AWARD NUMBER: W81XWH-13-1-0306

TITLE: Prenatal Antidepressants and Autism Spectrum Disorder

PRINCIPAL INVESTIGATOR: Charles Vorhees

CONTRACTING ORGANIZATION: Children's Hospital Medical Center
Cincinnati, OH 45229-3026

REPORT DATE: September 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2014	Annual	1Sept 2013-31Aug2014

4. TITLE AND SUBTITLE
Prenatal Antidepressants and Autism Spectrum Disorder

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0306

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Charles Vorhees, Ph.D.

E-Mail: Charles.Vorhees@cchmc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Children's Hospital Medical Center

Cincinnati, Ohio 45229

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT According to the CDC Autism Spectrum Disorder (ASD) prevalence has risen to 1/66, but its causes remains unclear. Croen et al. (2011) reported an increase in ASD in children whose mother's took SSRI antidepressants (ADs) during pregnancy. We are testing this hypothesis in rodents. The study is a 2-year long experiment to be decoded and analyzed in YR02. Groups treated during gestation and lactation are: saline (SAL), citalopram (CIT), fluoxetine (FLX), bupropion (DUP), and a newly added positive control group given valproic acid (VPA) on E12 only. There will be 20 litters/group (except CIT10 with 10) for a total of 110 litters (x8 offspring/litter=880 progeny). All ADs caused unexpected skin irritation which is being successfully treated with a 1.5% topical lidocaine spray. Current number of sperm-positive females enrolled: SAL=12, CIT10=10, CIT5=11, FLX5=11, BUP15=12, and VPA500=10; total=66 litters (~528 offspring), leaving 44 more litters yet to be enrolled. Litters completed: SAL=6, CIT10=5, CIT5=4, FLX=6, BUP=6, and VPA500=3; total=30, leaving 80 litters still in testing or yet to be enrolled (totally 640 progeny). Given the current rate of enrollment, the last litter is to be enrolled in February 2015 and the last litter to finish testing by the end of March 2015.

15. SUBJECT TERMS
Antidepressant drugs, selective serotonin reuptake inhibitors, citalopram, fluoxetine, bupropion, valproic acid, autism spectrum disorder, developmental disorders, prenatal effects, neurodevelopment, rat, behavior, cognitive effects in rats

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	10	USAMRMC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

Project: W81XWH
Progress Report for YR01
Project Title: ***Prenatal Antidepressants and Autism Spectrum Disorder***

Performance Site: Neuroscience Research Laboratories (MLC 7044), Division of Neurology, Location R, laboratories R-1447-1453 and Behavioral Suite R-5280A-B
Cincinnati Children's Research Foundation, Cincinnati Children's Hospital Medical Center
3333 Burnet Ave., Cincinnati, OH 45229-3039
PI: Charles V. Vorhees, Ph.D., Professor

1. INRODUCTION

According to the CDC, Autism Spectrum Disorder (ASD) now occurs at a prevalence of 1/66, however, its causes remain unclear. ASD has been linked to genetic, drug, environmental, and other causes but proof of these remains indeterminate. It is evident that ASD is not one entity but a collection of disorders with a range of etiologies. Multiple genes (e.g., Pten), including some associated with serotonin, have been linked to ASD; so too have drugs (thalidomide, valproic acid, and misoprostol), and recently so have Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants (ADs). ASD has also been linked to prenatal infection (e.g., Rubella) and to a number of environmental agents. Some of these may turn out to be combination effects. It is unlikely that any one will explain all cases of ASD. More realistically, solutions will arise by deconstructing individual contributions to ASD and then reassembling them to determine if there are interactions that when acting in synergistic fashion account for more cases. Unfortunately, we are at the building-block stage. At this stage we need to investigate each promising factor we can identify and rule it in or out. The data of Croen et al. (Croen et al., 2011) that prenatal SSRIs are associated with ASD fits with other data implicating serotonin in ASD. Accordingly, our hypothesis is that prenatal SSRI exposure causes ASD-like behavioral changes in rodents. If correct, this will allow us to determine the connection between these effects and serotonin. In addition, we will test a non-SSRI that we hypothesize will not cause the same effects and therefore serve as a safer alternative for use during pregnancy. Hypothesis/Objective: Prenatal treatment with SSRIs (we will use citalopram because it is the most selective serotonin transporter inhibitor and fluoxetine because it is the prototype SSRI) will result in social deficits, repetitive behavior, compulsiveness, increased sensitivity to stereotypy, reduced learning and memory, reduced cognitive flexibility, impaired sensory filtering, and biochemically will alter serotonin synthesis, reuptake, and receptor expression in exposed offspring and that these changes will be persistent into adulthood, whereas the same exposure to a non-SSRI (we will use bupropion, which is a dopamine transporter (DAT) and norepinephrine transporter (NET inhibitor)) will not cause the same effects. The project is now progressing well, but we encountered some start-up problems as denoted below.

