Title: Effect of Bumetanide on Neural Correlates of Emotion Recognition in Youth with Autism Spectrum Disorder: An Event-Related Potential Study

Running Title: Effect of Bumetanide on Emotion Recognition in Autism

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ABSTRACT

Objectives: Individuals with autism spectrum disorder (ASD) have impairments in emotion processing including recognizing emotional faces. There is a major need for medication to improve core symptoms of ASD. Bumetanide is one of the most recently used drugs in some studies in ASDs to address this need. This study aimed to evaluate the effect of bumetanide on the brain response of youth with ASD while they were recognizing emotional faces using the event related potentials (ERPs).

Methods: Fifteen youth with ASD aged between 7 to 16 years were evaluated using the Childhood Autism Rating Scale (CARS), Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL), Social Responsiveness Scale (SRS), Wechsler Intelligence Scale for Children-Revised Form (WISC-R), and common blood tests. The electrical brain response was measured while they were doing a Facial Emotional Recognition Task (FERT). After 3 months of treatment, they were assessed again in terms of core symptoms and ERPs.

Results: The behavioral problems of the participants decreased significantly based on CARS. With regards to behavioral performance on FERT, the accuracy of detecting emotions increased and reaction time decreased significantly. The amplitude of N170, EPN, and N250 increased and latency for N170 and N250 decreased significantly in some electrodes. There were no serious side effects.

Conclusion: In this study, bumetanide improved behavioral symptoms as well as the ability to recognize emotions. Also, brain function was improved based on the ERP components. So, it can be concluded that bumetanide can be used safely in children and adolescents with ASD to improve the main symptoms of the disorder.

Keywords: Bumetanide, Autism Spectrum Disorder, Facial emotion recognition, Event-Related Potentials.

Introduction

Autism Spectrum Disorder (ASD) is considered as a neuro-developmental disorder that is identified by a deficit in social communication and having repetitive behaviors (American Psychiatric Association (APA), 2013). In recent years, the number of children recognized with ASD has increased and reports indicate that ASD affects nearly 1% of the population (Baio, 2012; Dilip Jeste et al., 2012). This disorder is characterized by an inability in social interaction, including difficulty in making friendships and lack of understanding others' emotions and minds (Kaland, Smith, & Mortensen, 2007). Impairment in comprehending others' mental states plays a significant role in social and communicative deficits in ASD (Baron-Cohen et al., 1996). According to Haviland and Lelwica (1987), the ability to identify and distinguish emotional expressions develops in typically developing infants from 10 weeks of age (Haviland & Lelwica, 1987) and continues during childhood (Herba & Phillips, 2004). This ability is delayed in children with ASD and is evaluated using the tasks which assess facial emotion recognition, vocal intonation, and body language (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001; Yirmiya, Sigman, Kasari, & Mundy, 1992). The behavioral studies reported a failure in this ability and reduced accuracy in detecting emotions through faces, especially negative ones (Ashwin, Chapman, Colle, & Baron-Cohen, 2006; Corden, Chilvers, & Skuse, 2008; Wallace, Coleman, & Bailey, 2008)(Rump, Giovannelli, Minshew, & Strauss, 2009) as well as voices (Baker, Montgomery, & Abramson, 2010). This impairment is supposed to be a core deficit in autism spectrum disorder (Philip et al., 2010).

In event related potentials studies, smaller amplitudes and increased latencies were compatibly reported to emotional expressions in patients with ASD compared to those of the control group and confirmed the facial emotion processing deficits in ASDs. According to these studies, statistically significant differences were found between the groups mainly in P1 and N170 amplitudes and latencies, in which those with ASD represented delayed latencies and lower amplitudes to emotional stimuli compared to typically developing individuals (Apicellaa, Siccaa, Federicob, Campatellia, & Filippo

Muratoria, 2012; Batty, Meaux, Wittemeyer, Rogé, & Taylor, 2011; O'Connor, Hamm, & Kirk, 2005).

Accordingly, emotion recognition, especially in the face, demonstrates an ordinary intervention target, which highlights the need for explaining sensory and cognitive processes that uphold social cognition, especially emotion recognition in ASD (Akechi et al., 2010; Lerner, McPartland, & Morris, 2013).

Social cognition deficiency in this population remains the main treatment challenge (Weiss & Harris, 2001). On the other hand, the European Medicine Agency or Food and Drug Administration has not so far found approved and effective medication for ASD that significantly improves the core symptoms, especially social interaction and emotion recognition (E. Lemonnier, Villeneuve, Sonie, Serret, Rosier, Roue, Rondeau, et al., 2017). Only two approved drugs are available for treating autism-related irritability, including risperidone (5-16 years old) which is regarded as a serotonin type 2A (5-HT2A) and dopamine type 2 (D2) receptor antagonist (McCracken, 2002) and aripiprazole (6-17 years old) which is regarded as a partial dopamine (DA) D2 and 5-HT1A agonist, and a 5-HT2A antagonist (Wink, Erickson, & McDougle, 2010). Regardless of their side effects (Fung et al., 2016; E. Lemonnier, Villeneuve, Sonie, Serret, Rosier, Roue, Rondeau, et al., 2017), none of these drugs seems to reduce the core symptoms of ASD (E. Lemonnier, Villeneuve, Sonie, Serret, Rosier, Roue, Rondeau, et al., 2017). Bumetanide, as a diuretic and chloride cotransporter antagonist, has been recently proposed for the treatment of ASD (E Lemonnier et al., 2017; Mollajan, Joghataei, & Tehrani-Doost, 2019). Based on previous evidence, the level of chloride and GABAergic signaling changes in ASD and some other developmental disorders (Ben-Ari, 2015; Blaesse, Airaksinen, Rivera, & Kaila, 2009; Nardou et al., 2011). Bumetanide has been widely used in adults since 1975 and children since 1986 for the treatment of some disorders such as broncho-pulmonary dysplasia, hypertension, congestive heart failure, and nephrotic syndrome. Bumetanide restores low $(Cl^-)_I$ levels and shifts GABA from excitation to inhibition (Ben-Ari, 2015). According to a previous study, the maternal infusion of bumetanide in ASD animal models reduced the physiological levels $(Cl^-)_I$ in the offspring, and regulated electrical and behavioral parameters (Eftekhari et al., 2014; Tyzio et al., 2014). Based on these observations, the

