activity in thalamocortical networks (Lilienfeld, 2005). This leads to the induction of neural plastic changes.

To verify and extend the positive changes in behavior reported in previous autism work with neurofeedback (Pineda et al., 2008; Coben et al., 2009; Jarusiewicz, 2003), the following study was designed to compare the effects of this type of training methodology on high-functioning children with ASD compared to typically-developing matched controls. Participants received a total of 30 hrs or training and were assessed with a large variety of cognitive assessment tools.

2. Methods

2.1 Participants

Eighteen individuals diagnosed with autism (17 male, 1 female; right-handed), as well as twelve typically developing (TD) individuals (10 males; 2 females) were initially recruited for the study. One participant was diagnosed as low-functioning and was not included in the analyses. An additional autistic child and one TD child completed pretraining assessments but did not complete the 20 weeks of training. Hence, post-training analyses included 16 participants with autism (age 6-17; M = 9.8 $\pm$ 3.3 years) and 11 TD (age 6-17; M = 11.2 $\pm$ 3.5 years). Autism participants were recruited through Valerie's List, a listserv of families and professionals in the San Diego autism community. Parents were asked to provide an outside diagnosis, which was verified by a licensed clinical psychologist not

associated with the research. In most cases this involved administration of the Autism Diagnostic Observation Schedule – Generic (ADOS-G), the Autism Diagnostic Interview-Revised (ADI-R), and the Wechsler Abbreviated Scale of Intelligence (WASI) (see Table 1). Based on the results of these assessments and clinical judgment all 16 children met criteria for *Autism Spectrum Disorder*. All were considered high-functioning, defined as having age appropriate verbal comprehension abilities and an IQ greater than 80. No children were diagnosed with attention deficit hyperactivity disorder (ADHD) and only one child had experienced epileptic-like seizures prior to the study. TD participants scored within the normal range on a standardized test of intelligence, had no neurological or psychological disorder, and were matched on chronological age and gender with a participant in the ASD group. All participants and their guardians (for children under 18 years of age) signed the informed consent form before participating. The protocol was approved by the University of California, San Diego's Institutional Review Board and the study has been performed in accordance with the ethical standards described in the 1964 Declaration of Helsinki.

	IQ Verbal	IQ Non Verbal	IQ Full	ADI COM	ADI SOC	ADI REP	ADOS COM	ADOS SOC	ADOS C+S	ADOS REP
ASD	105.4	108.1	107.5	12.5	20.2	4.8	3.7	9.2	12.8	2.2
TD	114.7	109.7	116	-	-	-	-	-	-	-

Table 1. Mean scores on the WASI, ADI, and ADOS

2.2 Cognitive assessments

All participants received a series of electrophysiological, cognitive, behavioral, and parental-assessments before and after 30 hours of PIRT over a 20 week period. These included a mini-Quantitative EEG (mini-QEEG), Suppression Indices for all frequencies, including mu suppression index (MSI), evaluation of imitation and sustained attention, and parental assessments.

2.2.1 Mini-QEEG

Participants were asked to remain still and to relax for one minute intervals while EEG was recorded from two sites at a time over six intervals for a total of twelve EEG sites. Participants were given a short break after each minute of recording. Mini-QEEG is used to assess the asymmetry and coherence of the scalp-recorded EEG at rest and provides an electrophysiological profile indicating the degree of functional connectivity between different pairs of electrodes that reflect processing in distinct cortical areas.

2.2.2 Suppression indices

The mu suppression index (MSI) is an electrophysiological tool developed by Oberman et al (Oberman et al., 2005) to measure the changes in mu power during the observation of

biological or non-biological movement that is either goal- or non-goal oriented. Similar indices for other EEG frequencies (delta, theta, SMR, beta, and gamma) were computed in addition to the MSI. Recordings were taken while participants viewed silent action videos (120 seconds each) on a computer monitor while performing a continuous performance attention task (counting the number of pauses in the action). A baseline, non-biological "Ball" condition consisted of two light gray balls (32.9 cd/m^2) on a black background (1.0 cd/m^2) moving vertically towards each other, touching in the middle of the screen, and then moving apart to their initial starting position. The ball stimulus subtended 2^o of visual angle when touching in the middle of the screen and 5^o at its maximal point of separation. Experimental, biological movement conditions incorporated simple and complex non-goal and goal-directed movements. Simple non-goal directed movement included a hand opening and closing (Hand). This motion was visually equivalent to the trajectory taken by the bouncing ball in the "Ball" video and subtended 5^o of visual angle when open and 2^o when closed. Simple goal-directed movement included a hand extracting a crayon from a crayon box using a precision grip (Crayon), and complex goal-directed movement included three individuals playing catch with a small ball (Social). Videos were presented at a distance of 48 cm. The hand, crayon, and crayon box were medium gray (8.6 cd/m^2) on a black background (3.5 cd/m^2). The social video was in color.

2.2.3 Imitation tasks

Imitation abilities were assessed using the Apraxia Imitation Scale (AIS), developed by De Renzi and normed in the general public (De Renzi E. et al., 1980). This test measures imitation ability of arm/hand, finger, and general movements of varying complexity. The experimenter demonstrates each movement and participants are instructed to attempt to copy the movement exactly. Each movement is repeated three times.

2.2.4 Sustained attention

Participants were administered the visual form of the Test of Variables of Attention (TOVA) to measure sustained attention. The TOVA is a computerized visual continuous performance test for the diagnosis and treatment of children and adults with attentional disorders (Greenberg & Waldman, 1993). The visual form of the TOVA has been normed in the general population.

2.2.5 Parental assessment

One of the parents for each of the ASD participants completed the Autism Treatment Evaluation Checklist before and after training. ATEC calculates four subscale scores in which ASD individuals have known deficits: speech/language communication, sociability, sensory/cognitive awareness, and health/physical/behavior, as well as a total score. These are weighted according to the response and the corresponding subscale. The higher the subscale and total scores, the more impaired the individual. Participants in the TD group were not administered the ATEC.

2.3 EEG recording

The resting EEG from twelve scalp electrode sites was recorded for the mini-QEEG using a Brainmaster 2.5W system and Mini-Q software. The sites were recorded in pairs with a sampling rate of 256 Hz referenced to mastoids and grounded at Fpz. The MSI was

extracted from EEG recorded from the C3 site over the left hemisphere and C4 over the right hemisphere at a sampling rate of 256 Hz, referenced at the mastoids and grounded at Fpz. PIRT required the use of BioExplorer software, a Brainmaster 2.5W system, and a five electrode configuration. EEG from the C4 over the right hemisphere was recorded using a sampling rate of 256 Hz, referenced to the right ear lobe, and grounded at the left ear lobe. EMG was recorded from the right trapezius muscle of the shoulder and referenced to the left trapezius muscle. The EMG was bandpass filtered for 30-60 Hz, a frequency range previously found to be sensitive to movement artifact.

