

FINAL COMPREHENSIVE REPORT
THE MATERNAL AND CHILD HEALTH RESEARCH PROGRAM
Predictors of effects of propranolol on language & connectivity in autism

I. Introduction

A. Nature of the research problem. Autism is a condition characterized by impaired social interaction, impaired communication, and repetitive and restricted behaviors with the onset before three years of age. Autism spectrum disorder (ASD) is the term utilized to include autism as well as Asperger syndrome, where early language development is relatively preserved. The incidence has been reported as high as 1 in 88 for ASD, and some evidence suggests that it is increasing. Available pharmacotherapeutic interventions for autism are mostly directed at treating the various psychiatric manifestations, including agitation, anxiety, repetitive and obsessive behaviors, and depression. Pharmacotherapy research directed at the core features of autism (language, social interaction, and repetitive and restricted behaviors) is only beginning to reveal potential approaches. Unfortunately, most of these interventions are quite expensive, such that even if they are successful, access may be limited. The proposed study will investigate a cost-effective pharmacological intervention aimed at improving performance on language tasks.

B. Purpose, scope, and methods of the investigation. Our purpose is to examine how markers of sympathetic reactivity predict response to propranolol in autism. Drugs that address the core features of autism are lacking. Agents that decrease NE activity in the brain have shown some benefits in language and social communication, as well as behaviors in autism, in uncontrolled case series studies. Our preliminary work has suggested that agents which decrease NE activity in the brain, such as propranolol, benefit individuals with autism in tasks that require semantic network flexibility. Furthermore, propranolol, unlike other agents under exploration for treatment of core features of autism, is available in a generic form and is inexpensive, increasing its availability for underserved patients. Therefore, our first specific aim was to examine the effect of propranolol in autism on a range of language tasks, and to determine who is most likely to respond, examine whether this response is predicted by sympathetic reactivity as indicated by Galvanic skin response and heart rate variability. We also wished to assess effects on social interaction. We accomplished this by performing cognitive testing on an off propranolol in patients with autism, and determined whether sympathetic reactivity predicted the response to drug. Also, since recent research has demonstrated that the interaction between activated brain regions is decreased in autism, as measured by functional connectivity MRI (fcMRI), we explored the effect of propranolol on fcMRI. We accomplished this by examining fcMRI during task performance on and off propranolol in patients with autism, and determined whether sympathetic reactivity predicted connectivity response to drug. In our pilot experiment we had showed an increase in connectivity on a word categorization task with propranolol. Therefore, our second specific aim was to compare the effect of propranolol on functional connectivity in autism patients with high and low sympathetic reactivity as assessed by Galvanic skin response and heart rate variability.

C. Nature of the findings. As predicted, we demonstrated a significant beneficial effect of propranolol on social interaction and on several cognitive measures including our language tasks, and for many of these tasks, the improvement with propranolol was greatest among those with the greatest sympathetic reactivity on our psychophysical measures. On the imaging aim, analysis is ongoing. However, preliminary analysis suggests a significantly greater task-related functional connectivity after propranolol rather than after nadolol or placebo for verbal processing.

II. Review of the Literature

Most pharmacotherapeutic approaches to autism have been directed at specific psychiatric manifestations. Research into pharmacotherapy for cognitive impairments is only beginning to

be explored. In previous research, benefits in language and social behaviors have been reported with beta-adrenergic antagonists including propranolol in uncontrolled case series studies (Ratey et al, *J Autism Devel Disord*, 1987; 17:439-46). A number of researchers have demonstrated findings suggestive of increased noradrenergic activity in autism (Barthelemy et al, *J Autism Devel Disord*, 1988; 18:583-91). A more recent study countered this (Martineau et al, *Dev Med Child Neurol*, 1992; 34:593-602). However, regardless of the ambient activity of the noradrenergic system in autism, our previous research into the neuropsychological effects of propranolol, as well as the impairments in context utilization known in autism, suggest the potential for cognitive benefits in autism from propranolol. Our pilot data supported such a benefit in this cognitive domain (Beverdors et al, *Neurocase*, 2008; 14:378-83; Beverdors et al, *Cogn Behav Neurol*, 2011; 24:11-7), and our imaging data suggested that functional connectivity is also affected (Narayanan et al, *Brain Imag Behav*, 2010; 4:189-97).

Therefore, to improve our understanding of the cognitive effects of propranolol in autism, we examined a range of tasks affected, with particular attention to language and social interaction. As mentioned above, previous research suggests that language benefits do occur in autism with propranolol (Ratey et al, *J Autism Devel Disord*, 1987; 17:439-46). We also examined whether functional connectivity may serve as an imaging marker for the cognitive effect of propranolol, and to better understand the effects of the drug. In this manner, we determined the extent of language benefits from a putative pharmacological intervention, and began to examine how this benefit occurs, in order to provide evidence for subsequent hypothesis-driven clinical trials. Furthermore, these neuropsychopharmacological and imaging techniques could also be applied to other cognitive pharmacotherapeutic agents in order to better understand their roles in the treatment of autism. We also are helping to define which patients are most likely to respond to propranolol, using measures that indicate increased noradrenergic reactivity. Finally, as propranolol is an inexpensive agent, unlike other agents under study for autism, better understanding of its effects are likely to result in greater access to care among the underprivileged.