effects of bumetanide on five neonates with autism were evaluated and the results showed the improvement of the behavioral symptoms of infantile autistic syndrome with no serious side effects (Eric Lemonnier & Ben-Ari, 2010). The results of another study confirmed these findings and demonstrated that bumetanide significantly ameliorated the symptoms of ASD. The side effects of the drug in this study were limited to mild hypokalaemia which was managed by potassium supplement (E Lemonnier et al., 2012). Moreover, an open trial study on seven adolescents and young adults with autism indicated improvement of emotional face recognition and the activation of involved brain regions (Hadjikhani et al., 2015). Hadjikhani et al., using fMRI and eye-tracking, also found that the administration of bumetanide normalized the amygdala activation level during constrained eye contact with dynamic emotional face stimuli in individuals with autism spectrum disorder. Furthermore, data on eye-tracking revealed that the amount of time allocated to the spontaneous eye gaze increased during a free-viewing mode of identical face stimuli by administering bumetanide. These results represented that bumetanide probably improves the specific aspects of social processing in autism (Hadjikhani et al., 2018)

Based on the results of recent studies on the effect of bumetanide in these patients, especially at the behavioral level, the present study aimed to evaluate the effect of this drug on facial emotion recognition and emotion processing, as well as changes in neuronal functions using ERPs. We wanted to see if the medication could improve emotion recognition and whether the neural correlates of emotion processing would be changed after the treatment.

Material and methods

Participants

Twenty boys were recruited for this study from the child and adolescent psychiatry clinics, who were diagnosed with autism spectrum disorder (ASD) level 1- needing support based on DSM-5 (high-functioning ASD (ASD-HF), pre-DSM-5 nomenclature) and were within the age range of 7-16 years. Among the participants, five cases were excluded the study before ending the study because of some behavioral disturbance in

their behaviors such as aggression and restlessness due to stopping the previous medications (n=2) and before the follow up phase because of not having enough cooperation for using the drug regularly and continuing the plan (n=3). All participants were right-handed, possessed normal or corrected to normal vision, and reported no history of critical neurological and medical problems such as epilepsy, brain injury, or metabolic disorders. Moreover, they had no liver dysfunction, renal disease, cardiac problem, and allergy to sulfonamides. All participants had IQ scores above 70 based on the Wechsler Intelligence Scale for Children (WISC-R).

Measures

Childhood Autism Rating Scale (CARS): It contains 15 items each scored 1-4 ranging from normal, mildly abnormal, moderately abnormal to severely abnormal, that collectively adds up to a total score of 15-60 and 30 is the cutoff rate for diagnosing mild autism. Additionally, scores within the range of 30-37 represent mild to moderate autism whereas those between 38 and 60 demonstrate severe autism (Schopler, Reichler, DeVellis, & Daly, 1980) .

The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL): This scale is a semi-structured diagnostic interview that is used to evaluate current, past, and lifetime diagnostic status in children and adolescents within the age range of 6-18 years (Kaufman et al., 1997) . Kaufman et al. reported various components of the K-SADS, and the psychometric characteristics of its Persian version were well confirmed for most psychiatric disorders (Shahrivar, Kousha, Moallemi, Tehrani-Doost, & Alaghband-Rad, 2010).

Social Responsive Scale (SRS): It was designed to determine the presence of impairments in reciprocal social behaviors (Constantino et al., 2006). This 65-item rating scale measures the severity of each behavior ranging from mild to severe, and produces a total and five subscale scores. This scale can be filled out by parents, teachers, or other frequent care providers in 15-20 min. This questionnaire can be

applied in various ways such as a measure of the severity of social impairment in children at risk of ASD and a general population screening instrument. In addition, SRS can be utilized for behavioral, genetic, and intervention evaluation studies. The Persian version of this questionnaire was validated in a community sample of Farsi speaking individuals (Tehrani‐Doost et al., 2018).

Wechsler Intelligence Scale for Children-Revised (WISC-R): The measure is individually administered to children in the age range of 6-16 years to evaluate their intelligence levels (Wechsler, 1949). It includes two verbal (i.e., information, comprehension, Mathematics, numbers, similarities, and vocabulary) and non-verbal (i.e., picture arrangement, picture completion, object assembly, block design, coding, and Maze) intelligence scales. The Persian version of this questionnaire was validated in a community sample of Farsi speaking individuals (Shahym, 1991). The verbal intelligence scale was used in this study.

Event-Related Potential (ERP) task: This task was developed using different faces expressing sadness, happiness, and neutral status (4 men and 4 women) selected from the Radboud face database (Lang, 1997). The selected faces were validated in terms of their emotions in 60 normal individuals with the same age range and gender. All the face stimuli covered a visual angle of about $5.5^{o} \times 7.5^{o}$ and were presented on a computer screen in front of a gray background. The task in this study was designed by the eevoke^{TM} software and had a total of 144 stimuli and each facial expression (i.e., happy, sad, and neutral) was repeated 48 times. The task included 3 blocks, 48 stimuli per block which were randomly presented to avoid the lack of interest in the subjects. The presentation time of each stimulus included 2000 ms, followed by continuously presenting a blank screen with a white fixation cross at the center for 1000 ± 300 ms. The participants were requested to carefully look at the stimuli while maintaining fixation, and to avoid the other body movements or taking a deep breath.