2.4 Plasticity-Inducing Rehabilitation Training (PIRT)

Both ASD and TD groups received a total of 30 hours of PIRT in either 30 minute sessions three times a week or 45 minute sessions twice a week for approximately 20 weeks. Children played a variety of video games, such as racecar, asteroids, and jigsaw puzzle completions during this training. Both groups received feedback focused on the high mu band (10-13 Hz) recorded over the right sensorimotor area (C4) and referenced to the right earlobe, as well as on EMG activity (30-60 Hz) recorded from the right trapezius muscle referenced to the left trapezius muscle. Feedback was given based on satisfying two conditions: 1) power in the mu band exceeded a specified threshold, and 2) power from the muscle electrodes fell below a specified threshold. When both criteria were met, the video game progressed (e.g., car moved along the track) and a pleasant tone sounded. When the criteria were not met, visual and auditory feedbacks paused. Thresholds for both EEG and EMG channels were set as a function of the levels in the initial mini QEEG analysis and subsequently raised as a function of the preceding session. All participants viewed a computer screen displaying two threshold bars on the left and right side of a video game window. The left threshold bar corresponded to power in the 10-13 Hz band and the right threshold bar corresponded to power in the EMG band. Participants were instructed to make the mu band display larger in order to exceed a threshold bar, while making the EMG band display fall below a threshold bar. In order to help children stay focused, the experimenter encouraged them to pay attention to the game to meet these goals.

EMG feedback was included in the design for two reasons. First, it ensured that children could not advance in the game by producing movement-induced power increases in the entire EEG spectrum. Second, it allowed us to distinguish improvement effects as a function of EEG modulation, modulation of autonomic nervous system activity, or placebo effects. Because the comparison was between ASD and TD groups, nonspecific effects, such as child-trainer interaction would not explain any significant differences.

3. Results

Mixed ANOVAs were used to analyze all the data, including training performance, EEG power, suppression indices, imitation, TOVA, ATEC, and mini-QEEG results. Step down ANOVAs and post hoc comparisons with Bonferroni corrections were performed on data with significant effects. The Greenhouse-Geisser correction for degrees of freedom was applied. A summary of the results can be seen in Table 2.

3.1 Training performance

A behavioral performance measure was computed during training based on the number of hits achieved during each session multiplied by the mu rhythm threshold level established

Assessment tool	Effects of training
TOVA	No change
ATEC	• Total scores ↓ • Scores of sociability subscale ↓ • Scores of sensory/cognitive awareness subscale ↓
Imitation	• Under imitation errors ↓ • No imitation errors ↓ • Over imitation errors ↑
Absolute power	• Overall absolute power ↓ • Beta and gamma frequencies ↓ • Theta and mu frequencies ↑
Mu Suppression	• Social > Hand > Crayon • All frequencies ↓
EEG coherence	• Delta, theta and mu coherence ↑ • SMR, beta, high beta and gamma coherence ↓

Table 2. Summary of Results

for that session (Hits/Mins*MuThr). Sessions were then aggregated into phases with Phase1 including the first 5 training sessions as a baseline, Phase 2 included sessions 6-20 and Phase3 included all sessions after session 20. These data were analyzed using phases (3) as a within subjects factor and group (ASD, TD) as a between subjects factor.

A highly significant main effect of phase occurred, with Phase1 exhibiting the smallest hit rate (96.4), Phase2 with a higher hit rate (132.5), and Phase3 showing the highest rate (150.6), $F(2,52) = 20.8$, $p < 0.001$. There was also a significant interaction between phases x group, $F(2,26) = 5.04$, $p < 0.05$. Step-down one-way ANOVAs confirmed that the only significant difference between groups occurred in Phase3 ($p < 0.01$). As can be observed from Fig. 1, both groups demonstrated increases in their performance with PIRT, although the TD group profiles a steeper learning curve, while the ASD group appears to plateau in the later sessions.

3.2 Behavioral assessments

3.2.1 Sustained attention

The various subscales of the TOVA (attention deficit hyperactivity disorder or ADHD scores, errors of omission, errors of commission, time response, variability reaction time, and signal detection) were analyzed using a repeated measures ANOVAs including training (pre, post) and subscales (6) as within subjects factor and group (ASD, TD) as a between subjects factor.

A highly significant main effect of subscales indicated that standard scores for errors of commission (95.3) and reaction times (95.1) were larger than errors of omission (87.6), reaction time variability (84.2), and signal detection (88.8), $F(4,104) = 7.15$, $p < 0.001$. Stepdown ANOVAs for each subscale indicated that the only one reaching statistical significance between groups were errors of omission where the mean ASD score was lower (82) compared to that for the TD group (93), $F(1,26) = 4.57$, $p < 0.05$. Analysis of z-scores produced a highly significant main effect of subscales, $F(5,130) = 12.24$, $p < 0.001$ indicating that the ADHD (-1.87) and reaction time variability (-1.05) had the largest z scores compared

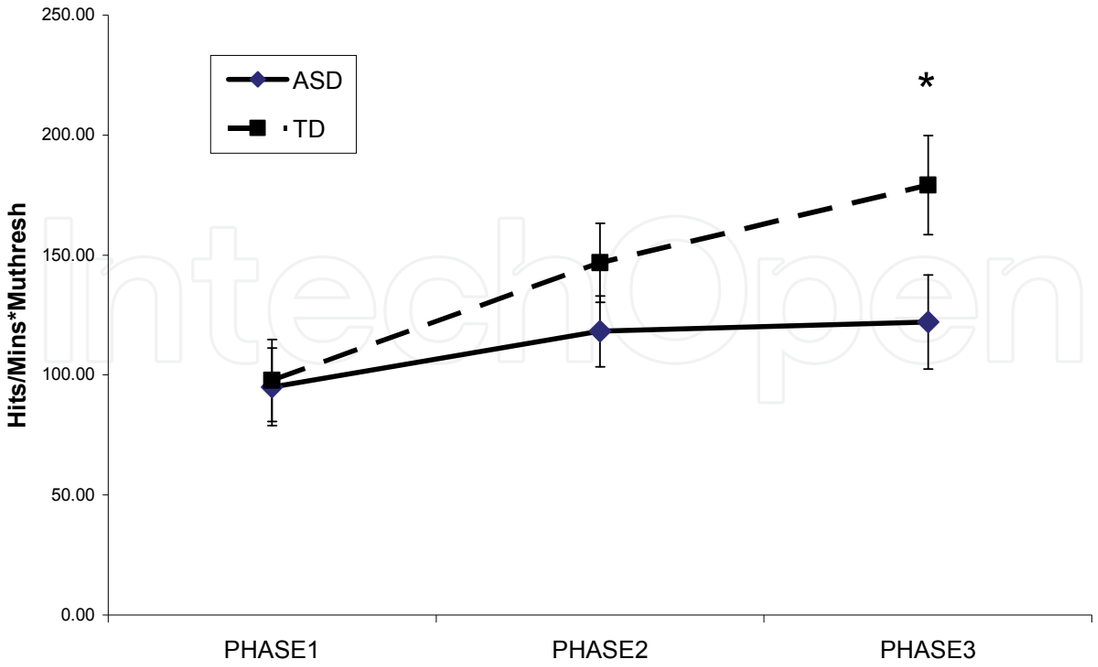


Fig. 1. Behavioral learning curves for ASD and TD groups during plasticity-inducing rehabilitation training. Performance measure was computed based on the number of hits achieved during each session multiplied by the threshold level of the mu rhythm for that session (Hits/Mins*MuThr). Illustrates scores aggregated into sessions 1-5 (Phase1), sessions 6-20 (Phase 2) and sessions after session 20 (Phase 3).