III. Study Design and Methods

A. Study design. Our first specific aim was to examine the effect of propranolol in autism on a range of language and social tasks, and to determine who is most likely to respond, examine whether this response is predicted by sympathetic reactivity as indicated by Galvanic skin response and heart rate variability. We accomplished this by performing cognitive testing on an off propranolol in patients with autism, and determined whether sympathetic reactivity predicted the response to drug. Also, since recent research has demonstrated that the interaction between activated brain regions is decreased in autism, as measured by functional connectivity MRI (fcMRI), we explored the effect of propranolol on fcMRI. We accomplished this by examining fcMRI during task performance on and off propranolol in patients with autism, and determined whether sympathetic reactivity predicted connectivity response to drug. In our pilot study, funded by NAAR, we showed an increase in connectivity on a word categorization task with propranolol. Therefore, our second specific aim compared the effect of propranolol on functional connectivity in autism patients with high and low sympathetic reactivity as assessed by Galvanic skin response and heart rate variability. Our hypothesis was that functional connectivity and language would improve more with propranolol in patients with the highest degree of sympathetic reactivity. Our long term goal is to utilize these studies to help to develop future cost-effective, rational pharmacotherapy to help in the treatment of individuals with autism, which can be optimized on an individual basis.

B. Population studied

In Aim 1, 20 patients with ASD participated, as confirmed by ADI-R, IQ >85, ranging in age from 15-31 (16 age 24 or less, 4 age 25 or above); of the 20 there were 19 males and 1 female; of the 20, 19 were Caucasian (2 Hispanic and 17 non-Hispanic), and 1 was American Indian.

In Aim 2, for the autism group, 15 with ASD participated, as confirmed by ADI-R, IQ >85, ranging in age from 15-31 (13 age 24 or less, 2 age 25 or above); of the 15 there were 13 males and 2 females; of the 15, 12 were Caucasian (2 Hispanic and 10 non-Hispanic), 1 was African American, and 1 was American Indian.

In Aim 2, for the control group, 15 participated, IQ >85, ranging in age from 16-30 (12 age 24 or less, 3 age 25 or above); of the 15 there were 13 males and 2 females; of the 15, 12 were Caucasian (12 non-Hispanic), 2 were African American, and 1 was Asian.

C. Sample selection. All autism subjects were recruited from the Thompson Center for Autism and Neurodevelopmental Disorders at the University of Missouri – Columbia. The Thompson Center is a comprehensive, multidisciplinary center that provides diagnostic, treatment and surveillance services for close to 1,000 adults and children with autism spectrum disorders (ASD) and their families each year. Subjects for this study ranged from 15 to 31 years of age as these tasks are predominantly standardized for this age range among youth and adolescents and young adults. Since the cognitive effects of propranolol in individuals without neurodevelopmental diagnoses has already been fairly extensively studied, and our primary interest in this study is to examine the effect of propranolol in autism for potential development of future clinical trials, a control group without neurodevelopmental diagnoses was not incorporated into the design of Aim 1. However, as the effects of propranolol on imaging markers is not established for any population, aside from our one pilot study in ASD, a control group is incorporated into the design of Aim 2 in order to determine the specificity of this imaging effect in ASD. Inclusion/exclusion criteria are below

INCL/EXCL CRITERIA	EXCLUSION FOR DRUG	EXCLUSION FOR IMAGING
ADI-R/GADS and DSM-IV for autism	diabetes	metallic implants
Full scale IQ at least 85, verbal reactive	airway disease	exposure to metal foreign bodies
Native English speaker	unstable thyroid disease	pacemakers
No interacting drugs	bradyarrhythmias	claustrophobia
Non ASD LD (ADD, dyslexia etc)	unexplained syncope	any other risk for MRI exposure
No other major psych diagnosis	pregnancy	pregnancy
No other neuro diagnosis, TBI depression		
No allergy to adhesives.		

D. Instruments used (predicted outcome measures in italics)

SYMPATHETIC REACTIVITY

Galvanic Skin response and heart rate variability

COGNITIVE TASKS purpose

- | | |
|-------------------------------------|--|
| 1. CRA | verbal problem solving (access to semantic network) |
| 2. <i>Anagrams</i> | <i>verbal problem solving (access to semantic network)</i> |
| 3. <i>Word fluency (categories)</i> | <i>verbal fluency (also access to semantic network)</i> |
| 4. Word fluency (letters) | verbal fluency (access to phonological network) |
| 5. HVLТ | verbal memory |
| 6. RCF | spatial memory |
| 7. Stroop | attention, set shifting |
| 8. SDFMS, BAI, SCAS | mood, anxiety |
| 9. <i>GSOM</i> | <i>social interaction</i> |

IMAGING MEASURES

Task-related functional connectivity during *CRA*, *word categorization*, face-matching, n-back, and *word fluency*, resting state connectivity, diffusion tensor imaging.