ERP Recording: ERPs were recorded using the ANT (Advanced Neuron Technology) system with 32 Ag/AgCl sintered electrodes mounted on an elastic cap following the 10/20 international system. The averaged mastoids were employed as the reference and grounded to the Fz channel. Furthermore, two electrodes were applied to monitor vertical eye movements in order to remove offline artifacts, followed by keeping the impedance below 5 κΩ throughout the procedure. Then, raw data were filtered on-line with a band-pass from 0.01 to 100 Hz and sampled at 250 Hz. Next, the acquired data resembled offline at a 250-Hz sampling rate. Moreover, baseline corrections were performed approximately 200 ms before the stimulus onset in order to confirm the quality of the recording. The recording condition was in line with the required standards (i.e., under the electrical shield and at a sound-attenuated and dim-lighted room).

Common Blood Test: These tests included Alkaline Phosphatase (ALP), Uric Acid (UA), and Creatine, sodium, potassium, and the kidney and liver functions of the participants.

Procedure

First, the participants were interviewed by a child and adolescent psychiatrist and diagnosed as ASD-HF (level 1-needing support) based on DSM-5 criteria. Then, they were evaluated using the CARS and K-SADS-PL, as well as SRS. The WISC-R was utilized to evaluate intellectual abilities as well. The blood tests were also performed on the participants. The study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1395.4537), and a consent form was obtained from the participants' parents before initiating the study. The participants were off medication one week before the study except for melatonin at a dose of 3 mg. To perform the ERP experiment, each participant was requested to sit with a 60-cm distance from the monitor screen (a 19w monitor, LG F900P with a 100-Hz screen refresh rate) and was instructed to fix his head on a chin rest and watch the monitor screen. After the evaluations, bumetanide was prescribed at 1 mg daily (0.5 mg twice a day) for three months based on the protocol of previous studies (E. Lemonnier et al., 2012; E. Lemonnier,

Villeneuve, Sonie, Serret, Rosier, Roue, Brosset, et al., 2017). The medication adherence and probable adverse effects of the medication were assessed asking the parents based on the number of pills used, and using common blood test periodically at weeks 1, 6, and 12 of the trial in case of the adverse effects. The CARS, SRS, and the blood tests were repeated at weeks 6 and 12 again. The ERP experiment was repeated less than one week after the intervention.

Data measure and analysis

The latencies and amplitudes of traditionally analyzed channels for N170 (P7, P8) (Eimer, 2000; Eimer & Holmes, 2007; Luo, Feng, He, Wang, & Luo, 2010; Pesciarelli F, 2011), Early Posterior negativity (EPN) (Cp5, Cp6, P3, P4) (Junghöfer, Bradley, Elbert, & Lang, 2001; Rellecke, Sommer, & Schacht, 2013; Schupp et al., 2004), and N250 (C3, C4, Fc1, Fc2, F3, F4) (Eimer, 2000; Harms, Martin, & Wallace, 2010; Luo et al., 2010; Pesciarelli F, 2011) as the primary variables and other exploratory electrode regions for this components as the secondary variables were evaluated in this study. The time epochs selected for these components included 130-250 MS for N170, 150-250 MS for EPN, and 215-350 MS for N250. The data were analyzed using the MATLAB software (version 2014b) integrated with EEGLAB and ERPLAB. Then, the acquired data was approached the offline at a 250-Hz sampling rate and digitally filtered offline from 0.1 to 30 Hz. The continuous electroencephalographic data were divided into 1000-ms epochs starting 200 ms before and 800 ms after the stimulus onset. The eye-movements were corrected by applying Independent Component Analysis (Makeig & Enghof, 2000). Next, the remaining artifacts were further eliminated by a semi-automatic rejection, removing epochs exceeding ± 100 μV in each channel. The mean rejection rate of the epochs was 7.66% and there were no significant differences between the conditions.

Statically analysis

The paired t-test and the repeated measures ANOVA including two factors; emotion (3 levels: happy, sad, and neuter) and treatment (2 levels: pre-test and post-test) were conducted to find the main effect of emotion, treatment, and their interactions on

latencies and amplitude for each electrode separately. P-values were corrected by the Bonferroni correction.

Results

Characteristics of the participants are shown in table 1and 2.

The results of the childhood autism rating scale (CARS) and social responsiveness scale (SRS)

With regard to CARS variables, the repeated measures analysis revealed a significant amelioration of the total score during and after the intervention (P<0.05). The total score of SRS failed to differ markedly during and after treatment with bumetanide (P>0.05) although it decreased due to the intervention (Table 2).

Behavioral data of ERP recording

Response Accuracy (RA): The repeated measures analysis showed that the main effect of emotion on the correct response was significant (P=0.000, F=13.121). Based on the pairwise comparisons, happy faces were significantly detected more compared to sad (P=0.000) and neutral faces (P=0.003). Additionally, the main impact of treatment was significant on RA while the interaction between emotion and treatment demonstrated no significant effect (Table 3).

Reaction Time (RT)

RT of All Responses (RT_AR): The main effects in RT_AR were not significant in any of the emotional states (P>0.05) according to the ANOVA-RM results.

RT of Correct Responses (RT_CR): Based on ANOVA results, the main effect of emotion in RT_CR was significant (P=0.020, F=4.490) whereas no one was significant in the pairwise comparison with Bonferroni correction between the emotional states. In addition, the main effects of treatment (P=0.258, F=1.391)

and interaction between emotion and treatment (P=0.146, F=2.066) were not significant regarding this variable (Table 3).