to intermediate scores for errors of omission (-0.82) and signal detection (-0.75), while reaction time (-0.32) and errors of commission (-0.19) showed the lowest scores. Errors of omission was the only subscale that produced a statistically significant z-score difference, with the ASD group exhibiting a z-score further from the mean (-1.2) than the TD group (-.5), $F(1,26) = 4.56$, $p < 0.05$.

3.2.2 Imitation

Scores on the AIS were used to compute four levels of accuracy: no imitation, under imitation, correct imitation and over imitation. Sessions were videotaped and later analyzed by three independent raters. Lack of imitation of a movement was scored as a 0 (no imitation), partial imitation was given a 0.5 score (a score $\geq$ 0.5 but < 1 was considered under imitation), accurate imitation was scored as a 1, while excessive imitation was given a 1.5 score (anything > 1.0 was considered over imitation). These scores were then subjected to a non parametric analysis using the Kruskal-Wallis analysis of ranks with factors of imitation type (no imitation, under imitation, correct imitation, over imitation) and training (pre, post) as within subjects factors and group (ASD, TD) as a grouping factor.

Analysis of the AIS indicated that pre-training ranking of no imitation scores was significantly different between groups, with ASD showing a larger number of no responses (17.97) compared to the TD group (9.88), χ^2 (1, N = 28) = 7.12, $p < .01$. These differences disappeared post-training, χ^2 (1, N = 28) = 0.164, $p = .69$. A similar difference occurred pre-training for correct imitation scores. ASD children had less correct responses (11.83)

compared to TD children (18.33), χ^2 (1, N = 28) = 4.78, p < .05. In contrast, following training the differences were not statistically significant in that the ASD (13.47) group had about the same level of responding as the TD group (15.88), χ^2 (1, N = 28) = 0.64, p = .42. No differences occurred between groups for under- and over-imitation responses.

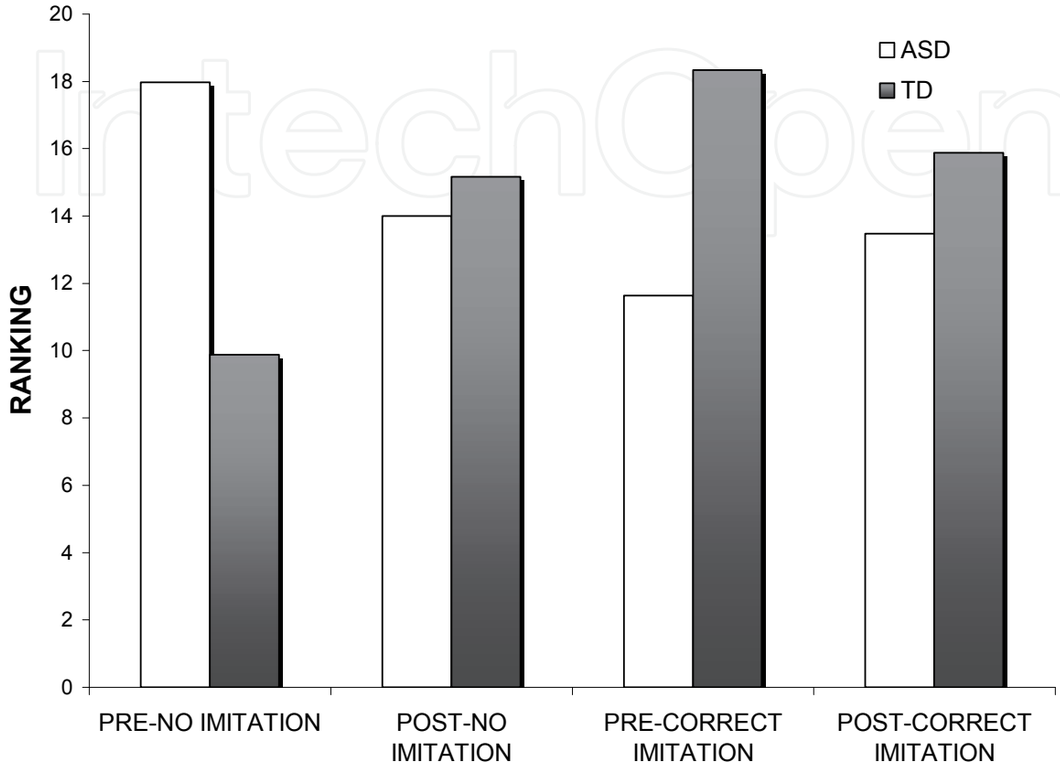


Fig. 2. Performance during the Imitation tasks for ASD and TD groups. Note that ASD had more non responses (no imitation errors) than the TD group before but not following training. Similarly, ASD had statistically fewer correct imitation responses before but not following training.

3.2.3 ATEC parental assessments

Each of the four dimensions of symptoms measured by the ATEC, which includes speech/language communication, sociability, sensory/cognitive awareness, health/physical/behavior contain descriptions of behaviors that are rated on a scale of 1-5. Each score on these dimensions, as well as the total score, were converted to a percentage of the highest possible score for that dimension. These percentile scores were then analyzed using a repeated measures ANOVA with training (pre,post) and subscales (5) as within subjects factors.

There was a highly significant main effect of subscales in which scores on the sociability subscale were the highest and therefore indicated the most impairment, while speech/language communication were the lowest and indicated the least impairment, F(4,60) = 33.0, p < 0.001. There was also a significant main effect of training on all scores such that post-training produced a lowering of scores (0.20) relative to pre-training (0.25), F(1,15) = 4.73, p < 0.05. Finally, there was a significant training x subscales interaction, F(4,60) =

3.78, $p < 0.05$. As illustrated in Fig. 3, training produced a reduction in all scores, with the greatest changes occurring in the sociability and sensory/cognitive awareness dimensions.