In addition, we added a positive control group to the study post-award using prenatal exposure to valproic acid (VPA), a drug associated with causing ASD in children and an ASD-like phenotype in rats. In addition, the initially planned dose of citalopram proved to be problematic and was therefore reduced by 50%. Citalopram and fluoxetine, and to a lesser extent bupropion, unexpectedly caused skin irritation which, in some rats, progressed to skin lesions. Reducing the dose of citalopram and treating the skin with an aerosol topical lidocaine solution controlled the problem. The study has enrolled 66 litters, but 10 of these were in the Cit10 group which has since been discontinued, therefore, we have enrolled 56 litters in the remaining groups. The main study has 5 groups: SAL, CIT5, FLX5, BUP15, and VPA500. Our target is 20 litters/group, hence, 100 litters. If we include the 10 Cit10 litters, since we are testing them, that changes the target number of litters to 110. At the current rate of breeding we are enrolling about 2 pregnant females/week. Therefore, 110 – 66 = 44 more pregnant females will be needed to complete enrollment. Assuming no losses, 44 litters ÷ 2/week = 22 more weeks of breeding. Starting from today's date (8/11/14) and projecting ahead 22 weeks results in a date of January 12, 2015 to enroll the last pregnant female, however, factoring in current period sperm-positive females

that do not deliver, litters with insufficient numbers or imbalanced sex ratios that fail to meet our enrolment criterion moved our projected last enrollment litter to approximately February 15, 2015.

2 KEY WORDS

Antidepressant drugs, selective serotonin reuptake inhibitors, citalopram, fluoxetine, bupropion, valproic acid, autism spectrum disorder, developmental disorders, prenatal effects, neurodevelopment, rat, behavior, cognitive effects in rats.

3. OVERALL PROJECT SUMMARY

Investigative team: Lindsey Burns, RAII was lead person for animal husbandry, drug treatments, colony maintenance, and behavioral testing with assistance from Jo-El Gilbert (RAII when the project began, now RAIII), and Mary Moran, Senior RA, for statistical analyses; Michael Williams, Ph.D., consultant/collaborator on planning experiments, overseeing progress, and discussing findings during with the project team; a postdoctoral fellow (initially Jennifer Daily, Ph.D., and now Sarah Jablonski, Ph.D. who replaced Jennifer when Jenniefer accepted a position at a clinical trials company, MedPace Inc. in June 2014). Dr. Jablonski is supported from NIH training grant T32 007051 for which Dr. Vorhees is PI. Dr. Jablonski will be the lead person for the immunohistochemistry (IHC) and Western blot analyses; and Arnold Gutierrez, Neuroscience Graduate Student (paid from other sources) will participate in the cellular and behavioral aspects of the project to add to his education by participating in this project while working toward his Ph.D. In June 2014 Ms. Burns took a new position at Assurex Inc.; we are currently searching for a replacement and have made an offer to one interviewed candidate. Jo-El Gilbert has now taken over Ms. Burns' duties as lead technician on this project. Ms. Moran retired June 2014 and Ms. Gilbert is taking over her duties as well. The replacement for Ms. Burns will be an entry-level RAII and perform many of the behavioral tests, animal husbandry and drug treatment under Ms. Gilbert's supervision, assisted by Mr. Gutierrez and Dr. Jablonski.