ERP components

Figures 1A and 1B show the grand averages of ERP waves of the three emotional states in pre- and post-treatment.

N170

Peak Amplitude (PA): The results of ANOVA-RM in the PA of this component showed that the main effect of interaction between emotion, and treatment was significant in P7 electrode (P=0.008, F=5.720) and according to the t-test result, it increased significantly in sad faces (P=0.05, T=2.056). However, the main effects of emotion and treatment were not significant in this electrode (P>0.05). The PAs of the other electrodes were not significant (P>0.05).

Peak Latency (PL): The PL of this component was not significant in any of the electrodes (P>0.05), the details of which are provided in Table 4.

EPN

Peak Amplitude: Based on the ANOVA-RM for PA, the main effect of treatment was significant for Cp1 (0.047, F=4.719) and its mean difference in the post-test was greater than the pre-test. Contrarily, the main effects of emotion and interaction between emotion and treatment were not significant (P>0.05) in this electrode. Further, the PAs of the other electrodes were not significant.

Peak Latencies: The main effect of emotion was significant (P=0.000, F=495.591) for Cp6 and the latency decreased in post-test compared to pre-test in all emotional states, but the main effects of treatment and interaction between emotion and treatment were not significant in this electrode (P>0.05). Eventually, the PL of this component was not significant in the other electrodes as well (P>0.05). The related data are presented in Table 4.

N250

Peak Amplitude: The analysis of the results regarding this component revealed that the main effect of interaction between emotion and the treatment in P3 was significant in terms of PA based on ANOVA results (P=0.011, F=5.372). The results of t-test showed that it increased significantly in happy faces (P=0.05, T=2.070). However, the main effects of emotion and treatment were not significant (P>0.05) in this electrode. Furthermore, the PA of the other electrodes were not significant respecting this component (P>0.05).

Peak Latencies: With regard to the ANOVA results, the main effects of treatment in P3 (P=0.018, F=7.141) and Pz (P=0.002, F=14.079) were significant and decreased in the post-test, but the main effects of emotion and interaction between emotion and treatment were not significant in these channels (P>0.05). As regards Fc2, the main effects of treatment (P=0.031, F=5.730) and interaction between emotion and treatment (P=0.05 F=3.261) were significant and based on the results of the t-test (Table 3), there was a decrease in the mean differences in the sad (P=0.048, T=-2.168) and happy (P=0.003, T=3.514) faces.

Figures 3A, 3B, and 3C show ERP scalp distributions of the N170, EPN, and N250 components respectively.

In this study, the results of the blood tests showed no significant changes of blood serum potassium before and after the intervention, indicating that bumetanide failed to increase the risk of hypokalemia. Moreover, the adverse events of bumetanide only included mild diuresis which was not salient.

Discussion

Based on previous studies, bumetanide medication which alters the GABAergic system from excitation to inhibition(Ben-Ari, 2015; Blaesse et al., 2009; Nardou et al., 2011), can reduce the core symptoms of autism spectrum disorder. One of the main deficits of this disorder is the impairment of social interaction which is caused by deficits in

understanding others mental states including their emotions through the facial expressions (Baron-Cohen et al., 1996, 2001; Kaland et al., 2007; Yirmiya et al., 1992). Moreover, based on the Hadjikhani's findings, bumetanide can improve emotion recognition and alter brain activation related to this function in people with ASD (Hadjikhani et al., 2018, 2015). According to these findings we hypothesized that bumetanide could change neural responses based on action potentials related to facial emotion recognition in individuals with ASD. Therefore, we conducted
the present study to evaluate the effect of bumetanide on facial emotion recognition and neural correlates of boys within the age range of 7-16 years old with ASD level 1 (needing support) according to DSM-5 classification. The evaluations included CARS, K-SADS-PL, WISC-R, SRS, ERP recording, and common blood tests. The CARS, SRS, and the blood tests were repeated at weeks 6 and 12 and ERP recording was repeated 12 weeks after treatment. The blood tests of the participants had no significant changes during the intervention and no obvious side effects were reported for medication.

Effects of bumetanide treatment on core symptoms of ASD

In this study, the total score of CARS showed a significant improvement in terms of clinical and general behavioral symptoms. These findings confirmed our hypothesis indicating that bumetanide decreases the clinical and behavioral aspects of ASD and ameliorates its core symptoms. This is consistent with the results of Lemonnier and Ben-Ari. (2010), Lemonnier et al. (2012, 2013, and 2017), and Hadjikhani et al. (2015).

Effects of bumetanide on behavioral data of ERP recording

Response Accuracy (RA): Based on the results, the main effect of emotion was significant on response accuracy (without considering the effect of bumetanide) so that the happy faces were recognized more than sad ones. This means that this medication can improve detection of emotional faces especially happy ones and confirmed one of our hypotheses. This finding is consistent with the results of some studies which reported impaired detecting negative emotions in individuals with

ASD (Ashwin et al., 2006; Corden et al., 2008; Wallace et al., 2008). Considering feature-based processing in ASD (Behrmann, Thomas, & Humphreys, 2006), these individuals have more difficulty in recognizing sadness compared to happiness which can be recognized by the upturned mouth alone (Harms et al., 2010). This was interpreted that processing of the mouth region could be sufficient for detecting happiness compared to the other emotional states (Adolphs et al., 2005; Uljarevic & Hamilton, 2013).

It was found in this study that response accuracy increased after treatment with bumetanide regardless of the type of emotion. This finding is consistent with the study results which showed that bumetanide increased response accuracy and reduced reaction time in an emotional matching task along with improving emotion recognition and increased activity of the brain regions involved in emotion processing (Hadjikhani et al., 2018, 2015; Vlaskamp, 2015).