E. Statistical techniques employed

For Aim 1, within subject comparisons are performed in this crossover study comparing task performance between drug conditions with ANOVA, accounting for test order, and a regression of drug response with GSR and HRV for determination of sympathetic effects. Similar analysis for Aim 2 using the functional connectivity product of image analysis, and compared to placebo as well as to nadolol, to control for peripheral blood pressure and heart rate effects.

IV. Detailed Findings

Aim 1: In the just completed analysis of the first 18 of 20 subjects, we have found a significant benefit from propranolol as compared to placebo for the number of anagrams solved ($F(1,14) = 15.215, p = 0.002, \eta_p^2 = 0.521$) (Fig 1) and for the solution latency for anagrams ($F(1,17) = 6.538, p = 0.020, \eta_p^2 = 0.278$) (Fig 2).

Figure 1 Anagram latency

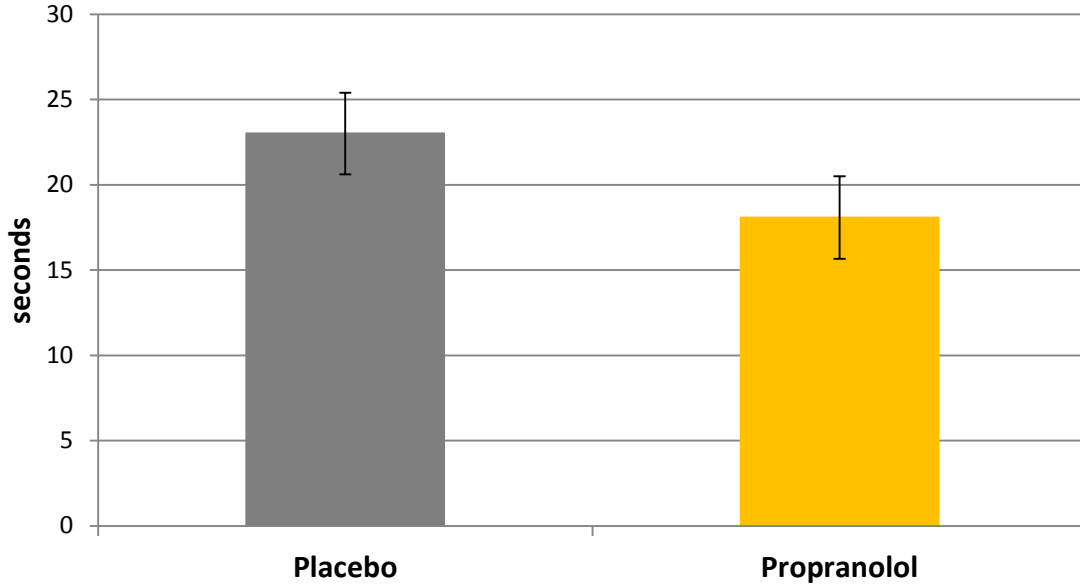
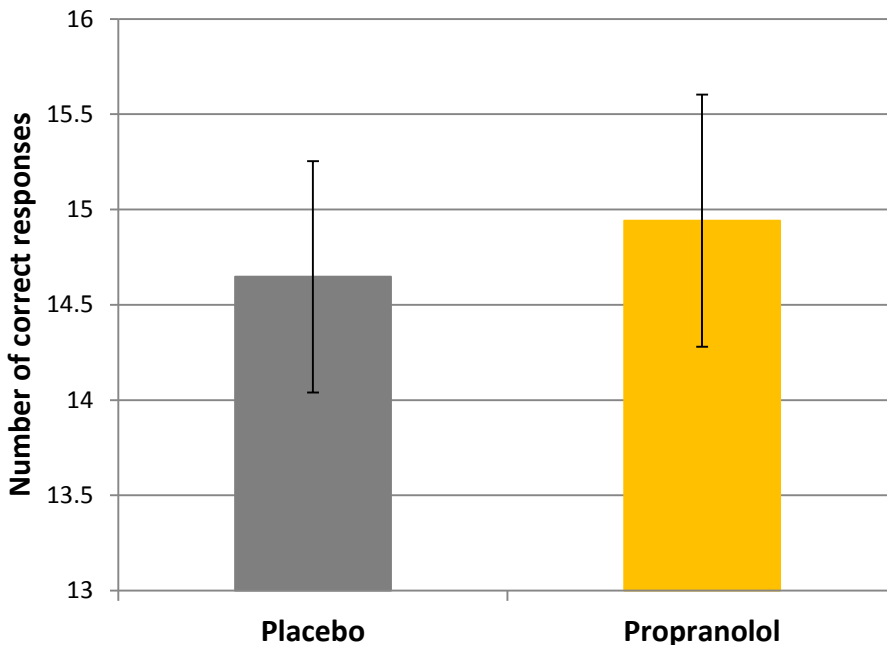