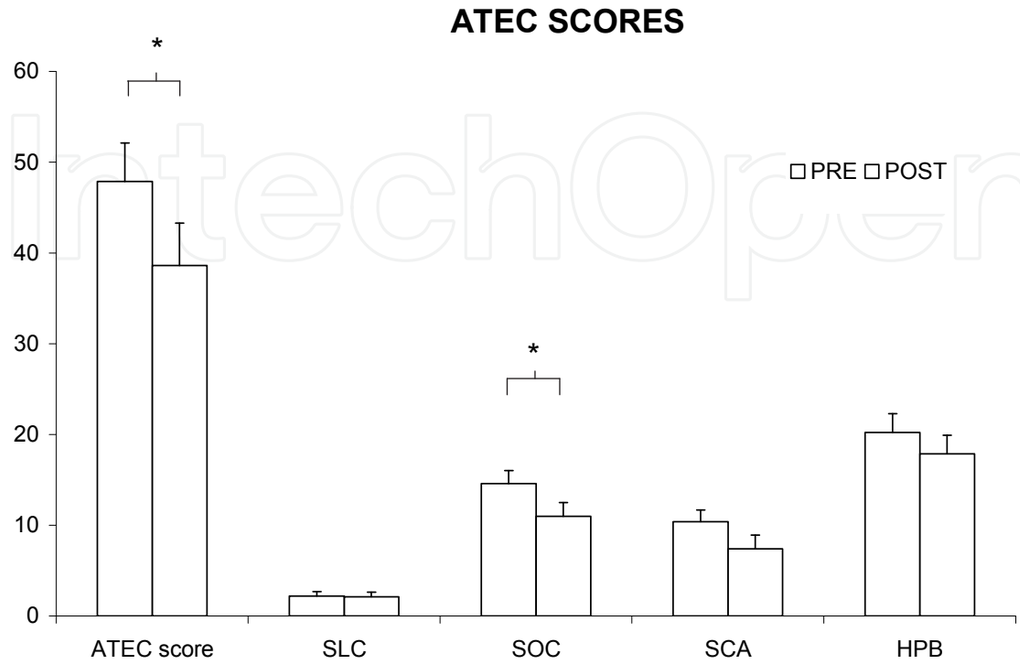


Fig. 3. Scores on the Autism Treatment Evaluation Checklist (ATEC). Questionnaire was filled out by the same parent before and following the 20 weeks of training. Graph depicts the total score (ATEC score), as well as scores on the speech/language communication (SLC), sociability (SOC), sensory/cognitive awareness (SCA), and health/physical/behavior (HPB) subscales.

3.3 Electrophysiological results

3.3.1 Absolute power

The first and last ten seconds of EEG recorded during the observation of movement in the various video conditions were eliminated in order to remove attentional transients due to initiation and termination of the stimulus. The remaining one-minute segments were combined with data from the same conditions resulting in two, one-minute segments of data per condition. Eye blinks and movement artifacts were removed automatically as well as manually prior to analyses. A Fast Fourier Transform was performed on the edited data set (1024 points) using cosine windowing to control for artifacts resulting from data splicing. Absolute power at scalp locations corresponding to premotor cortex (C_3, C_Z, and C_4) was compared to baseline condition. Absolute power was analyzed using training (pre, post-), video type (crayon, hand, social), and frequencies (delta, theta, mu, SMR, beta, gamma) as within subjects factors and group (ASD, TD) as a between subjects factor.

As illustrated in Fig. 4, there was a main effect of video type such that experimental conditions (Hand, Crayon, Social) were significantly reduced in power from baseline (Ball), $F(3,78) = 8.24$, $p < 0.05$. Pairwise comparisons confirmed that responses to the Social video varied significantly from baseline ($p < 0.01$), while Hand ($p = 0.06$) and Crayon ($p = 0.92$) were not significant. Additionally, a video type x training interaction indicated that while

the effect of training was to reduce power, the largest reduction occurred in the baseline condition, $F(3,78) = 3.65$, $p < 0.05$. No differences occurred between groups. Step down analysis for each video type revealed that the responsiveness to the Ball condition reflected a marginally significant effect of training, $F(1,26) = 4.08$, $p = 0.054$. That is, participants displayed reduced mu power to the baseline Ball condition post-training. A highly significant training x frequencies interaction, $F(5,130) = 14.7$, $p < 0.001$ indicated that the decrease was primarily in the beta and gamma frequencies. Highly significant training x frequencies interactions also occurred for responses to the Hand ($F(5,130) = 9.68$, $p < 0.001$), Crayon ($F(5,130) = 10.3$, $p < 0.001$), and Social videos ($F(5,130) = 8.55$, $p < 0.001$). In all these cases, power increased post-training, primarily for theta and mu frequencies, while decreases occurred primarily for beta and gamma bands.

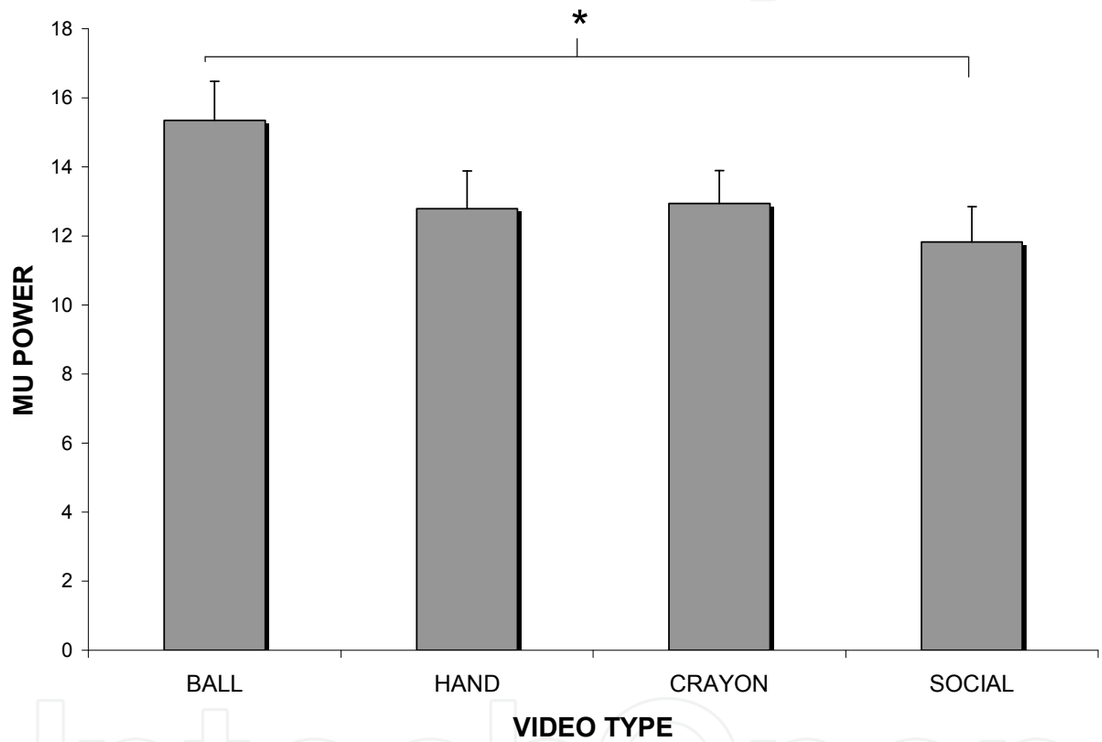


Fig. 4. Mu power in response to the observation of movement in the various videos (Ball, Hand, Crayon, and Social). The Ball video was used as the baseline condition. Note the decrease in mu power in the experimental conditions relative to baseline.