The Reaction Time of All Responses (RT_AR) and correct responses (RT_CR): According to our findings, the main effect of emotion was not significant among the emotional states in terms of the RT_AR and RT_CR. This can be interpreted that bumetanide did not change the time spent to recognize emotions through faces. It was proposed that this duration would be increased after treatment because of slow response time in recognizing emotions in individuals with ASD.

The main effects of treatment and interaction between emotion and treatment were not significant regarding these variables, meaning that the intervention had no significant effect on the response time. This may be due to the small sample size.

Effects of bumetanide on ERP components

Data analysis showed that the peak amplitude and the peak latency of ERP components including N170, EPN, and N250 were significantly differ in some electrodes between pre and posttreatment.

N170 component

Peak Amplitude: Regarding the PA of N170, a significant increase was observed in P7 electrode after treatment with bumetanide. It has been shown that individuals with ASD had smaller N170 amplitude compared to normal people (Lerner et al., 2013; O'Connor et al., 2005; Wong, Fung, Chua, & McAlonan, 2008). Therefore, it seems that bumetanide has led to more neural response while recognizing sad expression. This finding is in line with the Hadjikhani`s results when studied brain activation in individuals with ASD after bumetanide treatment. They found an increase brain activity in regions involved in processing emotional face expressions including the inferior occipital cortex and the fusiform cortex where is equivalent to P7 increased amplitude seen in our study (Hadjikhani et al., 2015).

Peak Latency: The PL of N170 was not significant in any channels. It seems that bumetanide had no significant effect on speed of neural response while watching emotional faces. The behavioral responses mentioned above are consistent with neural responses which are reflected in peak latencies of the ERPS.

EPN Components

Peak Amplitude: Based on the result, the PA of EPN was significantly increased in Cp1 after treatment. This finding shows that bumetanide increased the neural brain activity while recognizing emotions which led to increased accuracy in detecting facial expressions.

Peak Latency: The PL of this component was significantly decreased in Cp6 channel just in neutral faces.

EPN is the emotion-sensitive early posterior negativity that arises over temporo-occipital electrodes about 150 to 300 ms after the stimulus onset. It is related to the increased perceptual encoding of emotional stimuli in the visual cortex (Rellecke, Sommer, & Schacht, 2012). Studies on children with autism indicated a weak and delayed appearance of this component in these patients

(Faja, Dawson, Aylward, Wijsman, & Webb, 2016; Monteiro, Simões, Andrade, & Castelo Branco, 2017; Rellecke et al., 2013). Based on the findings of the current study, it seems that bumetanide can increase the neural activity and cognitive effort in temporo-occipital region while approaching emotions.

N250 component

Peak Amplitude: The results showed that the PA of N250significantly increased in P3 channel while recognizing happy faces. It means that bumetanide can increase neural responses in individuals with ASD while detecting positive emotion which leads to increased accuracy of detecting emotions.

Peak latency: Based on the results of this study, bumetanide treatment decreased significantly the latency of N250 in P3, Pz, and Fc2 electrodes. Moreover, the significant decreases were shown for happy and sad faces in Fc2 channel based on the significant interaction between emotion and treatment in repeated measure analysis. This finding demonstrated that using bumetanide can increase speed of neural response in youth with ASD while viewing happy and sad faces which causes more rapid recognizing these emotions.

The N250 is considered for marking higher-order face processing such as affect decoding. This ERP component reflects the earliest evaluation of emotion processing involved in facial emotion perception (Lerner et al., 2013). Other researches showed that it is associated with decoding emotional concepts and is evoked by observing an emotionally expressive face (Balconi & Pozzoli, 2008; Carretié, Martín-Loeches, Hinojosa, & Mercado, 2001; Wynn, Lee, Horan, & Green, 2008). In the present study, the amplitude of this component increased in P3, especially in happy faces while its latency significantly decreased for sad and happy faces in P3, Pz, and Fc2 after the intervention. It means that bumetanide can increase neural involvement and rapid response in reaction to emotional faces and recognizing the content of

facial expressions. Based on previous studies it has been found a decrease in amplitude and an increase in latency of this component in individuals with autism spectrum disorder (Jeste & Nelson, 2009; Lerner et al., 2013). Therefore, it seems that bumetanide can increase neural activity and its response speed in individuals with ASD while evaluating emotions.

Conclusion

To the best of our knowledge, this study was the first one to evaluate the effect of bumetanide on facial emotion recognition and its neural correlates in youth with autism spectrum disorder (ASD) using the event-related potentials (ERP). The findings showed that using bumetanide improved the clinical and behavioral aspects of ASD according to the total score of the Childhood Autism Rating Scale. Furthermore, the results demonstrated an increase in the amplitude as well as a decrease in the latency of ERP components including N170, EPN, and N250 in some channels while viewing happy and sad faces. Thus, it seems that using bumetanide can increase the ability of emotion recognition and alter the neural activity of involved brain areas. These results confirmed previous findings regarding the improvements of symptoms and brain function of individuals with ASD after bumetanide treatment.

The results of this study should be considered in light of some limitations including the small sample size and the absence of placebo control group. It is recommended to do a multi-center double blind placebo-controlled study with larger sample size.

Conflict of interests

The authors declare that they have no conflict of interests.