Figure 2 Anagram number solved



Also, we have found a significant beneficial effect of propranolol as compared to placebo for the GSOM, suggesting improvement in social interaction ($F(1,17) = 10.987, p = 0.004, \eta_p^2 = 0.407$) (Fig 3). This appeared to be driven by trends on the sharing of information ($F(1,17) = 3.150, p = 0.094, \eta_p^2 = 0.156$) (Fig 4) and nonverbal communication subscores ($F(1,17) = 3.400, p = 0.083, \eta_p^2 = 0.167$) (Fig 5).

Figure 3 GSOM score

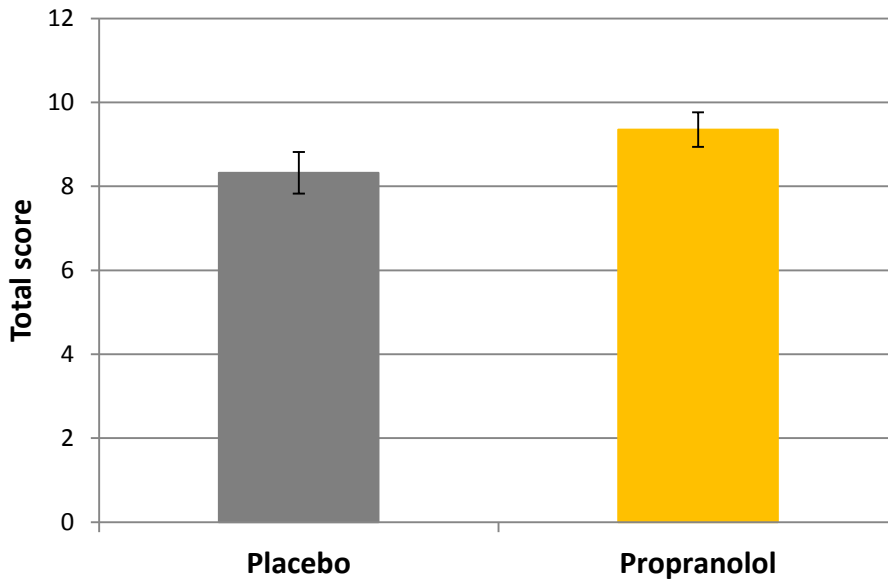


Figure 4 GSOM sharing information

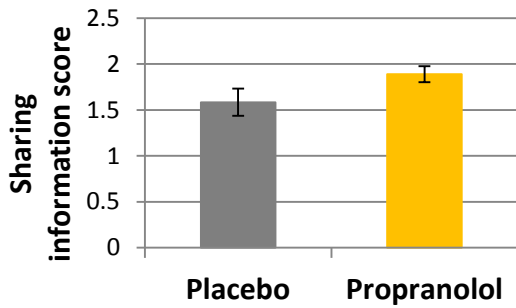
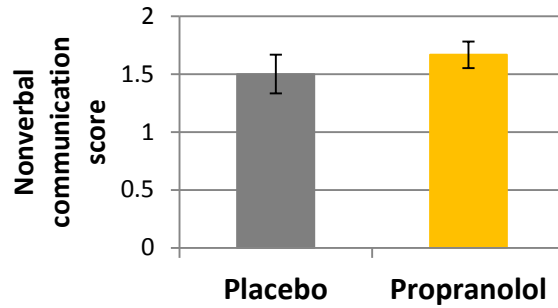
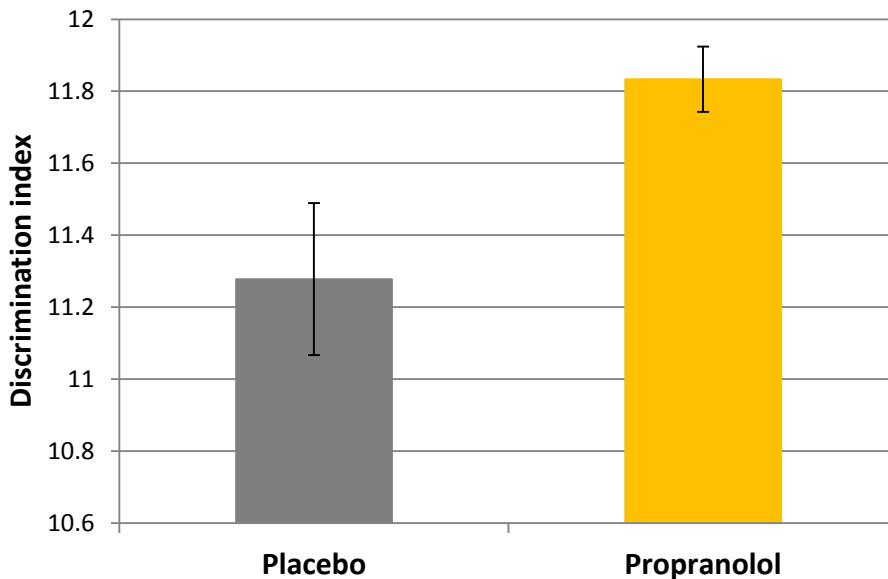


Figure 5 GSOM nonverbal communication



We also found a significant beneficial effect of propranolol on discrimination on the verbal memory task ($F(1,17) = 6.538, p = 0.020, \eta_p^2 = 0.278$) (Fig 6).