Statement of Work:

Specific Aim-1 (with minor corrections): Four groups of pregnant rats were planned to be treated during prenatal and neonatal development with saline (SAL), citalopram (CIT), fluoxetine (FLX), or bupropion (BUP) using established, clinically relevant doses with offspring tested as adults for ASD-associated behavior including social, emotional, repetitive/compulsive, sensory-filtering, and cognitive ability and flexibility. IACUC approval was obtained from Cincinnati Children's IACUC for this project. We have obtained DOD ACURO approval subsequent to IACUC approval after a second around of changes by amendment to both animal oversight committees. During the startup phase we ordered drugs from Toronto Research Chemicals, except for fluoxetine which we received by donation from Eli Lilly; we ordered antibodies and other reagents for the IHC experiments from appropriate vendors. Once we received ACURO approval, we ordered sires and the first round of females from Charles River, Raleigh, NC. Simultaneously, we developed a detailed set of experimental protocols, timelines for the implementation of the live-animal portion of the project, and logistical schedules for the testing of the animals. We placed a standing order for 5 females per shipment. Shipments will be spaced ~3 weeks apart. Female rats will be acclimated for 2 weeks prior to cohabitation with males. As females become pregnant (determined by sperm plug and counted as embryonic day (E0)) they are moved to maternity cages with extra bedding and inner enclosures to provide environmental enrichment. Near to and at parturition, females will be provided with Nestlets to use as nesting material and monitored twice daily for delivery; parturition will be designated postnatal day (P) 0. Drug treatments are administered by subcutaneous injection and begin on E6 and continue twice daily (spaced 6 h apart) through E21. No treatments are given on E22 or P0 to prevent interference with labor and delivery. On P1, pups will be briefly removed, sexed, weighed, and randomly culled to 4 males and 4 females per litter. Treatments will continue directly to the pups

from P1-20 at the same dose and frequency per day as given to the dams. Dams will be removed from litters on P28. Same sex littermates will remain together until P42 when they are separated into same sex pairs.

Gravid females are assigned to one of the treatment groups on a rotating basis until all cells of the study are filled. Any females that resorbs her embryos or otherwise fails to deliver will have a replacement added until we obtain 20 litters per group that successfully deliver and reach weaning. Body weights are taken twice daily during treatment and once per week thereafter. Offspring testing begins on P60. We have all the behavioral equipment on hand and have SOPs for the conduct of each test. We test 7 days per week including holidays so that test ages are exact for all offspring regardless of day of birth. Rats are maintained in barrier animal facilities in an AAALAC accredited vivarium under the jurisdiction of veterinarians who are all AAALAS board certified. The behavioral suite is located on R-5 directly adjacent to our animal housing rooms. The behavioral suite consists of 13 rooms. One room is necropsy (for tissue harvesting) and the remaining 12 rooms house behavioral tests: water mazes, acoustic startle, conditioning chambers, swim channel, social apparatus, elevated zero and plus mazes, forced swim test, tail suspension test, locomotor activity, and a Digital gait/stride analysis system (Mouse Specific). A few tests operate alternately in the same room.

Methods: Elevated Zero Maze, SDI Locomotor chambers, Cincinnati water maze, Morris water maze, Crawley Sociability apparatus for rats (Stoelting Instruments), SDI SR-LAB acoustic startle (6 chambers, one that has dual functionality for startle or tremor), 244 cm straight swim channel, cages for marble burying, and an 8-arm radial-arm water maze (RWM).

Progress on Aim-1

The design of this project is as a single, large 2-year experiment. Accordingly, since the experimental data are not intended to be analyzed until the end and are coded in order to keep personnel conducting the experiment blind to treatment groups, there are no results to report at this time. Instead, we report the progress of experimental implementation of the project, i.e., breeding, enrollment, treatment, survival, weaning, number of litters enrolled versus number of litters lost versus number of litters completing testing, and unexpected adverse effects.