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Table 1. Characteristics of the participants

Group	Age	Mean & SD	IQ
Intervention (15 boys)	7-16	9.187±2.245	86.904±13.118

Table 2. Characteristics of the participants based on CARS and SRS variables

Variable	Pre-test	Follow-up	Post-test	Main Effect of Time		
				Sig	Mean	Pairwise Comparison
CARS	Mean=29.873 SD=2.326	Mean=27.233 SD=2.903	Mean=25.340 SD=2.474	P-value= 0.000* F=34.478	Pre=29.873 F=27.233 Pos=25.340	Pre-Follow=0.001* Pre-Pos=0.000* Follow-Pos=0.007*
SRS	Mean=154.866 SD=19.877	Mean=146.466 SD=13.772	Mean=146.400 SD=16.021	P-value= 0.127 F=2.228	Pre=154.867 F=146.467 Pos= 146.400	No Sig

Table 3. Characteristics of the Participants in Terms of Response Accuracy and Reaction Time in ERP Task

Variable	Emotional State	ANOVA-RM							
		Pre	Pos	Main Effect of Emotion			Main Effect of Treatment		Main Effect of (E&T)
				Sig	Mean	Pairwise Comparison	Mean	Sig	
Correct Response	Sad	Mean = 59.996 SD = 22.919	Mean = 74.858 SD = 21.700	P-value* = 0.000 F=13.121	67.427	Sad-Hap = 0.000* Nut-Hap = 0.003*	Pre=67.033 Pos=84.118	P-value = 0.001* F= 15.961	P-value = 0.476 F= 0.763
	Nut	Mean = 64.440 SD = 26.452	Mean = 85.136 SD = 14.999		74.788				
	Hap	Mean = 76.662 SD = 24.066	Mean = 92.358 SD = 8.650		84.511				
RT of All Responses	Sad	Mean = 1335.67 SD = 223.991	Mean = 1316.64 SD = 289.844	P-value = 0.074 F= 3.244	1326.16	NO Sig	Pre=1272.09 Pos=1296.00	P-value = 0.624 F= 0.251	P-value = 0.448 F= 0.739
	Nut	Mean = 1294.41 SD = 268.817	Mean = 1292.05 SD = 184.764		1293.23				
	Hap	Mean = 1260.28 SD = 190.052	Mean = 1205.23 SD = 202.353		1232.75				
Reaction time of correct	Sad	Mean = 1464.26	Mean = 1439.15	P-value* =	1451.70	NO Sig	Pre=1433.69 Pos=1384.93	P-value= 0.258	P-value = 0.146

responses			0.020		F= 1.391	
		SD = 275.861	SD = 262.262	F= 4.490		F= 2.066
	Nut	Mean = 1503.40	Mean = 1385.40		1444.40	
		SD = 195.146	SD = 181.467			
	Hap	Mean = 1333.40	Mean = 1330.25		1331.83	
		SD = 223.926	SD = 277.982			

Table 4. The Results of ERP Components

Variable		Electrode		ANOVA-RM							T-Test	
				Pre	Pos	Main Effect of Emotion			Main Effect of Treatment		Main Effect of (E&T)		
						Sig	Mean	P.C	Mean	Sig			
N170	PA	P7	Sad	Mean = -3.013 SD= 3.512	Mean= -4.092 SD= 3.485	P-value = 0.192 F = 1.749	-3.403	No Sig	Pre = -3.646 Pos = -3.978	P-value = 0.601 F = 0.286	P-value = 0.008 F=5.720	P-value * = 0.059	T-value = 2.056
			Nut	Mean = -3.116 SD = 2.293	Mean = -3.689 SD = 3.771		-3.553					P-value = 0.461	T-value = -0.757
			Hap	Mean = -3.727 SD = 3.008	Mean = -5.230 SD = 4.640		-4.479					P-value = 0.137	T-value = -0.577
EPN	PA	Cp1	Sad	Mean = -5.277 SD = 3.501	Mean = -6.681 SD = 4.955	P-value = 0.728 F = 1.321	-5.979	No Sig	Pre = -5.113 Pos = -6.667	P-value * = 0.047 F = 4.719	P-value = 0.820 F=0.200	No Sig	
			Nut	Mean = -4.955 SD = 3.024	Mean = -6.305 SD = 3.316		-5.631						
			Hap	Mean = -5.104 SD = 3.922	Mean = -7.014 SD = 4.553		-6.060						
	PL	Cp6	Sad	Mean = -201.041 SD =	Mean = -200.260 SD = 34.890	P-value = 0.239 F= 1.509	200.651	No Sig	Pre = 204.167 Pos = 202.69	P-value = 0.870 F =	*P-value = 0.045 F=3.480	P-value =0.957	T-value= 0.055

				34.871					1	0.028			
			Nut	Mean = 206.771 SD = 32.125	Mean = 191.666 SD = 34.702		199.219					*P-value = 0.036	T-value = -2.319
			Hap	Mean = 219.791 SD = 27.82	Mean = 201.041 SD = 29.560		210.47					P-value = 0.155	T-value = 1.502
N250	PA	P3	Sad	Mean = -2.788 SD = 2.177	Mean= -3.164 SD = 2.272		-2.426					P-value = 0.563	T-value= 0.592
			Nut	Mean = -2.198 SD = 2.452	Mean = -3.265 SD = 2.283	P-value = 0.356 F = 1.070	-2.732	No Sig	Pre = -2.558 Pos = -2.865	P-value = 0.599 F = 0.290	P-value* = 0.011 F=5.372	P-value = 0.203	T-value = -1.337
			Hap	Mean = -1.619 SD = 1.848	Mean = -3.232 SD = 2.983		-2.977					P-value* = 0.057	T-value = 2.070
	PL	P3	Sad	Mean = 285.677 SD = 50.713	Mean = 266.145 SD = 46.685	P-value = 0.067 F = 3.428	275.912	No Sig	Pre = 280.295 Pos = 253.646	*P-value = 0.018 F = 7.141	P-value= 0.178 F=1.836	No Sig	
			Nut	Mean = 292.708 SD = 37.626	Mean = 246.614 SD = 37.983		269.622						