Figure 6 HVLT discrimination index



Analyses on the other tasks is ongoing, as is the analysis on the GSR. However, we have completed our first measure of heart rate variability, and as predicted, have found that the heart rate variability was a significant predictor for response to propranolol (difference between scores MCH Research Program Guidelines for Final Report 06/2010

on propranolol and score on placebo) for the verbal learning discrimination index ($F(1,16) = 4.021$, $p = 0.062$, $R^2 = 0.201$) (Fig 7) as well as the social outcome measure ($F(1,16) = 5.174$, $p = 0.037$, $R^2 = 0.244$) (Fig 8).

Figure 7 HVLT discrimination relation to baseline HRV

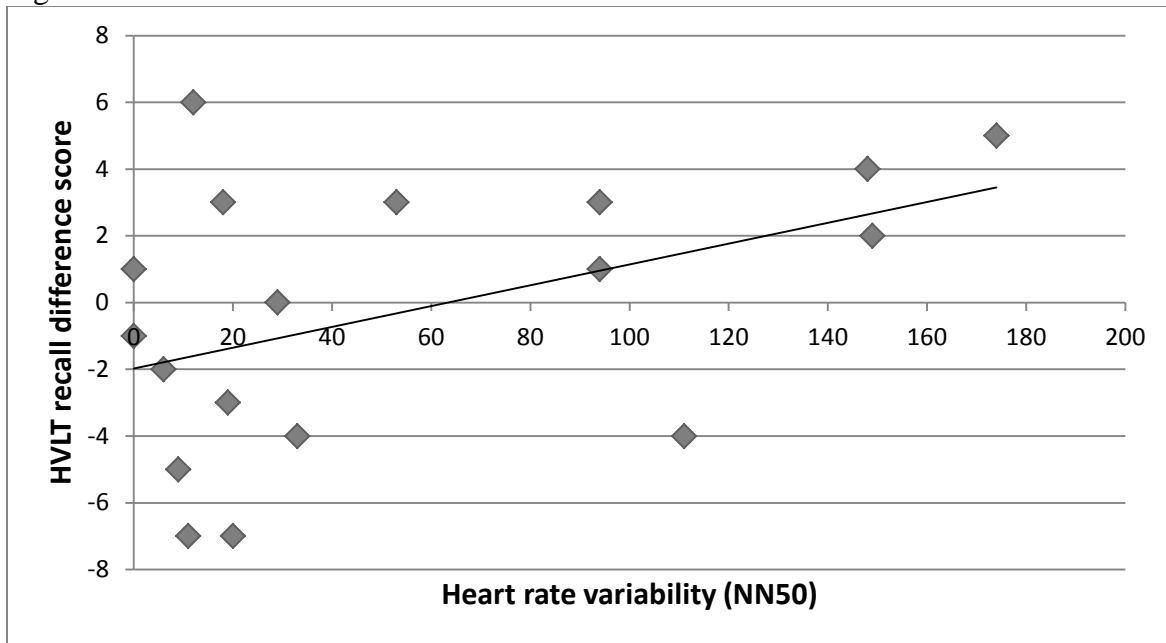
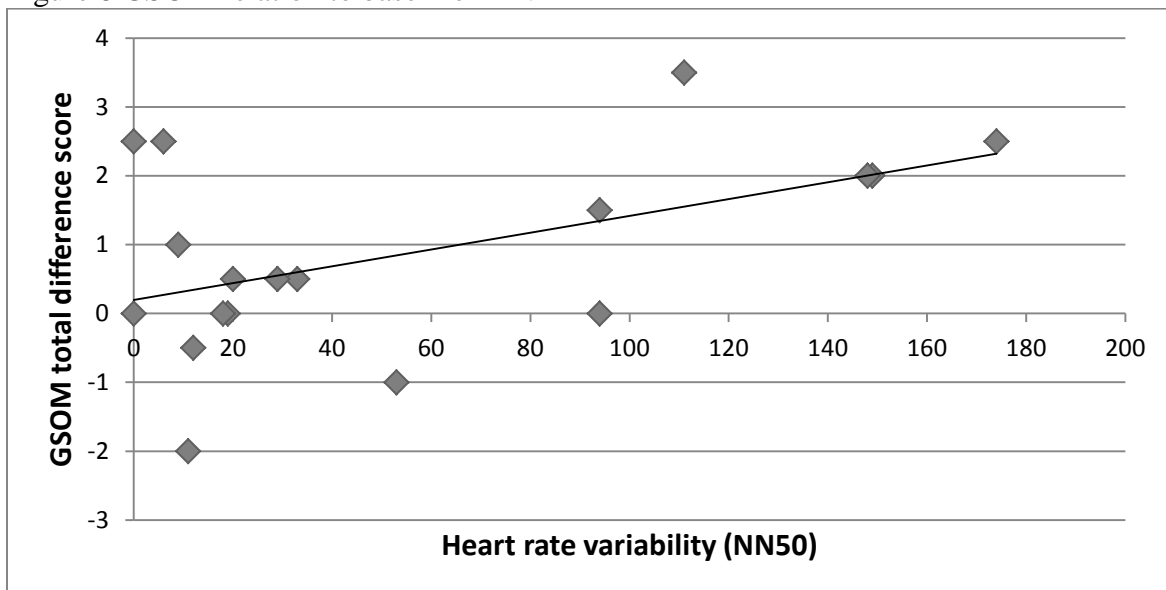
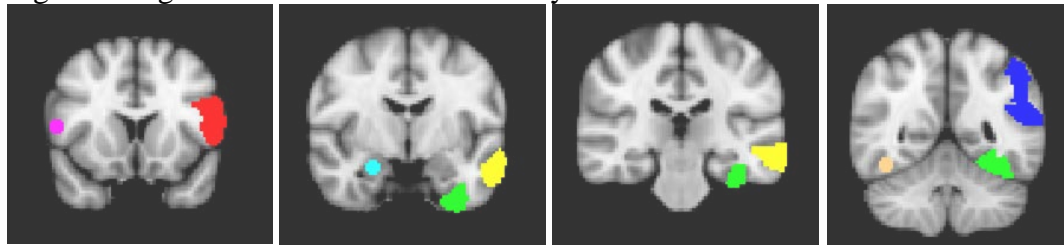