Prior to the start of the project, the lab discussed the merits of making a change to the study by adding a positive control in addition to the negative control already part of the plan. The consensus was that a positive control, to the extent that a satisfactory one could be found, would strengthen the study and its potential interpretation of any effects of ADs in relation to known ASD-associated phenotypes. Accordingly, the literature on induced ASD-like behaviors was searched and resulted in our selecting prenatal exposure to valproic acid (VPA). Prenatal VPA exposure is one of the strongest drug-induced associations in human studies at increasing the prevalence of ASD in children. In addition, the animal literature on prenatal VPA exposure has indicated a number of neurobehavioral outcomes with an ASD-associated phenotype. Accordingly, the study was expanded to include a group treated with VPA (600 mg/kg) on a single day (E12) as reported in several papers. The papers chosen for the model were those that used the same strain of rats we are using and similar behavioral outcomes as we use (Kerr et al., 2013;Kim et al., 2011;Kim et al., 2014). The initial dose was based on these studies and was 600 mg/kg on E12. Rats in this group were injected on all days other than E12 with saline, the same injection schedule that rats treated with ADs were subjected to in order to match groups for number of injections and handling. Because the study, planned as 20 (but up to 25) litters per group was now expanded from 4 to 5 groups, this lengthens the time-course of the study in order to prepare at least 100 litters compared with the original design for 80 litters. It is not feasible for this (or any) lab to get 100 or more rats pregnant in a short period of time, nor could we (or any lab) handle that many offspring (100 x 8 = 800 progeny) through the tests we propose. Therefore, we breed rats continuously and enroll 1-3 (on average 2) per week as they become pregnant. This method produces a steady-state workflow which is advantageous in terms of work capacity, but has the one limitation, which is that it prevents random assignment of dams to treatment groups. Instead, we assign females to groups on a rotation basis trying to keep the fill rate in each

group equal. This works well if all sperm positive females are pregnant, all pregnant females deliver, all litters have sufficient numbers of pups to meet the study requirements for enrollment, and all litters survive to adulthood for testing. Since this never occurs in practice, we rebalance the groups during enrollment depending on the outcomes of litters previously enrolled. By the end of the study, we plan on bringing each group to the required number recognizing that perfect balance in final numbers is unlikely which is why we set minimum targets but usually exceed them, hence the allowance of up to 25 females per group to ensure that we obtain at least 20 litters per group that complete all phases of the project.

Enrollment for this project began following ACURO final approval. We began breeding females in December 2013 and enrolled the first litter on or about December 17, 2013. Several noteworthy unforeseen problems emerged soon after the project began. First, the VPA positive control caused severe pregnancy loss. Either dams resorbed and never delivered or delivered and had small litters with small pups. This group also started late while we are determining what drug we would use as the positive control. Out of first 4 sperm positive females treated with 600 mg/kg of VPA on E12, 1 failed to deliver. Of the remaining 3 that did deliver, one had 1 live pup, 1 had 2 live pups, and one had too many pups die soon after birth so the litter was below our enrollment criterion, hence 4 dams produce no viable/enrollment-eligible litters. These results triggered a decision to reduce the VPA dose to 500 mg/kg and start this group over. Thus far, 10 sperm-positive females have been assigned to the VPA500 group. Of these, 8 have delivered and have had enough pups to be enrolled.

A second unexpected effect was that the AD treated dams (fluoxetine, citalopram, and bupropion) began developing skin irritation during pregnancy after about 10 days of treatment. This effect, once it emerged, did not subside even after parturition when the dams received no further injections. Rotating injection sites to new positions did not alleviate the problem. Moreover, the pups, after several weeks of receiving direct treatment postnatally also developed skin irritation. Scabs on dams and pups were generally not at the injection sites but appeared on the flanks and shoulders. These irritation zones appeared to be caused by scratching. In consultation with the chief veterinarian, Dr. Keller, several topical treatments were tried to alleviate the scratching. The product that proved most effective was a spray (Dermacool) containing 1.5% lidocaine as the active ingredient. In order to minimize skin irritation, we started treating all dams on E15 and all pups starting on P21 whether they had skin lesions or not. This greatly reduced the frequency and severity of skin problems in the AD-treated groups and appears to have no untoward effects.