3.3.2 Suppression indices

In order to control for individual differences in scalp thickness and electrode impedance, a ratio of absolute power between the experimental and baseline conditions was calculated. A log transform of these ratios was used since ratio data are inherently non-normal as a result of lower bounding. A log ratio less than zero indicates suppression, a value of zero indicates no suppression and a value greater than zero indicates enhancement. Suppression indices were analyzed using training (pre, post-), video type (crayon, hand, social), and frequencies (delta, theta, mu, SMR, beta, gamma) as within subjects factors and group (ASD, TD) as a between subjects factor.

There was an overall main effect of training on suppression indices with more suppression observed pre-training (-.17) compared to post-training (-.028), $F(1,26) = 6.09$, $p < 0.05$. There was also a main effect of video type in which the largest suppression occurred to the social video (-.129), followed by the hand (-.091) and then the crayon (-.079), $F(2,52) = 4.06$, $p < 0.05$. A main effect of frequencies occurred ($F(5,130) = 3.12$, $p < 0.05$), although pairwise comparisons did not show any that differed significantly. Nonetheless, a highly significant training x frequencies interaction, $F(5,130) = 7.43$, $p < 0.001$ indicated that all frequencies were reduced following training.

3.3.3 Mini QEEG

Covariance of power at two sites (amplitude coherence), asymmetry, and attention indices (theta/alpha, theta/SMR, theta/beta, mu/beta ratios) were measured as part of the mini-QEEG analysis and analyzed using repeated measures ANOVAs with frequency bands (delta, theta, mu, SMR, beta, high beta, gamma), electrode pairs (C3/C4, F3/F4, Fz/Cz, O1/O2, P3/P4, T3/T4) and training (pre, post) as within subjects factors and group (ASD, TD) as a between subjects factor.

In terms of asymmetry, there was an overall (across group and training) marginally significant effect of frequency bands, $F(6,156) = 2.95$, $p = 0.058$, with none of the pairwise comparisons reaching significance. Individual ANOVAs for each frequency band showed that only the delta frequency exhibited a main effect of electrode pair, $F(5,130) = 4.13$, $p < 0.05$ such that the main difference occurred between F3/F4 and both T3/T4 ($p < 0.01$) and O1/O2 ($p < 0.05$). In terms of amplitude coherence, there was a highly significant main effect of electrode pairs, $F(5,130) = 18.1$, $p < 0.001$ such that coherence at temporal (T3/T4) and parietal (P3/P4) sites were significantly different from all other pairs of electrodes. These differences varied across groups since there was a group x electrode pairs interaction, $F(5,26) = 2.79$, $p < 0.05$. There was also a highly significant main effect of frequencies, $F(6,156) = 478.5$, $p < 0.001$ as well as a frequencies x training interaction, $F(6,156) = 6.4$, $p < 0.01$. Step down analyses of the individual frequencies revealed that delta, theta, and mu coherence increased or tended to increase with training but SMR, beta, high beta, and gamma decreased or tended to decrease. Only the SMR data for both groups, as illustrated in Fig. 5, displayed a marginally significant training x group interaction, $F(1,26)=3.96$, $p = 0.057$. That is, following training the ASD group exhibited a statistically significant decrease in coherence compared to the TD group.

3.3.4 Frequency band ratios

Analysis of the ratios of the asymmetry data for the various frequencies revealed a main effect, with the theta/alpha ratio being closest to 1, while theta/SMR (1.05) and theta/beta (1.08) were both significantly larger, $F(3,78) = 4.41$, $p < 0.05$. These asymmetry ratios varied as a function of location since there was an electrode pair x ratio interaction, $F(15,390) = 3.57$, $p < 0.05$.

4. Discussion

Results from this study provide evidence for significant positive effects of plasticity-inducing rehabilitation training (PIRT) on the behavioral, cognitive, and electrophysiological indices of children with autistic spectrum disorders (ASD). The effectiveness of this type of operant training is supported by the fact that both ASD and typically-developing (TD)

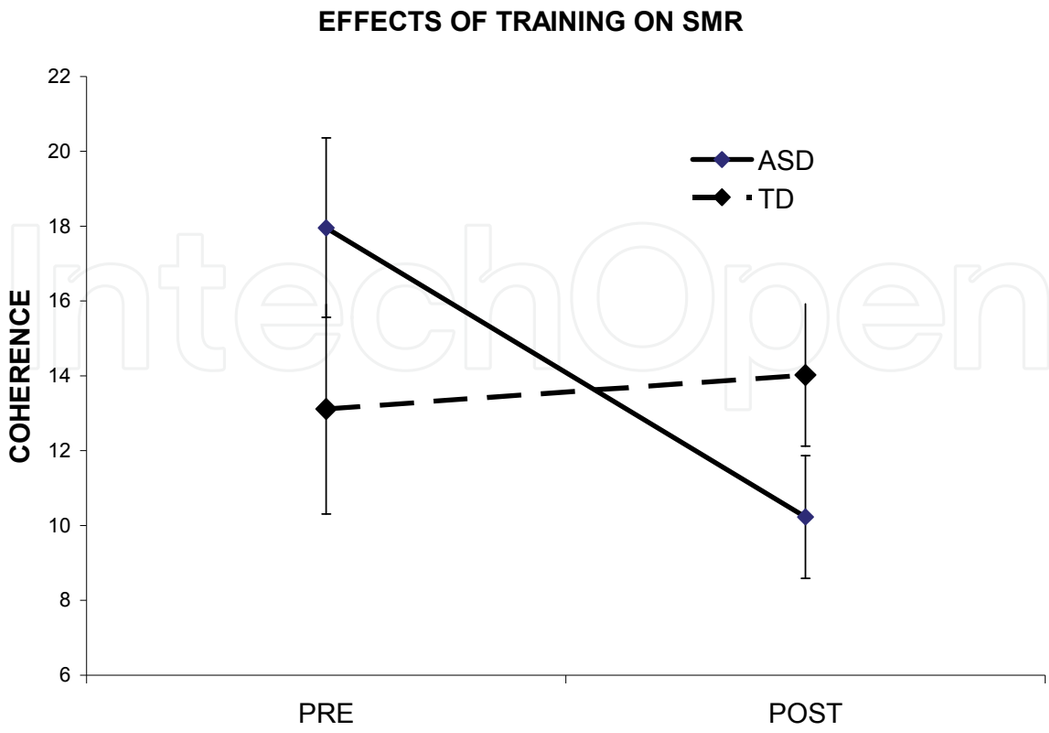


Fig. 5. Line graph showing the effects of training on the SMR.