Figure 8 GSOM relation to baseline HRV



For aim 2, fMRI analysis was done for imaging during our verbal tasks (verbal fluency, CRA) as well as an emotional processing task (matching faces demonstrating angry, fearful, or neutral expressions), and functional connectivity, or the interaction between brain regions was calculated. Connectivity was compared across drug conditions. As demonstrated in Figure 9, the a priori language regions were the left inferior frontal, left fusiform, left middle temporal, and left posterior parietal areas, and for emotional processing, the right inferior frontal, right amygdala, and right fusiform areas.

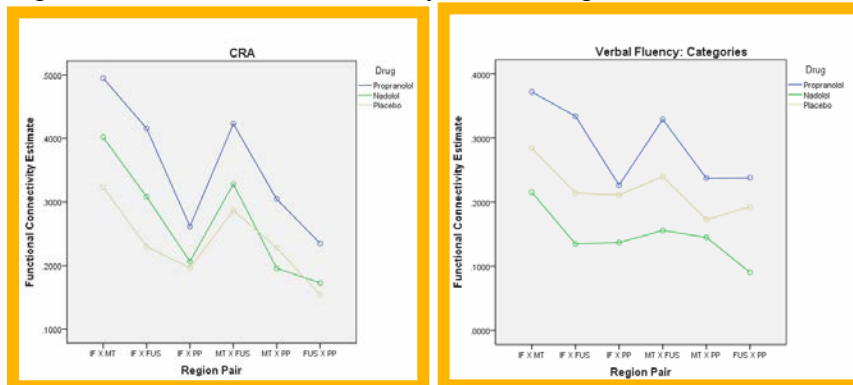
Figure 9 Regions of interest for fMRI study



- L Inferior Frontal
- R Inferior Frontal (pars opercularis)
- L Fusiform
- R Amygdala
- L Middle Temporal
- R Fusiform (fusiform face area, FFA)
- L Posterior Parietal (angular gyrus)

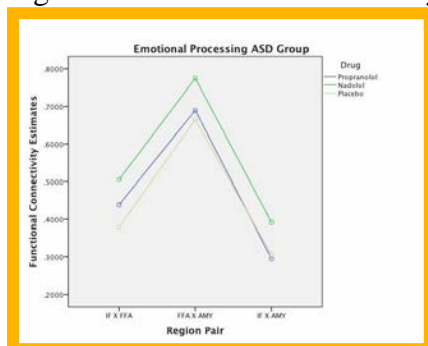
For the first 7 subjects analyzed, a significant main effect of drug was found in the ANOVA comparing all 3 drugs ($F=11.79$, $p<0.001$), with functional connectivity after propranolol being significantly greater than after placebo across all ROIs ($t(142)=4.66$, $p<0.001$) and significantly greater than nadolol ($t(142)=4.71$, $p<0.001$), with no difference between nadolol and placebo (Fig 10), as predicted based on our pilot study.