A third problem that developed was in the citalopram 10 mg/kg group. The plan was to treat this group with 10 mg/kg x 2 doses/day based on data we had using 7.5 mg/kg x 2/day given on P11-20 in which he saw no skin irritation or other adverse effects but resulted long-term cognitive deficits in the offspring as adults (Schaefer et al., 2013). However, when we gave 10 mg/kg x 2/day starting during pregnancy and continuing postnatally to P20, skin lesions developed in both the dams and the pups. Treatment with Dermacool, once we discovered this treatment, helped but this group continued to develop skin lesions more than did animals treated with fluoxetine or bupropion and some of the lesions were severe. In addition, the citalopram 10 mg/kg group offspring showed inhibited growth. Therefore, we decided to reduce the citalopram dose to 5 mg/kg. This brought this group's dose into alignment with fluoxetine. Originally, we planned on using 10 mg/kg for fluoxetine as well, but before the study started a further review of the literature and data shared with us by Eli Lilly caused us to reconsider the fluoxetine dose. Fluoxetine at 5 mg/kg/dose was predicted to be better tolerated, to be consistent with human doses, and data the from Eli Lilly based on their internal experience suggested that 10 mg/kg of fluoxetine would be toxic when given during pregnancy and/or lactation. The dose of bupropion is higher than for the two SSRIs at 15 mg/kg but this is based on clinical and animal data showing that a higher dose of this drug is required to obtain efficacy in humans and antidepressant effects in animals. Moreover, we find that this dose of bupropion is well-tolerated by the dams and offspring and this drug produces the fewest skin problems. In order to properly document the effects of 10 mg/kg of citalopram, this group has been continued until 10 litters were obtained that were successfully treated, delivered, reached testing age and have been or will complete the full testing scheme. Hence, going forward, the main groups of the study

are SAL, CIT5, FLU5, BUP15, and VPA500. At the time of this writing, 12 sperm-positive females have been assigned to the SAL group of which 11 have delivered litters that met the enrollment criterion, 1 is still pregnant; 11 sperm-positive females have been assigned to the CIT5 group of which 10 have been enrolled, and 1 is still pregnant; 11 sperm-positive females have been assigned to the FLU5 group of which 10 have been enrolled, and 1 is still pregnant;, 12 sperm-positive females have been assigned to the BUP15 group of which 11 have been enrolled, and 1 is still pregnant, and 10 sperm-positive females have been assigned to the VPA500 group of which 8 have been enrolled, and 2 are still pregnant.

Because behavioral testing is extensive and does not begin until offspring reach adulthood, only a limited number of litters have gone through the entire protocol and fished all tests. The breakdown of litters by group completing the entire protocol is as follows: SAL = 6, CIT10 = 5, CIT5 = 4, FLX = 6, BUP = 6, VPA500 = 3. In the coming months the CIT5 and VPA500 groups will have 2 litters assigned per rotation in order to catch these groups up to the others and rebalance the study.

After discussion of the behavioral protocol by the entire lab, the lab and PI made several adjustments. To reiterate the basic layout: On P1 each litter is randomly culled to 8, with randomized retention of 4 males and 4 females. Offspring within each litter are designated as M/F pairs A, B, C, or D. To maximize efficiency, control for litter effects, and minimize any given offspring from having an extended testing sequence, each of pair is placed into a different test sequence or path. The redesigned protocol has had these test series or paths slightly adjusted to the following:

Pair

A Straight swim channel, CWM-acq, CWM-shift (amended), Locomotor activity (3 phases: habituation with no treatment, activity following Saline injection, and activity following MK801 injection)

B Straight swim channel, MWM: Acquisition, acquisition-probe; reversal, reversal-probe; shift, shift probe (one 24 & one 72 h after the last training trial [72 h probe added), MWM-cued, Locomotor activity as above for pair-A except the challenge drug is (+)-amphetamine rather than MK801 [unchanged]

C Elevated Zero Maze (EZM), Locomotor activity (1 h only), Marble burying, Acoustic startle with prepulse inhibition (PPI), Crawley Social Preference test, Radial water maze (RWM) [new], FST (forced swim test)

D P60: half the offspring of this pair are transcardially perfused for brain IHC; and the other half have brains dissected and frozen for HPLC-ECD monoamine determinations [unchanged].

Specific Aim-2: As adults, pair-D will are euthanized and brains collected, dissected and markers of 5-HT analyzed in regions of interest (ROI) for serotonin (5-HT), 5-HT transporter (SERT), tryptophan hydroxylase-2 (TpH2) and selected 5-HT receptors (5HT1A, 5HT2, 5HT6 and 5HT7) by IHC. ROIs showing changes will be analyzed in separately prepared animals by Western blot using standard methods to semi-quantitatively estimate magnitude of effects. Alternate sections will be stained for tyrosine hydroxylase (TH), dopamine transporter (DAT), norepinephrine transporter (NET), and dopamine D1 and D2-like receptors (DAD1 and DAD2) to ensure that effects on 5-HT markers are specific and markers not predicted to be affected are in fact not affected.