children learned to modulate the amplitude of EEG mu rhythm oscillations (10-13 Hz) across the 20 weeks of training. Learning rates differed between groups, with ASD children exhibiting a more gradual learning curve and ultimately a leveling of their performance during the later stages of training. Such learning impacted a number of behavioral, cognitive and electrophysiological measures. Some of these measures, however, like the assessment of sustained attention using the Test of Variable Attention (TOVA) scale showed group differences in terms of errors of omission and response times but were not significantly affected by the training. While ASD children were significantly more impaired in sustained attention compared to the TD group, with larger differences from the mean than the control group, sustained attention was insensitive to PIRT. In contrast, in terms of the assessment of imitation skills, results indicated that ASD children exhibited significantly more no imitation responses than the TD group, as well as reduced levels of correct responses prior to training. Training had a significant impact on reducing the lack of imitation behaviors while increasing the tendency to correctly imitate. Positive behavioral changes were also noted by parents using the Autism Treatment Evaluation Checklist (ATEC) scale of participant's behaviors following training. Results suggest that the greatest impairment measured in the ASD group before training was in the sociability subscale. Training significantly reduced those scores, as well as scores on the sensory/cognitive awareness subscale. Reduction of such scores indicates behavioral improvement.

The present results are consistent with a variety of studies that have provided evidence that EEG neurofeedback training can be an effective form of intervention for ASD symptoms (Jarusiewicz, 2003; Pineda et al., 2008). All studies in the recent literature using this methodology have reported significant reductions in autistic symptoms following such training. In a single case study, Sichel et al. (Sichel, 1995) reported positive changes in all

DSM-IV-R diagnostic criteria for autism. Jarusiewicz (Jarusiewicz, 2003) reported an average of 26% improvement in the ATEC in 12 children diagnosed with autism, compared to 3% improvement in a control group. Pineda et al. (Pineda et al., 2008) reported decreased mu power and coherence, increased sustained attention ability, improved imitation ability, and improved scores on the Sensory/Cognitive subscale of the ATEC in children with ASD compared to a placebo group following training. Similarly, Coben et al. (Coben et al., 2009) validated EEG neurofeedback as a therapeutic modality for autistic children and argued that changes in connectivity anomalies may be related to the mechanism of action.

There was no distinction in the present study between ASD and TD groups in terms of electrophysiological responses to the variety of observed movement in the videos. Both groups displayed a gradient in such suppression with respect to the type of movements observed. The social video elicited the largest suppression, followed by the hand and then the crayon video. This is consistent with previous studies indicating a normal gradient of mu rhythm responsiveness as a function of sociability (Oberman et al., 2006). However, one difference between the current and previous results was the effect of training on mu rhythm suppression. Whereas in the earlier study, mu rhythm suppression increased with training, it decreased in the current study. One possible explanation for these differences may be that in the present study all frequencies, including the mu rhythm, showed an overall amplitude reduction in ASD children following training. Because the decreases were seen for all frequencies, it suggests a more general explanation of the results. That is, since suppression is typically computed as the ratio of experimental to the baseline condition, a training difference could result from changes in either one. The results indicated that the effect of training was to reduce power and the largest reduction occurred in response to the baseline Ball video. Additionally, while absolute power in response to Ball was reduced, responses to Hand, Crayon, and Social videos exhibited increases in power with training, especially for theta and mu frequencies, while decreases occurred primarily in the beta and gamma frequencies.

EEG asymmetry measures differed significantly between groups, but only delta frequencies showed a significant asymmetry between cortical areas, specifically those in the frontal and temporo-occipital regions. In contrast, measures of coherence indicated more widespread effects across all frequencies and especially between regions in temporo-parietal cortex and other electrode sites. PIRT also had a significant impact on amplitude coherence. Slower EEG oscillations (delta, theta, mu) tended to increase in coherence, while faster oscillations (SMR, beta, gamma) tended to decrease. Overall, the most significant difference occurred for the SMR frequency band.

EEG rhythmic oscillations are assumed to be generated by thalamocortical circuits and modulated by a variety of motivational, attentional, motor, and cognitive factors (Sterman, 1996). The PIRT intervention is grounded in the theory of operant conditioning and reinforcement in which participants are taught to modulate and volitionally control specific EEG frequencies. One explanation of how this methodology produces its behavioral and electrophysiological effects is by gaining access to and control over regulatory mechanisms that increase or decrease synchronous or desynchronous activity in thalamocortical networks (Lubar, 1997), leading to the induction of neural plastic changes.

Egner et al. (Egner et al., 2004) have argued that an assumption in the neurofeedback field that motivates clinical practice, especially the use of pre-training quantitative EEG assessments to formulate training protocols, is that "spectral EEG variables related to the operant learning of EEG frequency modulation mediate observed behavioural effects."

Although this assumption has had little support from the clinical research literature, several studies have provided evidence of beneficial effects of neurofeedback training on healthy human participants (Egner et al., 2002; Egner et al., 2004; Egner & Gruzelier, 2004). The present results are consistent with the general beneficial effects of such training on children on the autism spectrum but do not support the assumption that operant training contingencies result in corresponding enhancement/suppression of only the trained frequency components. Indeed, our results suggest a widespread nonspecific effect of training on most other EEG frequencies.

4.1 Conclusion

Sixteen high-functioning autistic spectrum disorder (ASD) participants and twelve typically-developing (TD) participants received twenty weeks of plasticity-inducing rehabilitation training (PIRT) centered on the high mu frequency band (10-13 Hz). Training had a significant impact on reducing the lack of imitation behaviors in ASD children while increasing correct imitation responses. While pre-training assessments indicated that the greatest impairment in the ASD group occurred in the sociability subscale of the Autism Treatment Evaluation Checklist, training reduced those scores as well as scores on the sensory/cognitive awareness subscale, indicating behavioral improvement. Furthermore, PIRT had significant and widespread effects on EEG coherence measures with slower EEG rhythms (delta, theta, and mu) increasing coherence with training, while faster rhythms (SMR, beta, and gamma) decreased coherence. These results suggest that operant conditioning of EEG mu rhythms can induce global neural changes with positive consequences on both the electrophysiology and behavior in high-functioning children on the autism spectrum.

5. References

Altschuler, E. L., Vankov, A., Wang, V., Ramachandran, V. S., & Pineda, J. A. (1998). Person see, person do: Human cortical electrophysiological correlates of monkey see monkey do cells. *J.Cogn Neurosci.*.

Baron-Cohen, S. (2009). Autism: the empathizing-systemizing (E-S) theory. *Ann.N.Y.Acad.Sci., 1156,* 68-80.

Baron-Cohen, S., Jolliffe, T., Mortimore, C., & Robertson, M. (1997). Another advanced test of theory of mind: evidence from very high functioning adults with autism or asperger syndrome. *J.Child Psychol.Psychiatry, 38,* 813-822.