Figure 10 Functional connectivity across drug conditions for verbal tasks (fluency, CRA)



There was no significant drug effect on functional connectivity during the emotional processing task (Fig 11).

Figure 11 Functional connectivity across drug conditions for emotional processing task



Analysis on the remaining subjects is ongoing, as is the analysis of control subject scans, to see if this imaging effect is specific to ASD, and the HRV and GSR analysis is also ongoing for the fMRI aim. Preliminary analysis is also suggesting that greater baseline functional connectivity is associated with greater response in improved task performance during the imaging study, but this analysis is still in process. Analysis of resting state connectivity, default network connectivity and diffusion tensor imaging is also in process.

V. Discussion and Interpretation of Findings

A. Conclusions to be drawn from findings

These results begin to suggest that propranolol may be beneficial for patients with ASD. Performance on language tasks (Fig 1, 2, & 6) and on social assessments (Fig 3, 4, & 5) improves after single doses of propranolol. This improvement is greatest in those subjects that have the greatest sympathetic reactivity at baseline (Fig 7 & 8). These results help guide future studies by providing the language and social outcomes most likely to respond in a subsequent clinical trial. Furthermore, propranolol increases functional connectivity in autism (Fig 9,10) towards the levels observed in healthy controls, an effect not observed in emotional processing (Fig 9 & 11). This provides a potential mechanism of action for the drug's effect. Baseline functional connectivity also appears to be related to the degree of response to propranolol. Analysis is ongoing regarding the other factors if interest. While these findings are of significant interest and are compelling, caution should be heeded not to overextend the implications of these results. Subsequent studies examining the impact of serial doses of propranolol would be necessary before beginning to infer the clinical impact, in order to confirm that the benefit of propranolol is not lost with repeated doses.

B. Explanation of study limitations

The main limitation of this study is the single dose nature of the study. The single dose challenge allows us to quickly and efficiently determine the effect of propranolol on a wide range of tasks that can subsequently be utilized in subsequent studies of serial doses. However, these serial dose studies would be necessary before establishing the clinical impact. A second limitation is that the study focused on higher functioning older patients, in order to allow more detailed assessment. The question would remain as to the generalizability of these findings to younger and lower functioning patients. Future studies should also examine a larger population.

C. Comparison with findings of other studies

Very limited information is available on the effects of propranolol. However, these findings are consistent with the results suggesting a social and language benefit from propranolol in an unblinded case series in ASD performed over 25 years ago (Ratey et al, J Autism Devel Disord, 1987; 17:439-46), and are consistent with the findings of our pilot studies examining the effects on verbal problem solving and word fluency in ASD (Beversdorf et al, Neurocase, 2008; 14:378-83; Beversdorf et al, Cogn Behav Neurol, 2011; 24:11-7), and with our pilot study suggesting increased functional connectivity with propranolol in ASD (Narayanan et al, Brain Imag Behav, 2010; 4:189-97). This is also consistent with a wide range of effects of propranolol on cognitive performance in healthy individuals in our previous work (for review, Campbell et al, Pharmacol Biochem Behav, 2008; 88:222-9; Alexander et al, J Cogn Neurosci, 2007; 19:468-78).

D. Possible application of findings to actual MCH health care delivery situations

These results will directly move the field forward in the advancement of current knowledge leading to improvements in interventions that address the behavioral health needs of children and adolescents with ASD, which is the objective of the MCH Autism Intervention Research program. Furthermore, as propranolol is available in a generic form, unlike almost all other agents currently under study for the core features of autism, this will improve access to care for the underserved. Finally, our use of markers to predict treatment response will result in progress towards individually optimized therapy for individuals with ASD.

F. Policy implications

There are no direct policy implications of this aspect of the work, but eventual progress towards clinical trials could result in policy implications for the state of the art for treatment of ASD.

G. Suggestions for further research

Based on the discussion above, the first subsequent step would be to perform a follow-up study to establish the effects of serial doses of propranolol. Confirmation of these effects would be necessary before moving towards a larger clinical trial. Second, effects in younger and lower-functioning subjects would be necessary to determine the generalization of these findings. Finally, it would be a significant breakthrough if we could establish whether propranolol, with its known anxiolytic effects as well as its cognitive effects, might augment the impact of current behavioral therapies for ASD, by improving the cognitive milieu while these therapies are being undertaken. Additionally, after our successful development of an animal model of the effects of propranolol on cognition, this also opens the door for animal model studies to determine the effects of beta-1 and beta-2 selective adrenergic antagonists, as propranolol affects both beta-1 and beta-2 receptors, for further future refinement of treatment.