	Hap	Mean = 262.500 SD = 42.948	Mean = 248.177 SD = 29.963		255.339						
Pz	Sad	Mean = 278.645 SD = 43.010	Mean = 273.697 SD = 42.948		267.188	No Sig	Pre = 273.090 Pos = 251.389	P-value* = 0.002 F = 14.079	P-value = 0.994 F=0.006	No Sig	
	Nut	Mean = 273.698 SD = 49.947	Mean = 252.604 SD = 48.915	P-value = 0.454 F = 0.661	263.151						
	Hap	Mean = 266.927 SD = 44.992	Mean = 245.833 SD = 38.795		256.380						
Fc2	Sad	Mean = 306.771 SD = 34.304	Mean = 282.291 SD = 41.693		309.636	No Sig	Pre = 308.941 Pos = 292.795	P-value* = 0.031 F=5.730	P-value* = 0.053 F=3.261	P-value =* 0.048	T-value = -2.168
	Nut	Mean = 299.218 SD = 47.079	Mean = 297.656 SD = 46.153	P-value = 0.221 F = 1.595	298.438					P-value = 0.878	T-value = -0.156
	Hap	Mean =322.396 SD = 31.245	Mean = 296.875 SD = 41.471		294.531					P-value =* 0.003	T-value = -3.514

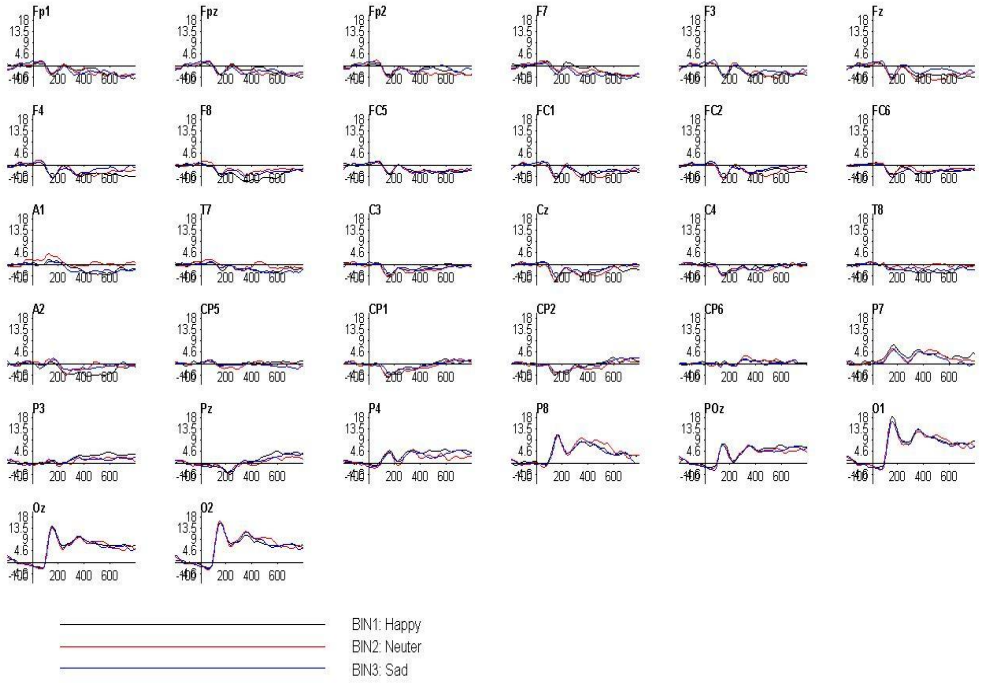
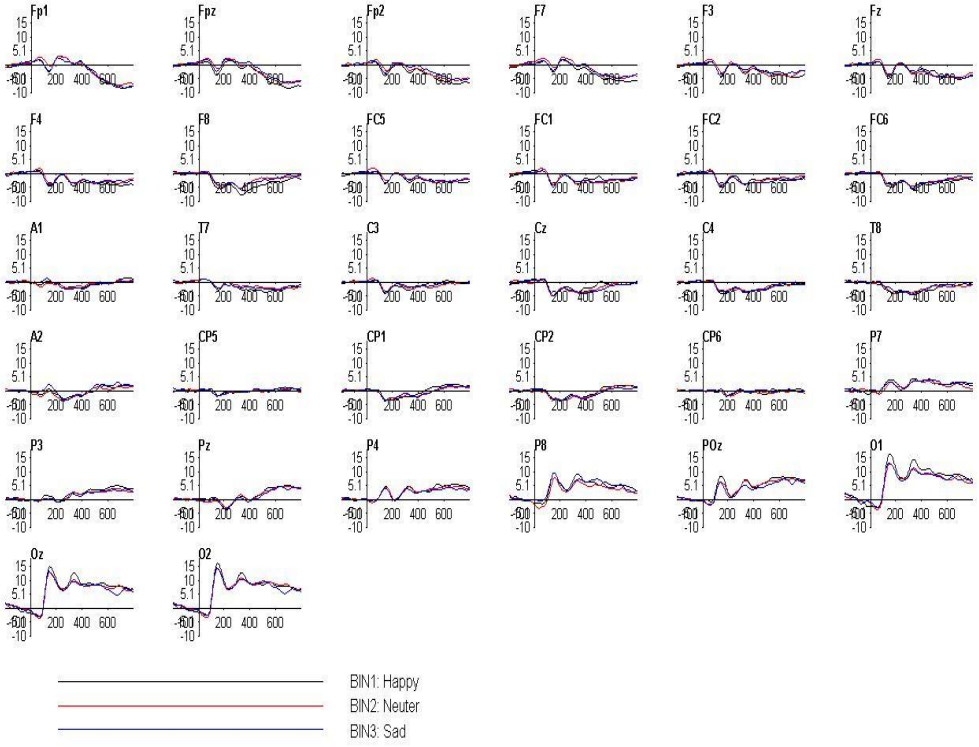
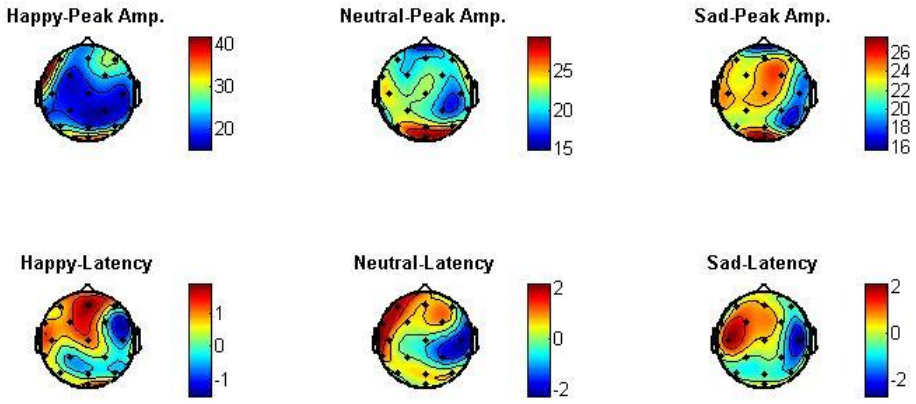