Baron-Cohen, S., Leslie, A. M., & Frith, U. (1985). Does the autistic child have a "theory of mind"? *Cognition, 21,* 37-46.

Blakemore, S. J., Frith, C. D., & Wolpert, D. M. (2001). The cerebellum is involved in predicting the sensory consequences of action. *Neuroreport, 12,* 1879-1884.

Buccino, G., Binkofski, F., Fink, G. R., Fadiga, L., Fogassi, L., Gallese, V. et al. (2001). Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. *Eur.J.Neurosci., 13,* 400-404.

Buccino, G., Binkofski, F., & Riggio, L. (2004). The mirror neuron system and action recognition. *Brain Lang, 89,* 370-376.

Buxbaum, L. J., Kyle, K. M., & Menon, R. (2005). On beyond mirror neurons: internal representations subserving imitation and recognition of skilled object-related actions in humans. *Brain Res.Cogn Brain Res., 25,* 226-239.

Carr, L., Iacoboni, M., Dubeau, M. C., Mazziotta, J. C., & Lenzi, G. L. (2003). Neural mechanisms of empathy in humans: a relay from neural systems for imitation to limbic areas. *Proc.Natl.Acad.Sci.U.S.A, 100,* 5497-5502.

Coben, R., Sherlin, S., Hudspeth, W. J., & McKeon, K. (2009). Connectivity-guided EEG biofeedback for autism spectrum disorder: evidence of neurophysiological changes. *Journal of Autism and Developmental Disorders*.

Cochin, S., Barthelemy, C., Roux, S., & Martineau, J. (1999). Observation and execution of movement: similarities demonstrated by quantified electroencephalography. *Eur.J.Neurosci., 11,* 1839-1842.

Courchesne, E. & Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Curr.Opin.Neurobiol., 15,* 225-230.

Dapretto, M., Davies, M. S., Pfeifer, J. H., Scott, A. A., Sigman, M., Bookheimer, S. Y. et al. (2006). Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. *Nat.Neurosci., 9,* 28-30.

De Renzi E., Motti, F., & Nichelli, P. (1980). Imitating gestures. A quantitative approach to ideomotor apraxia. *Arch.Neurol., 37,* 6-10.

di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., & Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. *Exp.Brain Res., 91,* 176-180.

Egner, T. & Gruzelier, J. H. (2004). EEG biofeedback of low beta band components: frequency-specific effects on variables of attention and event-related brain potentials. *Clin.Neurophysiol., 115,* 131-139.

Egner, T., Strawson, E., & Gruzelier, J. H. (2002). EEG signature and phenomenology of alpha/theta neurofeedback training versus mock feedback. *Appl.Psychophysiol.Biofeedback, 27,* 261-270.

Egner, T., Zech, T. F., & Gruzelier, J. H. (2004). The effects of neurofeedback training on the spectral topography of the electroencephalogram. *Clin.Neurophysiol., 115,* 2452-2460.

Fadiga, L., Buccino, G., Craighero, L., Fogassi, L., Gallese, V., & Pavesi, G. (1999). Corticospinal excitability is specifically modulated by motor imagery: a magnetic stimulation study. *Neuropsychologia, 37,* 147-158.

Gallese, V., Fadiga, L., Fogassi, L., & Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain, 119 (Pt 2),* 593-609.

Gastaut, H. J. & Bert, J. (1954). EEG changes during cinematographic presentation. *Electroencephalogr.Clin.Neurophysiol., 6,* 433-444.

Hari, R., Salmelin, R., Makela, J. P., Salenius, S., & Helle, M. (1997). Magnetoencephalographic cortical rhythms. *Int.J.Psychophysiol., 26,* 51-62.

Hickok, G. (2009). Eight problems for the mirror neuron theory of action understanding in monkeys and humans. *J.Cogn Neurosci., 21,* 1229-1243.

Hirshberg, L. M., Chiu, S., & Frazier, J. A. (2005). Emerging brain-based interventions for children and adolescents: overview and clinical perspective. *Child Adolesc.Psychiatr.Clin.N.Am., 14,* 1-19, v.

Iacoboni, M., Molnar-Szakacs, I., Gallese, V., Buccino, G., Mazziotta, J. C., & Rizzolatti, G. (2005). Grasping the intentions of others with one's own mirror neuron system. *PLoS.Biol., 3,* e79.

Iacoboni, M., Woods, R. P., Brass, M., Bekkering, H., Mazziotta, J. C., & Rizzolatti, G. (1999). Cortical mechanisms of human imitation. *Science, 286,* 2526-2528.

Jarusiewicz, B. (2003). Efficacy of neurofeedback for children in the autistic spectrum: A pilot study. *Applied Psychophysiology and Biofeedback, 28,* 311.

Just, M. A., Cherkassky, V. L., Keller, T. A., & Minshew, N. J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain, 127,* 1811-1821.

Kohler, E., Keysers, C., Umilta, M. A., Fogassi, L., Gallese, V., & Rizzolatti, G. (2002). Hearing sounds, understanding actions: action representation in mirror neurons. *Science, 297,* 846-848.

Leslie, K. R., Johnson-Frey, S. H., & Grafton, S. T. (2004). Functional imaging of face and hand imitation: towards a motor theory of empathy. *Neuroimage., 21,* 601-607.

Lilienfeld, S. O. (2005). Scientifically unsupported and supported interventions for childhood psychopathology: a summary. *Pediatrics, 115,* 761-764.

Lingnau, A., Gesierich, B., & Caramazza, A. (2009). Asymmetric fMRI adaptation reveals no evidence for mirror neurons in humans. *Proc.Natl.Acad.Sci.U.S.A, 106,* 9925-9930.

Lubar, J. F. (1997). Neocortical dynamics: implications for understanding the role of neurofeedback and related techniques for the enhancement of attention. *Appl.Psychophysiol.Biofeedback, 22,* 111-126.

Matson, J. L. (2006). Current status of differential diagnosis for children with autism spectrum disorders. *Res.Dev.Disabil.*.

Muller, R. A. (2007). The study of autism as a distributed disorder. *Ment.Retard.Dev.Disabil.Res.Rev., 13,* 85-95.

Muthukumaraswamy, S. D. & Johnson, B. W. (2004). Changes in rolandic mu rhythm during observation of a precision grip. *Psychophysiology, 41,* 152-156.

Muthukumaraswamy, S. D., Johnson, B. W., & McNair, N. A. (2004). Mu rhythm modulation during observation of an object-directed grasp. *Brain Res.Cogn Brain Res., 19,* 195-201.

Muthukumaraswamy, S. D. & Singh, K. D. (2008). Modulation of the human mirror neuron system during cognitive activity. *Psychophysiology, 45,* 896-905.

Niedermeyer, E. (1997). Alpha rhythms as physiological and abnormal phenomena. *Int.J.Psychophysiol., 26,* 31-49.