Methods: We have both a sliding-freezing stage microtome (Leica) and vibratome for brain tissue slicing for IHC and access to a core Cryostat. We have a Zeiss Z-1 Axio Plan Apotome fluorescence, bright/dark field microscope with Z-stack, automated stage and Zeiss software for stitching into composites. We have standard wet lab equipment for neurochemistry including Thermo-Fisher ESA HPLC with autosampler and UV/ECD detectors and software, analytical balances, centrifuges, MED64 brain slice electrophysiology rig, multiple PCR machines, Gel imager transilluminator, and Microtiter Plate Reader (Molecular Devices SpectraMax), and other lab equipment, including electrophoresis gear for Western blot analysis and densitometry semi-quantification.

Timeline (revised): We originally estimated it would take 48 weeks to enroll 80 females. Now with 110 litters (20/main groups = 100 + 10 CIT10 litters) in the design we estimate it will take longer for enrolment to be completed. Given that we have 66 litters enrolled and need 110 with an enrollment rate averaging 2/week, and allowing for make-up litters caused by litters where one or more pups die after enrollment, enrollment should be completed by mid-February 2015. Given 22 days of gestation, and testing that begins on P60 and ends on P85 (a total of 107 days or ~15 weeks) this indicates that the last litter should complete testing March 31, 2015.

Once the histology areas of interest are refined, the weeks after histological analysis will be for Western blot analyses on those regions assessed to be affected based on qualitative histology. Once the live animal portion of the project is complete, the study will be decoded and data extracted from software used to collect the data and moved to Excel files for cross-checking against records of notes made by technicians during testing of for irregularities during testing in their contemporaneous notes. Once satisfied that the data are correct, Ms. Gilbert will generate means and SEMs on all dependent variables. Then she and the PI will plan which ANOVA models are most appropriate to the data following the guidelines in the Statistical Analysis section of the Project Narrative. She and the PI will also determine the layout of graphs and tables (figures made using GraphPad). Significant findings and graphs will be reviewed as they become available by the project team at lab meetings. Based on findings, follow-up experiments will be planned and implemented, time permitting.

Progress on Aim-2: Tissues are being harvested on pair-D in each litter as they reach P60.

4. KEY RESEARCH ACCOMPLISHMENTS

As noted above, this is a single, long-term experiment with all groups coded to research personnel. The codes will not be broken until the live animal portion of the experiment is completed which will not be until 75% of the way through YR02, therefore, there are no results to report at this time. The project is progressing well and should be completed on time barring any unforeseen developments.

5. CONCLUSIONS

For the reasons outlined above, no results are yet available, hence no conclusions are possible at this stage of the project.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Since there are no results, there can be no publications, abstracts or presentations at this point of the project.

References

Croen LA, Grether JK, Yoshida CK, Odouli R, Hendrick V (2011) Antidepressant Use During Pregnancy and Childhood Autism Spectrum Disorders. Arch Gen Psychiatry.

Kerr DM, Downey L, Conboy M, Finn DP, Roche M (2013) Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav Brain Res 249:124-132.

Kim KC, Kim P, Go HS, Choi CS, Yang SI, Cheong JH, Shin CY, Ko KH (2011) The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. Toxicol Lett 201:137-142.

Kim KC, Lee DK, Go HS, Kim P, Choi CS, Kim JW, Jeon SJ, Song MR, Shin CY (2014) Pax6-Dependent Cortical Glutamatergic Neuronal Differentiation Regulates Autism-Like Behavior in Prenatally Valproic Acid-Exposed Rat Offspring. Mol Neurobiol 49:512-528.

Schaefer TL, Grace CE, Braun AA, Amos-Kroohs RM, Graham DL, Skelton MR, Williams MT, Vorhees CV (2013) Cognitive impairments from developmental exposure to serotonergic drugs: citalopram and MDMA. Int J Neuropsychopharmacol1-12.