Figure1. A. Grand Averages of the ERP components for the happy, sad, and neutral expressions in the pre-test

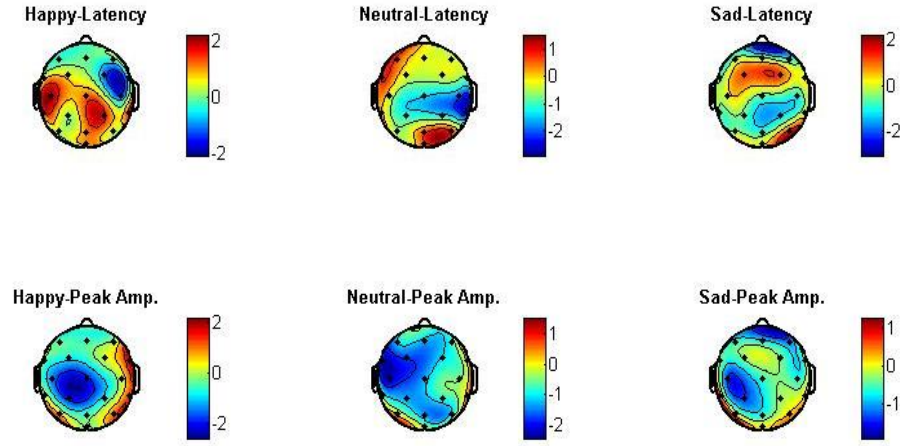


B. Grand Averages of the ERP components for the happy, sad, and neutral expressions in the post-test.

A.


Happy-Peak Amp.
40
30
20
Neutral-Peak Amp.
25
20
15
Sad-Peak Amp.
26
24
22
20
18
16
Happy-Latency
1
0
-1
Neutral-Latency
2
0
-2
Sad-Latency
2
0
-2

B.


Happy-Latency
2
0
-2
Neutral-Latency
1
0
-1
-2
Sad-Latency
2
0
-2
Happy-Peak Amp.
2
0
-2
Neutral-Peak Amp.
1
0
-1
-2
Sad-Peak Amp.
1
0
-1

C.

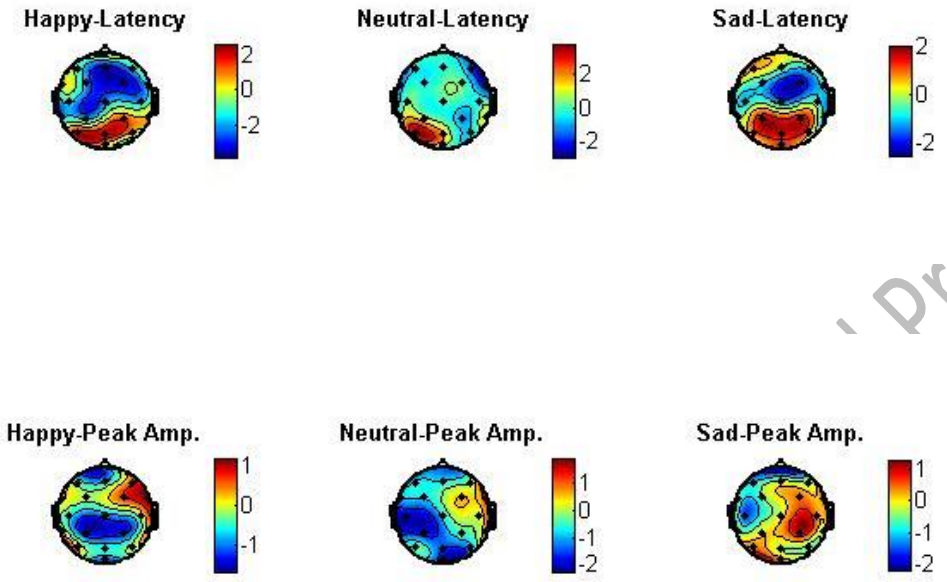


Figure 3. ERP scalp distributions of the A. N170, B. EPN, and C. N250 in each facial expression.