Oberman, L. M., Hubbard, E. M., McCleery, J. P., Altschuler, E. L., Ramachandran, V. S., & Pineda, J. A. (2005). EEG Evidence for Mirror Neuron Dysfunction in Autism Spectrum Disorders. *Cognitive Brain Research.*

Oberman, L. M., Pineda, J. A., & Ramachandran, V. S. (2006). The Human Mirror Neuron System: A Link Between Action Observation and Social Skills. *Soc.Cog.Affect.Neurosci.*.

Ozonoff, S., Pennington, B. F., & Rogers, S. J. (1991). Executive function deficits in high-functioning autistic individuals: relationship to theory of mind. *J.Child Psychol.Psychiatry, 32,* 1081-1105.

Petrides, M., Cadoret, G., & Mackey, S. (2005). Orofacial somatomotor responses in the macaque monkey homologue of Broca's area. *Nature, 435,* 1235-1238.

Pfurtscheller, G. & Aranibar, A. (1979). Evaluation of event-related desynchronization (ERD) preceding and following voluntary self-paced movement. *Electroencephalogr.Clin.Neurophysiol., 46,* 138-146.

Pineda, J. A. (2005). The functional significance of mu rhythms: translating "seeing" and "hearing" into "doing". *Brain Res.Brain Res.Rev., 50,* 57-68.

Pineda, J. A., Allison, B. Z., & Vankov, A. (2000). The effects of self-movement, observation, and imagination on mu rhythms and readiness potentials (RP's): toward a brain-computer interface (BCI). *IEEE Trans.Rehabil.Eng, 8,* 219-222.

Pineda, J. A., Brang, D., Hecht, E., Edwards, L., Carey, S., Bacon, M. et al. (2008). Positive behavioral and electrophysiological changes following neurofeedback training in children with autism. *Research in Austism Spectrum Disorders, 2,* 557-581.

Rizzolatti, G. & Craighero, L. (2004). The mirror-neuron system. *Annu.Rev.Neurosci., 27,* 169-192.

Rizzolatti, G. & Fabbri-Destro, M. (2009). Mirror neurons: from discovery to autism. *Exp.Brain Res.*.

Rogers, S. J., Hepburn, S. L., Stackhouse, T., & Wehner, E. (2003). Imitation performance in toddlers with autism and those with other developmental disorders. *J.Child Psychol.Psychiatry, 44,* 763-781.

Sichel, A. G. (1995). Positive outcome with neurofeedback treatment in a case of mild autism. *Journal of Neurotherapy, Vol 1 (1),* p. 60-p. 64.

Sterman, M. B. (1996). Physiological origins and functional correlates of EEG rhythmic activities: implications for self-regulation. *Biofeedback Self Regul., 21,* 3-33.

Umilta, M. A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C. et al. (2001). I know what you are doing. a neurophysiological study. *Neuron, 31,* 155-165.

Villalobos, M. E., Mizuno, A., Dahl, B. C., Kemmotsu, N., & Muller, R. A. (2005). Reduced functional connectivity between V1 and inferior frontal cortex associated with visuomotor performance in autism. *Neuroimage, 25,* 916-925.

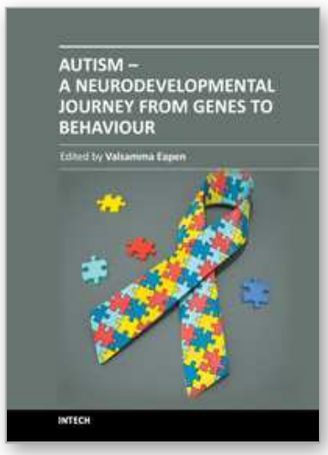
Volkmar, F. R., Klin, A., Siegel, B., Szatmari, P., Lord, C., Campbell, M. et al. (1994). Field trial for autistic disorder in DSM-IV. *Am.J.Psychiatry, 151,* 1361-1367.

Williams, J. H., Waiter, G. D., Gilchrist, A., Perrett, D. I., Murray, A. D., & Whiten, A. (2006). Neural mechanisms of imitation and 'mirror neuron' functioning in autistic spectrum disorder. *Neuropsychologia, 44,* 610-621.

Williams, J. H., Whiten, A., & Singh, T. (2004). A systematic review of action imitation in autistic spectrum disorder. *J.Autism Dev.Disord., 34,* 285-299.

Williams, J. H., Whiten, A., Suddendorf, T., & Perrett, D. I. (2001). Imitation, mirror neurons and autism. *Neurosci.Biobehav.Rev., 25,* 287-295.

Wohlschlager, A. & Bekkering, H. (2002). Is human imitation based on a mirror-neuron system? Some behavioural evidence. *Exp.Brain Res., 143,* 335-341.



Autism - A Neurodevelopmental Journey from Genes to Behaviour
Edited by Dr. Valsamma Eapen

ISBN 978-953-307-493-1
Hard cover, 484 pages
Publisher InTech
Published online 17, August, 2011
Published in print edition August, 2011

The book covers some of the key research developments in autism and brings together the current state of evidence on the neurobiologic understanding of this intriguing disorder. The pathogenetic mechanisms are explored by contributors from diverse perspectives including genetics, neuroimaging, neuroanatomy, neurophysiology, neurochemistry, neuroimmunology, neuroendocrinology, functional organization of the brain and clinical applications from the role of diet to vaccines. It is hoped that understanding these interconnected neurobiological systems, the programming of which is genetically modulated during neurodevelopment and mediated through a range of neuropeptides and interacting neurotransmitter systems, would no doubt assist in developing interventions that accommodate the way the brains of individuals with autism function. In keeping with the multimodal and diverse origins of the disorder, a wide range of topics is covered and these include genetic underpinnings and environmental modulation leading to epigenetic changes in the aetiology; neural substrates, potential biomarkers and endophenotypes that underlie clinical characteristics; as well as neurochemical pathways and pathophysiological mechanisms that pave the way for therapeutic interventions.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jaime A. Pineda, Heather Pelton, Oriana Aragon, Jia-Min Bai, Matt Erhart, Dane Chambers, Burcu Darst, Ernesto Enrique, Steven Gilmore, Stephen Johnson, Albert Anaya, Alicia Trigeiro, Dan T. Lotz, Nicholas Pojman, Tom Gamage and David Linderman (2011). Behavioral and Electrophysiological Characterization of Induced Neural Plasticity in the Autistic Brain, Autism - A Neurodevelopmental Journey from Genes to Behaviour, Dr. Valsamma Eapen (Ed.), ISBN: 978-953-307-493-1, InTech, Available from: http://www.intechopen.com/books/autism-a-neurodevelopmental-journey-from-genes-to-behaviour/behavioral-and-electrophysiological-characterization-of-induced-neural-plasticity-in-the-autistic-br

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

www.intechopen.com

IntechOpen

IntechOpen