VI. List of products

PUBLICATIONS AND PRESENTATIONS

6 abstracts presented at conferences

1. Hegarty JP II, Ferguson BJ, McKinley CR, Huddleston-Smith K, Christ SE, Beversdorf DQ. Beta-adrenergic modulation of functional connectivity during cognitive flexibility and verbal fluency tasks: a pharmacological autism spectrum disorder study. *Society for Neuroscience, 2012.*
2. Ferguson BJ, Zamzow RM, Reznicek EC, Lewis M, Christ S, Stichter J, Beversdorf DQ. Effects of beta-adrenergic antagonism on social interaction in autism spectrum disorders. *Cognitive Neuroscience Society, 2013.*
3. Hegarty JP II, Ferguson BJ, Zamzow RM, Christ SE, Mazurek MO, Beversdorf DQ. Cortical network flexibility during administration of beta-adrenergic antagonists: an autism spectrum disorder study. *Cognitive Neuroscience Society, 2013.*
4. Beversdorf DQ, Ferguson BJ, Zamzow RM, Reznicek EC, Lewis M, Christ S, Stichter J. Effects of propranolol on social functioning in ASD. *American Academy of Neurology, 2013 (platform presentation).*
5. Hegarty JP II, Ferguson BJ, Zamzow RM, Christ SE, Mazurek MO, Beversdorf DQ. Cortical network flexibility during facial and emotional processing: the effect of β -adrenergic antagonists. *International Meeting for Autism Research, 2013.*
6. Ferguson BJ, Zamzow RM, Reznicek EC, Lewis M, Christ S, Stichter J, Beversdorf DQ. Effects of propranolol on social functioning in autism spectrum disorder. *Combating Autism Act Initiative Meeting, 2013.*

Two more posters to be presented at Society for Neuroscience in November, and multiple submitted to the American Academy of Neurology and International Meeting for Autism Research in 2014.

9 talks

1. The Autism Research Institute Think Tank, Newark NJ April 30-May 1, 2012 (30 min): Effect of propranolol on neuropsychological and imaging markers in ASD
Audience approx. 50
2. Central Society for Neurological Research. French Lick, IN, September 17, 2011 (1 hour): Effect of propranolol on neuropsychological and imaging markers in ASD
Audience approx 10
3. Thompson Center Research Forum September 20, 2012 (1 hour): Translational Autism Research at the University of Missouri
Audience approx. 40
4. Neurology Grand Rounds, July 17, 2013 (1 hour): Effects of propranolol on social functioning in ASD.
Audience approx. 60
5. Central Society for Neurological Research. Grafton, IL, September 20, 2013 (1 hour): Effects of propranolol on social functioning in ASD
Audience approx. 4
6. (Rachel Zamzow-Dr. Beversdorf's graduate student) Thompson Center Research Colloquium August 1, 2013 (1 hour): Effects of propranolol on social and cognitive functioning in ASD
Audience approx 20

7. University of Missouri Developmental Psychology Brown Bag Seminar Series September 23, 2013 (1 hour): Effects of propranolol on social functioning in ASD
Audience approx 30
8. Psy Chi meeting, October 1, 2013 (1 hour)
Translational cognitive neuroscience and stress research in autism
Audience approx 90 undergraduates
9. (Rachel Zamzow-Dr. Beversdorf's graduate student) Life Sciences Fellowship Works in Progress Seminar September 12, 2013 (1 hour): Pharmacological Intervention and Network Organization in ASD
Audience approx 50 graduates

PLANNED PAPERS

Aim 1

Effect of propranolol on social behavior in ASD, and relationship to markers of sympathetic activity (graduate students Rachel Zamzow, Brad Ferguson, Patrick Hecht, faculty David Beversdorf, Micah Mazurek, Janine Stichter)

Effect of propranolol on language tasks in ASD, and relationship to markers of sympathetic activity (graduate students Rachel Zamzow, Brad Ferguson, Patrick Hecht, faculty David Beversdorf, Micah Mazurek)

Effect of propranolol on memory in ASD, and relationship to markers of sympathetic activity (graduate students Rachel Zamzow, Brad Ferguson, Patrick Hecht, faculty David Beversdorf, Micah Mazurek)

Aim 2

Effect of propranolol on functional connectivity during language tasks in ASD (graduate students John Hegarty, Rachel Zamzow, Brad Ferguson, faculty David Beversdorf, Micah Mazurek, Shawn Christ, Jeff Johnson, John Kerns)

Relationship between response to propranolol and baseline functional connectivity (graduate students John Hegarty, Rachel Zamzow, Brad Ferguson, faculty David Beversdorf, Micah Mazurek, Shawn Christ, Jeff Johnson, John Kerns)

Effect of propranolol on resting state connectivity (graduate students John Hegarty, Rachel Zamzow, Brad Ferguson, faculty David Beversdorf, Micah Mazurek, Shawn Christ, Jeff Johnson, John Kerns)

Effect of propranolol on default network connectivity (graduate students John Hegarty, Rachel Zamzow, Brad Ferguson, faculty David Beversdorf, Micah Mazurek, Shawn Christ, Jeff Johnson, John Kerns)

Possible paper examining the relationship with diffusion tensor imaging

Other papers are also possible